norden

Nordic Nutrition Recommendations 2004

Integrating nutrition and physicalactivity

NORD 2004: 13

Nordic Nutrition Recommendations NNR 2004

Integrating nutrition and physical activity

4th edition

Nordic Nutrition Recommendations 2004. 4th edition
Integrating nutrition and physical activity
Nord 2004:013

ISBN 10: 92-893-1062-6
ISBN 13: 978-92-893-1062-8

Graphic production and design: Kjell Olsson
Print: Scanprint as, Århus 2006
Copies: 1st 6,000; 2nd 4,000; 3rd 1,500
Printed on environmentally friendly paper.
This publication may be purchased from any of our sales agents
listed on www.norden.org/order
Other Nordic publications are available at www.norden.org/publications

Printed in Denmark

Nordic Council of Ministers
Store Strandstræde 18
DK-1255 Copenhagen K
Phone (+45) 3396 0200
Fax (+45) 3396 0202

Nordic Council
Store Strandstræde 18
DK-1255 Copenhagen K
Phone (+45) 3396 0400
Fax (+45) 3311 1870

www.norden.org

Nordic co-operation

Nordic cooperation is one of the world's most extensive forms of regional collaboration, involving Denmark, Finland, Iceland, Norway, Sweden, and three autonomous areas: the Faroe Islands, Greenland, and Åland.

Nordic cooperation has firm traditions in politics, the economy, and culture. It plays an important role in European and international collaboration, and aims at creating a strong Nordic community in a strong Europe.

Nordic cooperation seeks to safeguard Nordic and regional interests and principles in the global community. Common Nordic values help the region solidify its position as one of the world's most innovative and competitive.

The Nordic Food Policy Co-operation

The Nordic Committee of Senior Officials on Food Issues is concerned with basic Food Policy issues relating to food and nutrition, food toxicology and food microbiology, risk evaluation, food control and food legislation.

The co-operation aims at protection of the health of the consumer, common utilisation of professional and administrative resources and at Nordic and international developments in this field.

PREFACE

This 4th edition of the Nordic Nutrition Recommendations, NNR 2004, has been produced by a project group nominated by the Working Group on Diet and Nutrition, NKE, under the Nordic Committee of Senior Officials on Food Issues, EK-Livs. The project group was established in 2000.

The project group consisted of Wulf Becker, chair, Sweden; Åsa Brugård Konde and Eva-May Ohlander, secretariat, Sweden; Niels Lyhne and Agnes N. Pedersen, Denmark; Antti Aro and Mikael Fogelholm, Finland; Jan I. Pedersen, Jan Alexander, Sigmund A. Anderssen and Helle Margrete Meltzer, Norway; and Inga Þórsdóttir, Iceland. Brittmarie Sandström, Denmark, was a member of the group, but sadly passed away in October 2002. A working group on recommendations for children was established consisting of Inga Þórsdóttir, chair, Iceland; Olle Hernell, Sweden; Britt Lande, Norway; Olli Simell, Finland; Kim Fleischer Michaëlsen, Denmark; and Åsa Brugård Konde, secretariat, Sweden. The following experts were engaged for specific chapters: Nils-Georg Asp, Sweden (carbohydrates), Ingegerd Johansson, Sweden (caries, fluorine), Rune Blomhoff, Norway (vitamin A, antioxidants), Lars Dragsted, Denmark (antioxidants), Susanne Højbjerg Bügel, Denmark (copper), Lars Ovesen, Denmark (iodine), Maria Lennernäs, Sweden (eating pattern), and Ingibjörg Gunnarsdóttir, Iceland (pregnancy, lactation). Food-based recommendations were evaluated by a working group consisting of Ellen Trolle, Denmark; Liisa Valsta, Finland; Hólmfríður Þorgeirsdóttir, Iceland; Lars Johansson, Norway; and Heléne Enghardt Barbieri and Wulf Becker, Sweden.

Valuable comments and contributions were received from a large number of nutrition experts, national agencies and institutes in the Nordic countries in response to referrals in 2003 and 2004. A plenary discussion was held at the 8th Nordic Nutrition Conference in Tønsberg, Norway, in June 2004.

The Nordic Nutrition Recommendations NNR 2004 were officially approved by the Nordic Council of Ministers for Fishery, Agriculture, Forestry and Food, at their meeting on the 13th of August 2004.

This report consists of NNR 2004 and the comprehensive background documentation compiled by the project group.

DECLARATION OF INTERESTS

Members of the project group *Nordic Nutrition Recommendations 2000–2004* hereby declare that neither they nor any member of their immediate family has any direct or indirect interests relating to the work in the project mentioned above, other than the professional positions the group members hold that entitle them to membership of the said project group.

Jan Alexander *Norway*	Sigmund A. Anderssen *Norway*	
Antti Aro *Finland*	Wulf Becker *Sweden*	Mikael Fogelholm *Finland*
Niels Lyhne *Denmark*	Helle M. Meltzer *Norway*	Agnes N. Pedersen *Denmark*
Jan I Pedersen *Norway*	Inga Þórsdóttir *Iceland*	

CONTENTS

INTRODUCTION

The Nordic countries have for several decades collaborated in setting guidelines for dietary composition and recommended intakes of nutrients. Similarities in dietary habits as well as in the prevalence of diet-related diseases, such as cardiovascular disease, osteoporosis, obesity and diabetes, warranted a focus on the gross composition of the diet, *i.e.* the intake of fat, carbohydrate and protein as contributors to the total energy intake. Already in 1968 medical societies in Denmark, Finland, Norway, and Sweden published a joint official statement on 'Medical aspects of the diet in the Nordic countries' (*Medicinska synpunkter på folkkosten i de nordiska länderna*). The statement dealt with the development of dietary habits and the consequences of an unbalanced diet for the development of non-communicable diseases. Recommendations were given both for the proportion of fat in the diet and the fat quality, *i.e.* a reduced intake of total fat and saturated fatty acids and an increase in polyunsaturated fatty acids. Food-based guidelines were also included, aiming at an increased consumption of fruit, vegetables, potatoes, low-fat milk, lean meat and cereal products, and a limitation of the consumption of sugar and sugar-rich foods.

The first official Nordic Nutrition Recommendations (NNR), issued in 1980, emphasised a reduction in total fat intake to less than 35% of the energy consumed and an increased intake of carbohydrates and dietary fibre. Subsequent editions of NNR were published in 1989 (2nd edition) and 1996 (3rd edition) and included a further limitation of fat intake to 30% of energy intake, more detailed recommendations on fat quality and a focus on energy balance. NNR are an important basis for various uses in the area of food, nutrition and health policy, for formulation of food-based dietary guidelines and for diet and health-related campaigns.

In the present 4th edition, NNR 2004, the official Nordic Nutrition Recommendations are presented and summarized in *Chapters 1* and *2*. Subsequent chapters provide the scientific background documentation and some practical aspects of its derivation and use. The project group has reviewed and evaluated scientific work, national and international recommendations and expert reports. Changes have been introduced

only when strong scientific evidence has evolved since the 3rd edition. A Nordic perspective has been accounted for in setting the recommendations.

In the 4th edition a chapter on physical activity has been added and interaction with physical activity has been taken into account for the individual nutrient recommendations wherever appropriate. The chapter on energy requirement has been thoroughly reviewed and reference weights and physical activity levels updated. A new chapter on food-based recommendations has also been included. The chapters on eating pattern, antioxidants and breastfeeding have been expanded. Compared to the 3rd edition, the recommendations on the proportions of energy yielding nutrients remain virtually unchanged. The values for recommended daily intakes are in many cases also unchanged.

The recommended intake (RI) of vitamin A (women) has been lowered, while it has been increased for vitamin D (children and adults up to 60 y), vitamin C (adults) and folate (the same RI for all women of reproductive age; but increased for pregnant and lactating women). Regarding iron, the RI ranges for women of reproductive age included in the 3rd edition have been replaced by a single value. In many cases the values for infants and children are derived from adult data using either body weight or energy requirement as a basis for the estimations.

Recommendations for copper intake are introduced, while no recommended intakes are set for biotin, pantothenic acid, chromium, fluorine, manganese and molybdenum due to insufficient data, *i.e.* no change compared to the 3rd edition.

The primary aim of the NNR 2004 report is to present the scientific background of the Nordic nutrition recommendations and their application. In addition, the report can be used as a complement to textbooks in the field of nutrition, but it does not attempt to give a comprehensive overview of metabolism, physiology and clinical aspects of each nutrient or topic.

A secondary aim for NNR 2004 is to function as a basis for national recommendations adopted individually by the Nordic countries.

The 4th edition, the Nordic Nutrition Recommendations 2004 is published as a Nord report by the Nordic Council of Ministers. A summary and the main recommendations are also published in the *Scandinavian Journal of Nutrition* (2004; 48 (4): 178–187).

Aims and content of NNR

The Nordic Nutrition Recommendations NNR 2004 are to be interpreted as guidelines for the nutritional composition of a diet which provides a

basis for good health. The basis for setting recommendations is defined for each individual nutrient using available scientific evidence. As new scientific knowledge emerges with time, the NNR have to be reassessed when appropriate and should therefore not be regarded as definitive.

NNR are based on the current nutritional situation in the Nordic countries and are to be used as a basis for planning a diet which:

– satisfies the nutritional needs, *i.e.* covers physiological requirements for growth and function
– is a prerequisite for an overall good health and contributes to a reduction of risk for diet-associated diseases

NNR are primarily valid for groups of healthy individuals. For individuals with disease and for groups with special needs, the dietary composition may have to be adjusted accordingly.

NNR are valid for the average intake over a longer period of time, *e.g.* over at least a week, since the dietary composition may vary from meal to meal and from day to day. The recommended intakes refer to the amounts of nutrients ingested. Losses during food preparation, cooking etc. have to be taken into account when the values are used for planning diets.

NNR can be used:

– as guidelines for planning diets for groups
– as a basis for teaching and dietary information
– as a basis for food and nutrition policy
– as a basis for evaluation of dietary intake

NNR cover recommendations and criteria to be used in planning diets for groups and in evaluating dietary intakes. The recommendations include the following components, which are presented in *Chapters 1* and *2*:

1. Recommended intake of fat, carbohydrates and protein as percentages of total energy intake (E%). Recommendations on dietary fibre.
2. Recommended intake of vitamins and minerals. The values include a safety margin accounting for variations in requirement and physiological factors influencing the requirement, which makes it likely that a diet containing these amounts will be sufficient and adequate for practically the entire population.
3. Reference values for energy intake.
4. Recommendations for physical activity.
5. Recommendations for salt intake.

6. Recommendations for alcohol consumption.
7. Reference values for evaluation of nutrient intake.

Chapter 3 deals with the background and basis of the recommendations, while *Chapter 4* gives an introduction to the practical uses and applications of NNR. Subsequent chapters deal with the scientific background for establishing recommendations for each of the components included, as well as some additional topics such as breastfeeding, food-based dietary guidelines, eating pattern and antioxidants.

CHAPTER 1

Planning diets for groups

Recommended intake of fat, carbohydrates and protein as a percentage of total energy intake (excluding energy from alcohol)

Recommendations for adults and children from 2 years of age

FAT Intake of saturated plus trans fatty acids should be limited to approximately 10% of the total energy intake (E%). Trans fatty acids from partially hydrogenated fats should be limited as much as possible. Cis-monounsaturated fatty acids should provide 10–15 E% and polyunsaturated fatty acids 5–10 E%, including approximately 1 E% from n-3 fatty acids. Fat (calculated as total fat content including glycerol and other lipid components) should provide 25–35 E%. The population goal is 30 E% from fat, which should be used for planning purposes.

The fat composition of the diet should be modified, primarily by reducing the intake of both saturated and trans fatty acids. A reduction in the intake of foods rich in saturated fatty acids is generally accompanied by a reduction in the intake of cholesterol. The saturated fatty acids lauric, myristic and palmitic acid, trans fatty acids and cholesterol increase the serum LDL-cholesterol concentration, which is a strong risk factor for coronary heart disease. For the prevention of coronary heart disease, it is most important to reduce the intake of these fatty acids. In addition, moderation in total fat intake is important for the prevention of obesity. This makes it possible to increase the consumption of low-fat foods that are rich in nutrients and other compounds that may also be important for the prevention of cardiovascular diseases and cancer.

Essential polyunsaturated n-6 and n-3 fatty acids should provide at least 3 E%, including at least 0.5 E% from n-3 fatty acids. For pregnant and lactating women the essential fatty acids should contribute at least 5 E%, including 1 E% from n-3 fatty acids. An intake of polyunsaturated fatty acids exceeding 10 E% is not recommended, because this may theoretically increase the risk of lipid peroxidation. Furthermore, there are no health benefits associated with a higher intake. Cis-monounsaturated fatty acids (oleic acid) are almost as effective in lowering serum

LDL-cholesterol concentration as polyunsaturated fatty acids when substituted for saturated fatty acids.

CARBOHYDRATES AND DIETARY FIBRE Carbohydrates[1] should provide 50–60% of the total energy intake (E%). The population goal is 55 E% from carbohydrates, which should be used for planning purposes. For adults, the intake of dietary fibre should be 25–35 g/d, i.e. approximately 3 g/MJ. Refined sugars should not exceed 10 E%[2].

This recommendation means for most people an increase in the intake of both carbohydrates and dietary fibre. The increase should consist in the first instance of foods naturally rich in carbohydrates and dietary fibre, *i.e.* cereal products and potatoes, vegetables, fruit and berries, which are also good sources of vitamins and minerals. An adequate balance between carbohydrates and fat in combination with a high fibre content in the diet contributes to a reduced risk of developing overweight and associated diseases. An adequate intake of dietary fibre reduces the risk of constipation and can most likely contribute to protection against obesity and colon cancer. Intake of appropriate amounts of dietary fibre from a variety of foods is important for children as well. From school age the intake should gradually increase to reach the recommended level during adolescence. To ensure an adequate intake of essential nutrients and dietary fibre, especially in children and adults with a low energy intake, a limitation of the intake of refined sugars[2] is necessary. Limitation of the intake of refined sugars from drinks may be important to prevent obesity. It is recommended that the intake of refined sugars should not exceed 10 E%. Frequent consumption of foods rich in sugars should be avoided in order to reduce the caries risk.

PROTEIN Protein should provide 10–20% of the total energy intake (E%). The population goal is 15 E% from protein, which should be used for planning purposes.

Protein might provide less than 10 E%, but in order to achieve a varied diet and according to Nordic dietary habits the recommendation is 15 E% for planning purposes. This intake of protein should more than adequately meet the requirements for essential amino acids.

1. Carbohydrates including dietary fibre.
2. Refined sugars include sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations such as food components used as such or added during food preparation and manufacturing.

At very low energy intakes (< 6.5 MJ/d), a protein content providing more than 15 E% may be necessary for planning purposes.

Recommendations for children up to 2 years of age

Exclusive breastfeeding is recommended for infants during the first 6 months. Recommendations for intake of energy yielding nutrients for children 6–23 months are given in *Table 1.1.* The energy percent from fat should decline successively from the high level during the first year of life to reach the level recommended for children and adults at the age of 2 years. During the same period protein intake should increase from about 5 E% (the level in breast milk) to the recommended content of 10–20 E% for older children and adults.

n-6 fatty acids should contribute at least 4% of the total energy intake (E%) for children 6–11 months, and 3 E% for children 12–23 months of age. n-3 fatty acids should contribute at least 1 E% for children 6–11 months and 0.5 E% for children 12–23 months. During the first year, the intake of trans fatty acids from partially hydrogenated fats should be kept as low as possible. From 12 months the recommendation for older children and adults on saturated and trans fatty acids should be used.

Table 1.1 Recommended intake of fat, carbohydrates and protein expressed as g per MJ and as E% for children 6–23 months[a]. Within the age intervals, a gradual reduction in fat intake is recommended throughout the period from the highest value to the lowest, with a corresponding increase in carbohydrates

	g per MJ	Energy percent (E%)
6–11 months		
Protein	4–9	7–15
Fat	8–12	30–45
Carbohydrates[b]		45–60
12–23 months		
Protein	6–9	10–15
Fat	8–9	30–35
Carbohydrates[b]		50–55

a As exclusive breastfeeding is the preferable source of nutrition for infants < 6 months, no recommendations for fat, protein and carbohydrate intake are given. For non breast fed infants, it is recommended that the values for infant formula proposed by the European Commission Scientific Committee on Food (2003) be used. If complementary feeding has started at 4–5 months, the intakes recommended for 6–11 month old infants should be used.

b Intake of refined sugars should not exceed 10 E%.

Table 1.2 Recommended intake of certain nutrients, expressed as average daily intake over time, for use in planning diets for groups[a]. The requirements are lower for almost all individuals

Age years	Vit. A RE[c]	Vit. D[d] µg	Vit. E α-TE[e]	Thiamin mg	Riboflavin mg	Niacin NE[f]	Vit. B_6 mg	Folate µg	Vit. B_{12} µg	Vit. C mg
< 6 mo[b]	–	–	–	–	–	–	–	–	–	–
6–11 mo	300	10	3	0.4	0.5	5	0.4	50	0.5	20
12–23 mo	300	10	4	0.5	0.6	7	0.5	60	0.6	25
2–5 y	350	7.5	5	0.6	0.7	9	0.7	80	0.8	30
6–9 y	400	7.5	6	0.9	1.1	12	1.0	130	1.3	40
Females										
10–13	600	7.5	7	1.0	1.2	14	1.1	200	2.0	50
14–17	700	7.5	8	1.2	1.3	15	1.3	300	2.0	75
18–30	700	7.5	8	1.1	1.3	15	1.3	400	2.0	75
31–60	700	7.5	8	1.1	1.3	15	1.2	300[g]	2.0	75
61–74	700	10	8	1.0	1.2	14	1.2	300	2.0	75
≥ 75	700	10	8	1.0	1.2	13	1.2	300	2.0	75
Pregnant	800	10	10	1.5	1.6	17	1.5	500	2.0	85
Lactating	1,100	10	11	1.6	1.7	20	1.6	500	2.6	100
Males										
10–13	600	7.5	8	1.2	1.4	16	1.3	200	2.0	50
14–17	900	7.5	10	1.5	1.7	20	1.6	300	2.0	75
18–30	900	7.5	10	1.5	1.7	20	1.6	300	2.0	75
31–60	900	7.5	10	1.4	1.7	19	1.6	300	2.0	75
61–74	900	10	10	1.3	1.5	17	1.6	300	2.0	75
≥ 75	900	10	10	1.2	1.3	15	1.6	300	2.0	75

a Refers to the consumed amount, *i.e.* changes during preparation, cooking etc. must be accounted for.

b Exclusive breastfeeding is the preferable source of nutrition for infants during the first six months of life. Therefore, recommendations for single nutrients are not given for infants < 6 months. If breastfeeding is not possible, infant formula composed to serve as the only food for infants should be given (see *Chapter 5* on breast-feeding: Scientific Committee on Food, 2003). If complementary feeding has started at 4–5 months, the recommended intakes for 6–11 month old infants should be used.

c Retinol equivalents; 1 retinol equivalent (RE) = 1 µg retinol = 12 µg β-carotene.

d From 4 weeks of age, infants should receive 10 µg vitamin D per day as a supplement. Elderly people with little or no sun exposure should receive a supplement of 10 µg vitamin D_3 per day in addition to the dietary intake.

e α-tocopherol equivalents; 1 α-tocopherol equivalent (α-TE) = 1 mg RRR-α-tocopherol.

f Niacin equivalent; 1 niacin equivalent (NE) = 1 mg niacin = 60 mg tryptophan.

g Women of reproductive age are recommended an intake of 400 µg/day.

Table 1.2 *cont*. Recommended intake of certain nutrients, expressed as average daily intake over time, for use at planning diets for groups. The requirement is lower for almost all individuals

Age years	Calcium mg	Phosphorus mg	Potassium g	Magnesium mg	Iron[h] mg	Zinc[i] mg	Copper mg	Iodine µg	Selenium µg
< 6 mo[b]	–	–	–	–	–	–	–	–	–
6–11 mo	540	420	1.1	80	8	5	0.3	50	15
12–23 mo	600	470	1.4	85	8	5	0.3	70	20
2–5 y	600	470	1.8	120	8	6	0.4	90	25
6–9 y	700	540	2.0	200	9	7	0.5	120	30
Females									
10–13	900	700	2.9	280	11	8	0.7	150	40
14–17	900	700	3.1	280	15[l]	9	0.9	150	40
18–30	800[j]	600[j]	3.1	280	15[l]	7	0.9	150	40
31–60	800	600	3.1	280	15[l]/9[m]	7	0.9	150	40
61–74	800[k]	600	3.1	280	9	7	0.9	150	40
≥ 75	800[k]	600	3.1	280	9	7	0.9	150	40
Pregnant	900	700	3.1	280	–[n]	9	1.0	175	55
Lactating	900	900	3.1	280	15	11	1.3	200	55
Males									
10–13	900	700	3.3	280	11	11	0.7	150	40
14–17	900	700	3.5	350	11	12	0.9	150	50
18–30	800[j]	600[j]	3.5	350	9	9	0.9	150	50
31–60	800	600	3.5	350	9	9	0.9	150	50
61–74	800	600	3.5	350	9	9	0.9	150	50
≥ 75	800	600	3.5	350	9	9	0.9	150	50

h The composition of the meal influences the utilization of dietary iron. The availability increases if the diet contains abundant amounts of vitamin C and meat or fish daily, while it is decreased at simultaneous intake of *e.g.* polyphenols or phytic acid.

i The utilization of zinc is negatively influenced by phytic acid and positively by animal protein. The recommended intakes are valid for a mixed animal/vegetable diet. For vegetarian cereal-based diets, a 25–30% higher intake is recommended.

j 18–20 olds are recommended 900 mg calcium and 700 mg phosphorus per day.

k Supplementation with 500–1,000 mg calcium per day may possibly, to some degree, delay age-related bone loss.

l Menstrual flow and its associated iron losses may vary considerably among women. This means that some women require a larger iron supply than others. At an availability of 15%, 15 mg/d will cover the requirement of 90% of women of fertile age. Some women require more iron than the habitual diet can supply.

m Recommended intake for post-menopausal women is 9 mg per day.

n Iron balance during pregnancy requires iron stores of approximately 500 mg at the start of pregnancy. The physiological need of some women for iron cannot be satisfied during the last two thirds of pregnancy with food only, and supplemental iron is therefore needed.

Recommended intake of vitamins and minerals

The recommended intake (RI) of certain vitamins and minerals, expressed as average daily intake over time, is given in *Table 1.2.* The values for recommended intakes are intended for planning diets for groups of subjects of the specified age intervals. The values include a margin accounting for variations in requirement and physiological factors influencing the requirement, which makes it likely that a diet containing these amounts will be sufficient and adequate for practically the entire population. NNR do not cover all known essential nutrients, since the scientific basis for establishing recommendations was considered incomplete for some nutrients.

Recommendations for planning diets for heterogeneous groups

In planning diets for groups with a heterogeneous age and sex distribution, the amounts of nutrients per MJ given in *Table 1.3* are recommended. For each nutrient, the figures are based on the age and sex category of individuals 6–60 years old, for which the highest nutrient density is necessary to meet the requirements. These recommendations are not primarily aimed at pregnant and lactating women, nor at adult diets with a low energy level, *i.e.* less than 8 MJ per day. Furthermore, they are less applicable for planning diets with an energy level above 12 MJ per day, in which a lower density of many nutrients is sufficient.

A very low energy intake is defined as an energy intake below 6.5 MJ/d as a minimum daily energy intake necessary for providing adequate amounts of micronutrients from the diet. An energy intake of 6.5–8 MJ is considered a *low energy intake* with an increased risk of an insufficient intake of micronutrients.

A very low energy intake is related to either a very low physical activity level and/or to a low body weight. Low body weight is related to small muscle mass and thereby to low energy expenditure. Among healthy subjects, an actual very low habitual energy intake is probably rare – even among very inactive elderly subjects with physical activity level (PAL) of 1.4, the estimated energy requirements is 7–8 MJ/d (see *Chapter 9*). The age-related decrease in energy expenditure may result in very low energy intake in elderly subjects. Very low energy intake is also found among persons on slimming diets and among subjects with eating disorders, food intolerances, etc. When calculating the nutrient density for groups with *low* energy intake/requirement using RI in *Table 1.2,* the nutrient density may become unrealistically high. In that case the recommended nutrient density per MJ from *Table 1.3* should be followed and supplementation with a multi vitamin-mineral tablet should

be considered. For groups with a *very low* energy intake, the diet should always be supplemented with a multi vitamin-mineral tablet. The most effective way to prevent low and very low energy intake is to increase the physical activity level.

Table 1.3 Recommended nutrient density to be used for planning diets for groups of individuals 6–60 years of age with a heterogeneous age and sex distribution. The values are adapted to the reference person requiring the highest dietary nutrient density

		Content per MJ
Vitamin A	RE	80
Vitamin D	µg	1.0
Vitamin E	α-TE	0.9
Thiamin	mg	0.12
Riboflavin	mg	0.14
Niacin	NE	1.6
Vitamin B_6	mg	0.13
Folate	µg	45
Vitamin B_{12}	µg	0.2
Vitamin C	mg	8
Calcium	mg	100
Phosphorus	mg	80
Potassium	g	0.35
Magnesium	mg	35
Iron	mg	1.6
Zinc	mg	1.1
Copper	mg	0.1
Iodine	µg	17
Selenium	µg	4

Reference values for energy intake in groups

Both excessive and insufficient energy intake in relation to requirements lead in the long term to negative consequences for health. The individual's energy intake and expenditure should therefore be equal at adult age.

In *Table 1.4,* reference values are given for energy intake for groups of adults with two different physical activity levels. An active lifestyle, corresponding to PAL 1.8, is considered desirable for maintaining good health. An activity level of PAL 1.6 corresponds to a lifestyle with sedentary work and limited physical activity level during leisure time. The reference body weights used for calculation are based on Nordic populations. The original weights have been corrected so that all individuals fall within the normal range (18.5–25) of body mass index (BMI).

Individual recommendations for energy intake cannot be given due to the large variation between individuals with respect to metabolic rate, body composition and degree of physical activity.

Table 1.5 contains reference values for energy intakes in groups of children. The reference values for energy requirements in healthy infants and children up to 6 years are based on data from studies using the doubly labelled water technique. For children 6–17 years, the reference values are based on total energy expenditure using estimations of the basal metabolic rate (BMR) and physical activity level (PAL), with values for different activity levels from 10 to 17 years.

Table 1.4 Reference values for energy intakes in groups of adults with sedentary and active lifestyle[a]

Sex and age	Body weight[b]	REE[c]	*Sedentary* Sedentary work and limited physical activity in leisure time (PAL[d] = 1.6)	*Active* Sedentary work and regular physical activity in leisure time[e] (PAL[d] =1.8)
Women[f]	kg	MJ/d	MJ/d	MJ/d
18–30 y	62	5.9	9.4	10.7
31–60 y	63	5.8	9.2	10.4
61–74 y	63	5.3	8.5	9.5
≥ 75 y	62	5.1	8.2	9.3
Men				
18–30 y	76	7.7	12.3	13.8
31–60 y	77	7.4	11.8	13.3
61–74 y	74	6.6	10.6	12.0
≥ 75 y	73	6.0	9.6	10.8

a It should be noted that these estimations have a large standard error due to inaccuracy in estimation of both REE and PAL. Therefore, the results should be used only for estimation on group level. See *Chapter 9* on Energy for more details.

b Rounded values. The estimated reference weights are based on mean population weights in Denmark, Sweden and Finland, with adjustments for individuals outside BMI range 18.5–25. The values in the tables are thus estimations assuming that all individuals are at normal weight.

c REE = resting energy expenditure.

d PAL = physical activity level; total energy expenditure divided by basal metabolism (BMR).

e Corresponding to an energy expenditure of 60 minutes brisk walking daily.

f During pregnancy the energy requirement increases, mainly during the second and third trimesters. An increase in energy intake of approximately 1.5 MJ/d in the second trimester and 2 MJ/d in the third, is applicable for both activity levels provided that the level (1.6 or 1.8 PAL) is unchanged. During lactation the energy requirement increases by approximately 2 MJ/d for the reference woman provided that the level of physical activity is unchanged. For many pregnant and lactating women, the increased energy requirement is compensated for by a decreased amount of physical activity.

Table 1.5 Reference values for energy intakes in groups of children

Age	Average weight[a] kg	Estimated energy requirements[b] MJ/d
6–11 mo	9.1	3.2
12–23 mo	11.6	4.1
2–5 y	16.1	5.3
6–9 y	25.2	7.7
Girls		
10–13 y	38.3	8.6
14–17 y	53.5	9.6
Boys		
10–13 y	37.5	9.8
14–17 y	57.0	12.3

a Values for body weight in the group 0–5 years are primarily based on the mean of reference values from Denmark, Finland, Norway and Sweden. Recent values for growth at school age show increasing weight to height and a prevalence of overweight, therefore values for the age group 6–17 y are based on mean values from 1973–1977.

b Values for children 10–17 y are based on PAL 1.75/1.80 for boys and 1.65/1.70 for girls.

Recommendations on physical activity

Adequate physical activity contributes to the prevention of lifestyle-related diseases such as cardiovascular disease, osteoporosis and certain types of cancer. Daily physical activity is therefore recommended as part of a healthy lifestyle, together with a balanced diet.

Children and adolescents

There should be a minimum of 60 minutes of physical activity every day. The activity can probably be divided into shorter intervals of physical activity during the course of the day. Activities should be as diverse as possible in order to provide optimal opportunities for developing all aspects of physical fitness including cardio-respiratory fitness, muscle strength, flexibility, speed, mobility, reaction time and coordination.

Adults

The adult population should undertake a minimum of 30 minutes of daily physical activity of moderate intensity and/or vigorous intensity corresponding to an energy expenditure of about 630 kJ. This should be

in addition to the energy expenditure through normal inactive living. The activity can probably be divided into shorter intervals of physical activity during the course of the day, for instance intervals lasting about 10 minutes. An increase in activity beyond this duration and intensity will yield additional benefits. More physical activity (about 60 minutes daily) with a moderate and/or vigorous intensity may be needed for prevention of weight gain.

Recommendations on salt intake

A gradual reduction in the intake of sodium as sodium chloride is desirable. The population target is 6 g/d salt for women and 7 g/d for men, corresponding to 2.4 and 2.8 g/d of sodium, respectively. A further decrease to 5–6 g salt per day may have additional benefits.

The salt intake of children should also be limited and for children below 2 years of age the sodium density, expressed as salt, should not exceed 0.5 g/MJ, in order to prevent children becoming accustomed to a diet with a high salt content.

Recommendations on consumption of alcohol

The consumption of alcohol should be limited and not exceed approximately 10 g alcohol per day for women and 20 g per day for men. The energy contribution from alcohol should not exceed 5 E% in adults. Pregnant and lactating women, children and adolescents are recommended to abstain from alcohol.

CHAPTER 2

Reference values for evaluation of nutrient intake

Values for evaluating the adequacy of intake of vitamins and minerals

Table 2.1 gives values for the estimated average requirement (AR) and lower level of intake (LI) for certain vitamins and minerals. The values are intended only for use in assessing results from dietary surveys. Before comparing intake data with these reference values, it is crucial to check whether the intake data derived from a particular survey are suitable for assessing adequacy. More guidance on this subject and on how to use NNR in this context is given in *Chapter 4*.

The average requirement is the value to be primarily used to assess the risk for inadequate intake of micronutrients in a certain group of individuals. The percentage that has an intake below the AR indicates the proportion having an increased risk of inadequate intake.

Long-term intakes below the LI are associated with an increased risk of developing deficiency symptoms. There is a substantial uncertainty in several of these values. Thus, they should be applied with caution and if possible related to clinical and biochemical data. Furthermore, intake of nutrients above these values is no guarantee that deficiency symptoms could not occur in single individuals.

It must be emphasised that a comparison with AR and LI values can never decide whether intake is adequate or not, it can only indicate the *probability* that it is. That is because nutrient intake data are not absolutely true values, but are calculated using food composition tables and reported food consumption, both of which have a considerable error margin. Therefore, in order to find out whether an intake of a particular nutrient is adequate, biochemical measurements and thorough dietary assessment are necessary.

Table 2.1 Estimated average requirement (AR) and lower level of intake (LI) for certain vitamins and minerals for adults. The values are intended for use only in assessing results from dietary surveys. Long-term intakes below the LI are associated with an increased risk of developing deficiency symptoms. On the other hand, an intake of nutrients above these values is no guarantee that deficiency symptoms could not occur in single individuals

Nutrient		Women		Men	
		LI	AR	LI	AR
Vitamin A	RE	400	500	500	600
Vitamin D	µg	2.5[a]	–	2.5[a]	–
Vitamin E	α-TE	3	5	4	6
Thiamin	mg	0.5	0.9	0.6	1.2
Riboflavin	mg	0.8	1.1	0.8	1.4
Niacin	NE	9	12	12	15
Vitamin B_6	mg	0.8	1.0	1.0	1.3
Folate	µg	100	200	100	200
Vitamin B_{12}	µg	1	1.4	1	1.4
Vitamin C	mg	10	50	10	60
Calcium	mg	400	–	400	–
Phosphorus	mg	300	450	300	450
Potassium	g	1.6	–	1.6	–
Iron	mg	(5)[b, c]	10 (6)[b]	7	7
Zinc	mg	4	5	5	6
Copper	mg	0.4	0.7	0.4	0.7
Iodine	µg	70	100	70	100
Selenium	µg	20	30	20	35

a Primarily for individuals >60 years of age.

b ( ) Refers to post menopausal women.

c A lower level cannot be given for women of fertile age without considering the woman's iron status (determined by clinical-chemical/biochemical methods).

Values for the evaluation of high intakes of vitamins and minerals

For some nutrients, high intakes may cause adverse or even toxic symptoms. Upper intake levels (UL) have thus been established for some nutrients (*Table 2.2*). Prolonged intakes above these levels can, for certain nutrients, induce an increased risk of toxic effects (*e.g.* preformed vitamin A, vitamin D, iron and iodine). For other nutrients the adverse effects may be different and milder, *e.g.* gastrointestinal problems, interference with the utilization of other nutrients. The upper levels are not recommended levels of intake, but are maximum levels of daily chronic intakes judged to be unlikely to pose a risk of adverse health effects to humans. The upper levels are derived for the normal healthy population, and values are given for adults. For other life stages, *e.g.* infants

and children, specific data may exist for deriving specific values or such values could be extrapolated. To establish whether a population is at risk for adverse effects the fraction of the population exceeding the UL and the magnitude and duration of the excessive intake should be determined. There is a substantial uncertainty in several of the upper level values, and they must be used with caution for single individuals. UL values do not necessarily apply in cases of prescribed supplementation under medical supervision.

Table 2.2 Estimated upper levels (UL) for average daily intake of certain nutrients for adults. The upper levels are maximum levels of daily chronic intakes judged to be unlikely to pose a risk of adverse health effects to humans. The upper levels are derived for the normal healthy population. There is a substantial uncertainty in several of the upper level values, and they must be used with caution for single individuals. UL values do not necessarily apply in cases of prescribed supplementation under medical supervision

Nutrient		Upper intake level per day
Preformed vitamin A[a]	µg	3,000[b]
Vitamin D	µg	50
Vitamin E[c]	α-TE	300
Niacin[c]		
nicotinic acid	mg	10[d]
nicotinamide	mg	900
Vitamin B_6[c]	mg	25
Folic acid[c]	µg	1,000
Vitamin C	mg	1,000
Potassium[c]	g	3.7
Calcium	mg	2,500
Phosphorus	mg	4,000
Iron	mg	25[e]
Zinc	mg	25
Iodine	µg	600
Selenium	µg	300
Copper	mg	5

a As retinol and/or retinylpalmitate.

b Intake of retinol above 3,000 µg/d in pregnant women has been associated with an increased risk of foetal malformations. The upper tolerable level may not adequately address the possible risk of bone fracture in vulnerable groups. Postmenopausal women who are at greater risk for osteoporosis and bone fractures should therefore restrict their intake to 1,500 µg/day.

c In the form of supplements and fortification only.

d Not applicable to pregnant and lactating women.

e 10 mg in addition to habitual dietary iron intake.

CHAPTER 3

Nutrition recommendations – background and principles

There are two types of nutrition recommendations: 1) Traditional *nutrient recommendations* are based mainly on physiological data on the requirements of different nutrients, and 2) Recommendations on *energy distribution, i.e.* recommendations on the proportions of energy derived from fat and fatty acids, carbohydrates and protein, expressed as percentages of the total energy intake. The latter recommendations generally have a much wider and different background and imply advice on food intake aimed at prevention of several nutrition-related chronic diseases. In addition, the term *Dietary guidelines* is often used for recommendations at food level, but might also include nutrients. A preferred term is *food-based dietary guidelines,* originally used by FAO (1).

Nordic Nutrition Recommendations (NNR) constitute the scientific basis for practical planning of diets for various population groups and for the development of food-based dietary guidelines and advice. If the diet fulfils these recommendations, is varied, *i.e.* contains food from all food groups, and contains enough food to cover the energy requirements, the requirements for practically all nutrients will be covered. Exceptions are vitamin D, iron, iodine and folate in subgroups of the population. In diet planning it is therefore usually not necessary to calculate in detail the intake of the separate vitamins and minerals.

Background

Historically, the main objective of the nutrition recommendations was to prevent deficiency disorders. Vitamin and mineral deficiency diseases were common before these essential nutrients were discovered as vital components of the diet, *e.g.* iodine deficiency used to be widespread in inland communities in the Nordic countries and due to vitamin D deficiency, rickets crippled the lives of thousands.

Today, classical deficiency symptoms caused exclusively by too low dietary intake are very rare in the Nordic countries. Several Nordic dietary surveys have shown that the intake of vitamins and minerals in most cases is sufficient in relation to the recommendations, with the exception of vitamin D, iron, iodine and folate, which in some population groups have been found to be low. Low iron status is seen among children (2) and a varying proportion of women of fertile age (3). Marginal vitamin D status has been observed among children, adolescents, the elderly and some groups of immigrants (4–7). Folic acid supplementation is often recommended to women planning pregnancy.

In the 1970s, nutrition recommendations shifted their main focus from concentrating on the prevention of deficiency disorders to maintaining good health and preventing major chronic diseases, first and foremost coronary heart disease, cancer, diabetes and osteoporosis, but also other diseases. The present NNR 2004 have considered both data on the basal nutritional nutrient requirements and on clinical and epidemiological data about the relationship between diet and health.

For more than four decades, nutritionists in the Nordic countries have recommended that the balance between the macronutrients (energy-providing nutrients) should be changed. These recommendations emphasise for example a reduction in the consumption of fat, especially saturated fat, and refined sugars and an increased consumption of foods rich in complex carbohydrates and dietary fibre, for example bread, potatoes, vegetables and fruit. Similar recommendations have been published by international and national organisations during recent years (8–11).

General approach

The main objective of the recommendations is, on the basis of state of the art of knowledge, to ensure a diet that provides energy and nutrients for optimal growth, development, function and health during the whole life. All these factors are difficult to measure completely with currently available methods. Furthermore, health is difficult to define. Some of the indicators that are traditionally associated with good health, *e.g.* maximal growth (12) and early maturity, can be in conflict with other factors, such as the risk of certain forms of cancer (13, 14).

The establishment of a recommended daily intake for a certain nutrient consists of at least two main steps. The first step includes evaluation of the average requirement for the population group in question, judged by criteria that have to be set for every individual nutrient. The establishment of these criteria includes considerations about clinical

and biochemical deficiency symptoms, body stores, body pool turn-over and tissue levels. The nutritional requirements are influenced mainly by different biological factors like age, sex, growth, height, weight, pregnancy and lactation. In the second step an evaluation is made of a safety margin that should cover individual variations, as well as smaller unspecific increases in the requirement. The size of the safety margin depends on how much the requirement varies between individuals, but also on variations in bioavailability of the nutrient from the diet and potential negative effects of high intakes. Furthermore, the precision of the estimation of the requirement is taken into consideration.

Sub-clinical and clinical deficiency

Deficiency of a nutrient implies that the supply is so small that specific symptoms of disturbances in body functions emerge. During serious, manifest deficiency, overt clinical symptoms or signs such as bleeding of the gums during scurvy and neurological symptoms due to vitamin B_{12} deficiency arise.

It is also possible to measure the activity of enzymatic systems in which nutrients have a role as co-factors, or concentrations of nutrient in cells or fluids as a measure of tissue stores. Low activities or concentrations are usually associated with deficiency symptoms or impaired function. Moreover, it is possible to define an interval between manifest deficiency and optimal intake level, where clinical symptoms are more diffuse or do not exist at all. This level is sometimes called subclinical or marginal deficiency, or 'biochemical deficiency' (*Figure 3.1*). Such indicators are available only for a limited number of nutrients, *e.g.* vitamin D, iron, folate and B_{12}.

There is normally a transitional phase between a deficiency disease at a very low intake of the nutrient, through optimal conditions to toxicological effects at very high intakes. There is also a transitional phase between overt toxic effects at very high intakes and milder adverse effects at lower intakes.

Requirement and recommended intake

The concept 'requirement' is difficult to handle, partly due to the fact that the definition of the word 'requirement' is not always clear. The nutritional requirement could be defined as the absorbed amount of a nutrient that is needed to prevent clinical deficiency symptoms, or as the amount that maintains satisfactory body stores and tissue function. Usually 'requirement' means the smallest amount of a nutrient that is needed to prevent all physiological signs of insufficient nutrition

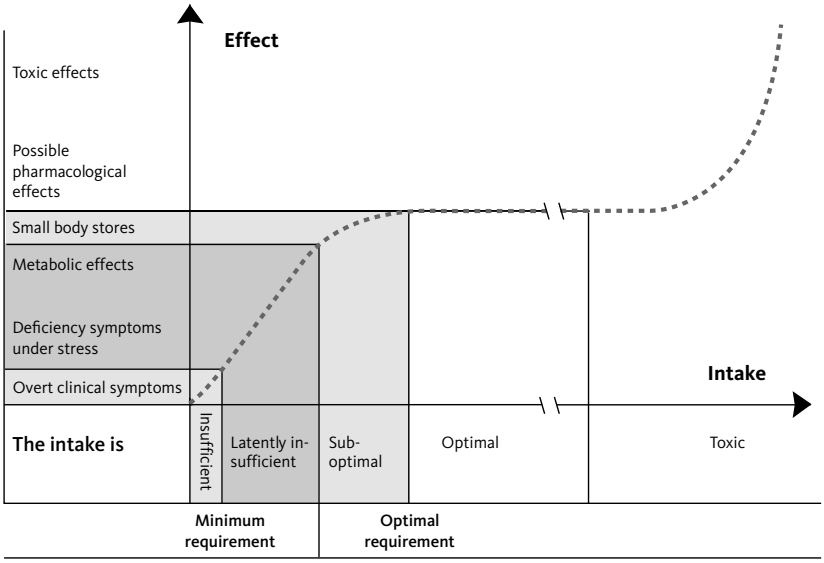

Figure 3.1 The theoretical relationship between intake of a nutrient and the effect on the organism

(judged from clinical signs or symptoms and/or with biochemical and physiological parameters) that can be attributed to an insufficient supply of that nutrient. In reality there is no fixed point for a requirement, rather there are transitional stages between manifest deficiency symptoms at a low intake via biochemical signs of low body stores to an optimal tissue level (*Figure 3.1*).

The requirement for a nutrient in a population can be described as a cumulated dose-response relationship (*Figure 3.2*). In the NNR, the term *average requirement* (AR) is used to define the intake of a nutrient that represents the average requirement for a defined group of individuals (*Figure 3.2*). The term 'average requirement' is also used by the EU Scientific Committee on Food (6). In the British (7) and US (2) recommendations, the term 'Estimated Average Requirement' is used for the same concept.

The lower intake level (LI) in NNR generally refers to the level below which an intake could lead to deficiency symptoms in some individuals, see *Figure 3.2*. Establishment of a 'lower intake level' is thus based on observations of individuals, and is in many cases based on criteria other than the average requirement.

In NNR the term 'recommended intake' (RI) refers to 'the amount of a nutrient that according to present knowledge can meet the known

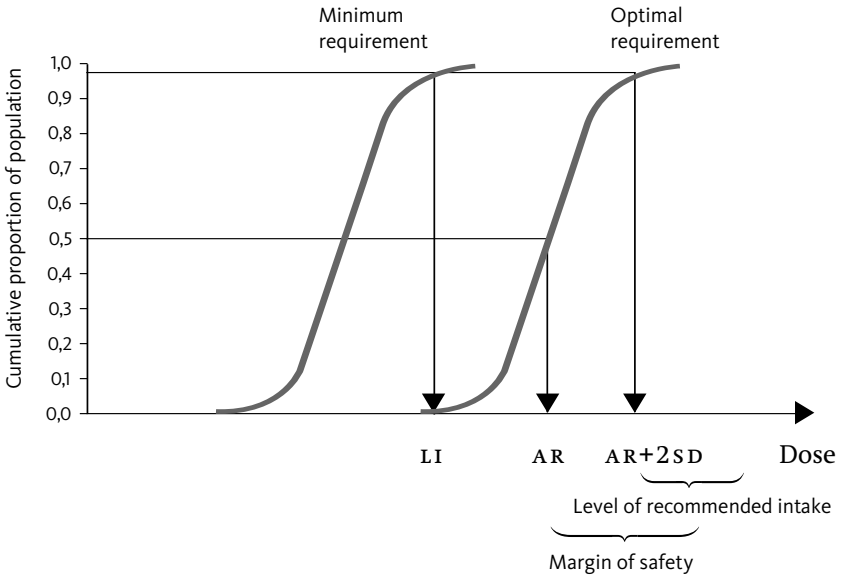

Figure 3.2 Relationships between levels of intake. Recommended nutrient intake (RI) is usually equal to the average requirement (AR) of a group of individuals plus a safety margin of two standard deviations (SD) or more. Lower intake level (LI) is similarly approximately the 97–98th percentile on the distribution curve of minimum requirement.

requirement and maintain good nutritional status among practically all healthy individuals'. This definition corresponds, in general, to definitions used by other expert committees, *e.g.* the US 'Recommended Dietary Allowance', the British 'Population Reference Intake', the EU Scientific Committee on Food 'Reference Nutrient Intake' and recommendations from the FAO/WHO (15).

In NNR the selected criteria for establishing the average requirement are usually based on data on biochemical markers of adequate nutritional status, and not on amounts that are sufficient to prevent clinical deficiency symptoms. In many cases preventive or interactive effects with other dietary factors are accounted for. Examples are the enhancing effect of ascorbic acid on iron absorption and the effect of folate on the homocysteine levels in blood. The recommended levels have been established as 'optimal or sufficient levels' in this context. This approach is applicable for most nutrients in the NNR.

Higher doses of certain vitamins and minerals may have pharmacological effects different from their primary nutritional effects. Generally, this concerns amounts that one cannot normally get from the diet.

High doses of nicotinic acid have been used as lipid-lowering agents and vitamin D_2 has been used in the treatment of hyperparatyreodism. Vitamin E has been used for the treatment of claucadio intermittens. The effect of fluoride on dental caries can also be considered a pharmacological rather than a nutritional effect. Such effects have not been taken into consideration in the establishment of the recommended intake.

The recommended intake corresponds to the consumed amount, which means that in planning, losses during handling, preparation, etc. have to be taken into consideration. The recommended intake is aimed at the average intake in a group expressed per day for a longer period, *e.g.* one week. The body can adapt and save nutrients when the intake is lower than the immediate requirement. The storage capacity varies and is highest for the fat-soluble vitamins (several months), while the stores of water-soluble vitamins (with the exception of vitamin B_{12}) usually last for about a month if the diet is completely lacking in these vitamins.

It is important to distinguish between the average requirement for a nutrient and the recommended intake. The recommended intake represents more than the requirement for the average person and also covers the individual variations in the requirement for the vast majority of the population group (*Figure 3.2*). Depending on the criteria used for setting the average requirement, the margin between AR and RI may vary. For some nutrients, where the variation in requirement is known, a statistical measure, usually 2 SD, is applied to cover the variations in the requirement. For other nutrients the margin is based on factors such as turn-over, size of body pool, interaction with other dietary factors and bioavailability. Generally the recommendations cover increased requirements, for example during short-term mild infections or certain medical treatments. It is important to note that the recommendations are intended for healthy persons. The recommended amounts are therefore usually not enough during long-term infections, malabsorption and various metabolic disturbances, or for treatment of persons with an insufficient nutritional status. Finally, the recommendations are as a rule only applicable if the supply of other nutrients and energy is adequate.

Recommendations in an international perspective

It has to be pointed out that the recommendations are not measurable quantities in a traditional scientific sense. They are the result of estimations and evaluations based on existing knowledge about human nutritional needs and health. Therefore they are not static but constantly have to be reviewed according to new knowledge.

Some nutrients or dietary components can influence the absorption, metabolism and excretion of other nutrients. The composition of the diet can therefore have an impact on the amount of a certain nutrient that has to be ingested to cover the requirement. For each nutrient it is therefore necessary to consider different possible interactions. This may lead to rather different recommendations in different parts of the world, due to the composition of the diet.

Differences in population structure and dietary habits are reasons for different recommendations in different countries. However, diverging interpretation of scientific data can also be a cause of such differences, as well as the frequency of updating the recommendations. The recommendations may therefore vary from one country to another and from one period to another for the same country. In spite of the above considerations, recommendations are surprisingly similar around the world.

Criteria for essentiality of vitamins and minerals

Contrary to what is generally believed, there is no general consensus in the scientific world as to which micronutrients should be classified as essential. This lack of consensus is reflected in the food regulations of the various countries, as they differ when it comes to trace elements allowed in food supplements[1].

The first classical criterion for defining a nutrient as essential for humans, is that:

a) A biological function for the nutrient is known.

Other criteria are that:

b) A person with an otherwise adequate diet demonstrates deficiency symptoms or reduced biochemical activity when the supply of the nutrient is insufficient in the diet or the endogenous production is inadequate,
c) These signs and symptoms disappear when the nutrient is reintroduced[2].

1. Examples: In the USA, vanadium is permitted in supplements, but not in Europe. In England, boron and germanium are permitted, but as far as we know not in the rest of Europe.

2. Codex Alimentarius defines an essential nutrient as follows: Any substance normally consumed as a constituent of food which is needed for growth and development and the maintenance of healthy life and which cannot be synthesised in adequate amounts in the body.

Following these criteria, there seems to be little disagreement today about which compounds belong to the group vitamins and major minerals. However, there is some controversy when it comes to which trace elements are considered essential[3]. This is partly due to the historical and scientific processes the elements have to go through before we consider them as essential. Not only the accepted essential elements, but also a large percentage of the non-essential elements in the periodic table are found in minute quantities in the body of mammals. A function may have been hypothesised for some and confirmed in a limited amount of animal studies, but not for humans. Only when clear deficiency signs or reduced biochemical activity are observed for humans are we willing to accept them as essential. It took 22 years from the discovery of selenium being essential to rats (1958) till it was accepted as essential to humans (in 1980), and a similar history applies to zinc and chromium. Diets deficient in arsenic, boron, bromine, lithium, silicon, tin or vanadium can induce deficiency signs in certain animals, and to some extent their function has been revealed (16). Nevertheless, as long as no specific biochemical function or clear deficiency signs have been observed in humans, they are not universally accepted as essential to us.

The uncertainty connected to some of the trace elements is reflected in this version of the NNR through the chapters on chromium, manganese and molybdenum, where no recommendations are given in spite of the recognition that they are essential for humans according to criterion a) above. As long as substantial evidence of fulfilment of criteria b) and c) are missing, we abstain from recommendations for these nutrients.

Reference values for energy intake

There is an important difference between the recommendations for energy intake and recommendations for vitamins and minerals. For some vitamins and minerals recommendations can be given with large margins, since the absorption can be limited or the excess broken down or secreted. The recommendations may therefore exceed the absolute requirements of the individual on a long-term basis. For the energy intake this is not the case. If the energy intake exceeds the energy

3. As a substance called vitamin sells better, there are examples of both natural and more or less synthetic compounds being sold as 'vitamins' to obtain better sales figures. Various flavonoids were sold as vitamin P with official acceptance until 1964, and the name still remains with several manufacturers, although erroneous. For several decades there were also substantial sales of the nonexistent 'vitamins' B_{13}, B_{15}, and B_{17}. As B_{17} (laetrile) turned out to be directly toxic, manufacturers seem to have become more careful in the past decade.

expenditure, the result will be overweight in the long-term. On the other hand, if the energy intake is lower than the expenditure, body stores will gradually be emptied. Generally energy intake and expenditure should be the same. There are mechanisms that help the body to save energy during low energy intakes and to increase expenditure during high energy intakes. However, the actual knowledge about these regulatory mechanisms is rather limited. In NNR the term *reference value* means the estimated energy requirement for a defined group of individuals with similar body size, age, sex and activity level.

Recommendations on macronutrients

In NNR the distribution of energy between the energy-providing nutrients, *i.e.* macronutrients, is emphasised. The background is that the current diet-related health problems mainly result from over-nutrition and nutritional imbalances, rather than the under-nutrition or deficiency illnesses that were more frequent before.

Most of the mechanisms that operate in the development of adult-onset degenerative diseases are fully functional immediately or soon after birth. All current evidence suggests that the risk factors that influence development of these diseases have a great time-based effect on development of the sedentary changes in childhood as well as in adults, suggesting that such preventive aspects in recommendations for children are probably at least as important as those given to the adult population. The important finding that most of the exogenous risk factors of diet-related degenerative diseases show a strong tracking, *i.e.* those who have proportionally high risk factor levels in early childhood have high levels also later in childhood and as adults, and also a strong clustering, *i.e.* several risk factors have a tendency to accumulate in the same subjects in childhood as well as in adults, further shows the importance of early recommendations for disease prevention.

The most important health problems are connected with an imbalance between energy intake and expenditure, high fat intake, especially saturated fatty acids, too little carbohydrates and dietary fibre and an excessive salt intake. The intake of refined sugar and alcohol is also high in some groups. These shortcomings of the diet may, especially in combination with a low physical activity, result in the development of diseases such as coronary heart disease, diabetes, high blood pressure, certain forms of cancer, overweight, constipation and dental caries.

The recommended energy distribution can be considered as 'optimal' in the dietary circumstances of the Nordic countries. The recommended levels are based on an overall (holistic) evaluation of our knowledge about the impact of diet on health. By following this advice, several

weak aspects of the diet can be corrected and provide a diet that, in combination with the recommendations on physical activity, would decrease the risk for several of our health problems. Thus, these recommendations have a completely different background than the classical nutrient recommendations.

Briefly, the recommendations imply a reduction in the share of the energy in the diet that comes from fat to about 30%. This should mainly be achieved by reducing the intake of saturated and trans fatty acids. A reduction in the fat content of the diet requires a corresponding increase in carbohydrates. The increase should mainly consist of foods that are naturally rich in carbohydrates and dietary fibre. The share of the energy intake that comes from refined sugars should be limited. The protein content of the diet is today satisfactory.

It is important to point out that the recommendations for macronutrients refer to an average intake for groups and should be considered as goals for dietary changes and dietary advice.

Dietary changes in accordance with the recommendations about the intake of fat, carbohydrates and protein as percentages of the total energy intake also result in an increased nutrient density of vitamins and minerals. The recommended intakes of micronutrients can therefore usually be met without dependence upon enrichment or supplementation, provided that the diet is varied and consumed in amounts that cover the energy requirement. Exceptions to this include iodine in some areas, iron for women of reproductive age and vitamin D in small children and the elderly.

Methodological considerations

Literature review

The documentation published in NNR 1996 was the starting point for the review of the literature, *i.e.* the search was concentrated on papers and other documents published after 1996 and was primarily performed using MEDLINE. Other important data sources included scientific reports and recommendations published by national and international institutions and expert groups, *e.g.* US Dietary Reference Intakes (17–19). For setting the upper levels of intake, opinions from the EU Scientific Committee on Food have been used as major sources, in addition to reports by national and international expert groups. National reports and articles published in Nordic languages were screened using Swemed+ and through networks such as the Nordic cooperation. Additional papers and reports have been identified during the work through *e.g.* reference lists. Where available, meta-analyses

and systematic reviews have been supplemented by papers on individual studies and investigations. Studies on Nordic population groups have been included where available.

The reference lists for the individual chapters include major key references used for the establishment of the recommendations, but do not intend to cover all literature that may be relevant to the basic issues of each subject, such as metabolism, etc.

Types of data used

A number of research methods are available when investigating nutrient, diet and health relationships – an overview is presented in *Figure 3.3*, *in vitro* studies and animal models generally generate knowledge about mechanisms of nutrient action and dose-effect relationships. Furthermore, such studies are used to establish upper intake levels when human data are missing. Experimental and observational studies in humans constitute the basis both for determining human nutrient requirements, diet and health relationships and for dietary guidelines. The separate chapters in this book clearly indicate which types of data are used in the different evaluations.

Nutrient requirements

Classical studies for assessing the average requirement of a specific nutrient are depletion-repletion studies in subjects fed adequate amounts of well-defined diets based on normal foods with graded levels of a nutrient. In many cases test diets have to be partially semi-purified or even based on liquid formula to enable a sufficiently low content of a nutrient to be provided. Chemical analysis of the dietary content of the nutrient is essential and the adequacy of the diet in general should be documented, *e.g.* in terms of stable body weights, or other valid measurements, *e.g.* energy intake : BMR ratios. The length of the study period should also be sufficient for achieving steady state with respect to the selected clinical or biochemical criteria.

Few studies, involving a limited number of nutrients, are available in which clinical symptoms have been induced. These are mainly classical depletion-repletion studies, *e.g.* on thiamin, riboflavin, niacin, vitamin B_6 and vitamin A. In these studies status or adequacy has been evaluated defining sufficient intakes when biochemical indicators have been normalised, *e.g.* plasma levels, urinary excretion or enzyme activities, in addition to clinical symptoms. Usually such studies include a limited number of subjects.

A number of studies have investigated biochemical indicators of status in normal subjects fed diets with graded levels of nutrients well

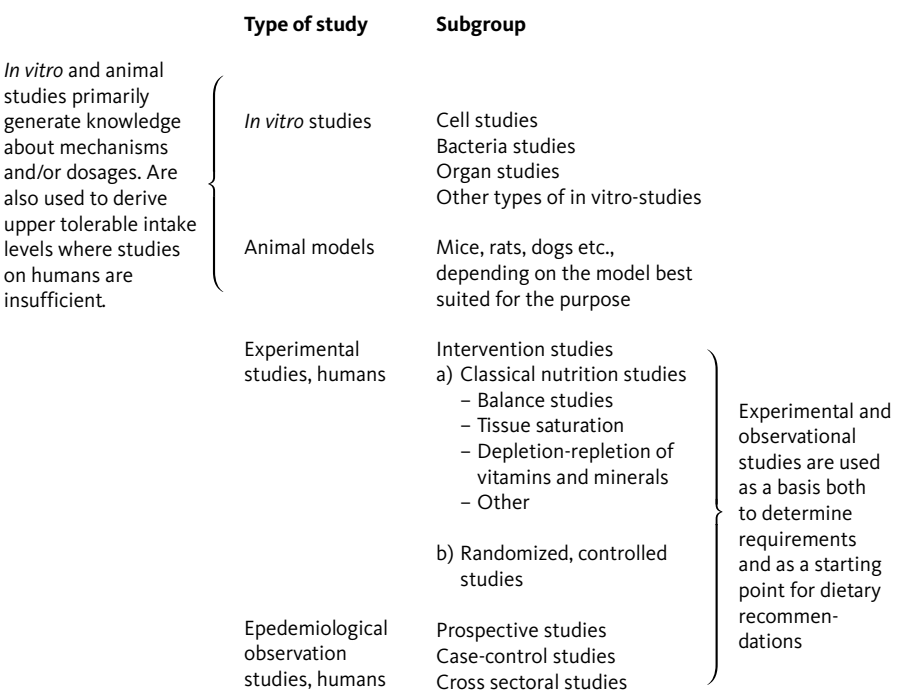

Figure 3.3 Types of studies used as a basis for nutrient and dietary recommendations

above the amounts necessary to induce clinical symptoms. Population studies, in which biochemical indicators of nutrient intake have been assessed and related to clinical or biochemical status, have been used in the absence of controlled studies.

Diet and health relationships

In addition to the basic requirements for nutrients to maintain body stores and functions, NNR also take into account effects of dietary intakes and diet composition on various health outcomes. In terms of nutrients this applies mainly to the energy-providing nutrients, *e.g.* fatty acid composition, but also to other components such as dietary fibre and to micronutrients such as sodium, potassium and some vitamins. The studies on which the recommendations are based include dietary intervention studies with defined diets, prospective cohort studies and case-control studies (*Figure 3.3*). Population studies linking a health outcome to dietary intake or a biochemical marker of intake have also been evaluated. The term 'health outcome' includes clinical endpoints such as coronary heart disease and markers of increased risk for disease, *e.g.*

serum cholesterol, insulin resistance, elevated blood pressure or bone density. Other biochemical markers of enhanced body functions, *e.g.* enzyme activities or immune response, have also been evaluated in relation to any health effects. The recommendations for physical activity are based on the same standards.

Weighing the evidence

Various criteria can be applied to evaluate the quality and strength of the scientific data that are produced by the various methods. Recommendations and dietary guidelines are never based on one finding or results from one single study. The clearest evidence is found between diet and health when observational studies are confirmed by experimental studies in humans and where at the same time there is a plausible biological explanation for the observations. A judgement is based on the consistency, strength and quality of separate studies evaluated against the total body of evidence within a subject (20).

There are today several classification systems when it comes to methodological evaluations of scientific studies, both internationally and in the Nordic countries. Meta-analysis of randomised, controlled studies or results from large, randomised, controlled studies are now required to see a relationship as convincingly established (21).

Although randomised, controlled studies have become an ideal which can successfully be applied to investigating single constituents of the diet, *e.g.* vitamins or minerals, they can rarely be applied for more than a short period of time in studies involving total dietary intervention, especially in large population groups. In total, dietary intervention studies are extremely expensive to conduct, but more importantly, they are not possible to conduct if the period of time from initiation to manifestation of a disease is long. This is the case *e.g.* with many cancers.

Observational studies therefore provide important evidence when evaluating the relationship between diet and human health. A lot of methodological work is being done to strengthen the results from such studies, in parallel to other work being done to strengthen evidence-based medicine. The WHO (22) uses a set of criteria for evaluating scientific evidence that was also applied in this version of NNR. A summary of the criteria is given in *Table 3.1.*

When assessing nutritional requirements and the health impact of dietary changes, it is essential that both the doses of a nutrient and the diet are objectively assessed. Hence, a major criterion for the studies used as evidence in NNR is that the total dietary intake during the study period is adequate for the subjects and that the intake of the compo-

Table 3.1 Strength of evidence

Criteria used to describe the strength of evidence according to WHO (22)
Convincing evidence. Evidence based on epidemiological studies showing consistent associations between exposure and disease, with little or no evidence to the contrary. The available evidence is based on a substantial number of studies, including prospective observational studies and relevant, randomised controlled trials of sufficient size, duration and quality showing consistent effects. The association should be biologically plausible.
Probable evidence. Evidence based on epidemiological studies showing fairly consistent associations between exposure and disease, but where there are perceived shortcomings in the available evidence or some evidence to the contrary, which precludes a more definite judgement. Shortcomings in the evidence may be any of the following: insufficient duration of trials (or studies); insufficient trials/or studies) available; inadequate sample sizes; incomplete follow-up. Laboratory evidence is usually supportive. The association should be biologically plausible.
Possible evidence. Evidence based mainly on findings from case-control and cross-sectional studies. Insufficiently randomised controlled trials, observational studies or non-randomised controlled trials are available. Evidence based on non-epidemiological studies, such as clinical and laboratory investigations, is supportive. More trials are required to support the tentative associations, which should also be biologically plausible.
Insufficient evidence. Evidence based on findings of a few studies, which are suggestive, but are insufficient to establish an association between exposure and disease. Limited or no evidence is available from randomised, controlled trials. More well-designed research is required to support the tentative associations.

nent being studied is adequately measured. Another criterion is that the study period is sufficiently long for the selected indicators or endpoints, based on available knowledge. Generally, stronger evidence is available from studies that involve biochemical markers of disease risk than from studies where clinical endpoints have been included. This is due to the difficulties in performing fully controlled dietary intervention studies and to such studies requiring a large number of subjects and long duration in order to obtain statistically measurable effects. Thus, the recommended intakes for those nutrients that are primarily based on diet-health relationships in many cases have to rely on a combined evaluation of evidence from different types of studies.

Data limitations

In spite of our careful review and analysis of existing data, the data were often scanty or drawn from studies that had limitations. Scientific judgement based on best available evidence has thus been the basis for

establishing values. The reasoning used is described in the separate chapters.

Generally, most available studies have been carried out on adults, while studies on infants and older children are relatively few. For many nutrients the recommendations for children have therefore been extrapolated from data for adults, using body weight, metabolic weight or energy requirement as a basis for the extrapolation. For infants below 6 months of age no specific RI is generally given, as breastfeeding during the first six months is recommended. In cases where breastfeeding is not possible, infant formula composed to serve as the only food for infants is recommended. The scientific basis for recommendations for the elderly has grown in recent years but the knowledge is still limited, especially concerning the very old (80+ years). Thus, recommendations for the elderly are often extrapolated from data for younger adults.

Establishment of the upper intake level (UL)

Adverse and toxic effects of nutrients

Like other chemical substances, vitamins and minerals may cause adverse and toxic effects if consumed in excessive amounts. Hence in addition to the risk associated with low intake and deficiency, there is also a risk associated with excessive intake and adverse and toxic effects. Thus, in addition to guidelines on nutritional requirements, advice is also provided on the upper tolerable level for each nutrient. When setting an upper level of intake, it is recognised that the nutritional requirement has to be taken into consideration to balance the risk of insufficient and excessive intake. The tolerable upper intake level (UL) was defined by the EU SCF as 'the maximum level of total chronic intake of a nutrient (from all sources), judged to be unlikely to pose a risk of adverse health effects to humans'. 'Tolerable intake' in this context denotes what is physiologically tolerable and is a scientific judgement as determined by assessment of risk, *i.e.* the probability of an adverse effect occurring at some specified level of exposure.

It should be noted that the upper intake level is not a recommended level of intake; it is an estimate of the highest level of intake that carries no appreciable risk of adverse health effects. The assessment of whether a population is at risk with respect to a high intake requires an assessment of the fraction of the population that exceeds the UL and by what magnitude and duration.

The basis for the upper level is a description of the capacity of a particular nutrient to cause potential adverse health effects. In this context the WHO definition (23) of an adverse health effect is used:

'a change in morphology, physiology, growth, development of life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase susceptibility to the harmful effects of other environmental influences. Decisions on whether or not any effect is adverse require expert judgement'.

The definition implies not only that overt toxic effects should be taken into account when evaluating high doses of nutrients, but also that more subtle effects, *e.g.* arising from antagonistic interactions between nutrients, may be considered adverse.

Upper intake level (UL)

The first step is to identify the nature of potential adverse effects of high intakes of a nutrient and secondly to describe at which doses these effects will occur in terms of severity and frequency. In principle, the same types of studies that form the basis for estimating nutritional requirement also form the basis for deriving the upper level. Several key issues such as evidence of adverse effects in humans, causality, relevance of experimental data (particularly those derived from studies in animals), mechanism of adverse effects, quality and completeness of the database and identification of distinct and highly sensitive subpopulations are addressed.

For nutrients, no risk of adverse effects is expected unless a threshold dose or intake is exceeded. The threshold for any given adverse effect varies among members of the population, as it does for any nutrient requirement. However, there are insufficient human data to establish distributions of thresholds for each adverse effect. In most cases the steps are identification of the critical endpoint, which is the adverse effect occurring at the lowest dose, and using a surrogate measure for the threshold. These are the following:

No Observed Adverse Effect Level (NOAEL), which is the highest intake of a nutrient with no observed adverse effects; and the
Lowest Adverse Effect Level (LOAEL), the lowest intake level with an observed adverse effect.

Based on these evaluations, an UL is derived taking into account the scientific uncertainties in the data by dividing the NOAEL by an uncertainty factor (UF). This factor should account for uncertainties in human inter-variability and, in the case of lack of adequate human data, an extrapolation from animals to humans, as well as other uncertainties or deficiencies in the data (*Figure 3.4*).

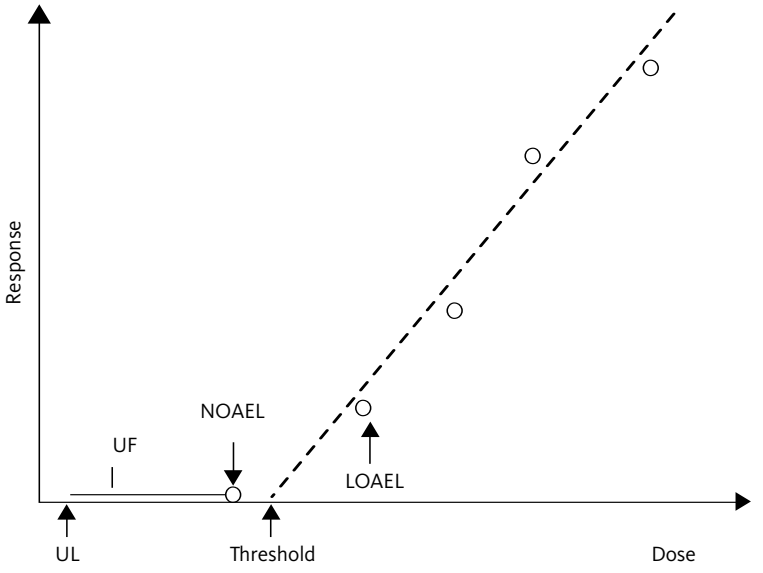

Figure 3.4 Derivation of upper intake level (UL)

Characterisation of the risk, which includes the description of the uncertainties associated with the UL estimate and scientific confidence, as well as an indication of the margin between the recommended intake and the UL or actual intakes is only briefly discussed for the individual nutrients.

General comments on the risk of adverse effects at high intakes of nutrients

Generally the risk of adverse effects due to high intakes of vitamins and minerals in the Nordic population is small at current intakes, although the margin between recommended intake and upper intake level for some vitamins and minerals is quite narrow.

In high doses, two of the fat-soluble vitamins (A and D) and all minerals are toxic as such, because the body has a limited capacity for secretion and breakdown. They are therefore stored, mainly in the liver but also in fat, muscle tissue, kidneys and bone. Deaths have been reported at very high intakes of vitamin A, D and iron. Overdoses of vitamin D to infants have led to irreversible calcification of the kidneys. At moderately high doses, preformed vitamin A may have a negative effect on bone health.

Water-soluble vitamins have generally been considered relatively harmless, even in higher doses and during longer periods. However, in

connection with increased attention to adverse effects, observations of negative effects of high intakes of some these vitamins have been documented.

It is extremely rare for acute poisoning to occur due to a high vitamin or mineral intake from ordinary foods. An example is an extraordinarily high consumption of liver from some Arctic animals, which can lead to vitamin A poisoning. High consumption of kelp may lead to iodine-induced goitre, as has been reported from *e.g.* Japan and Norway. Selenium poisoning has been reported from China in a situation where drought led to accumulation of the element in rice. Otherwise, there are probably no foods that have such a high vitamin or mineral content that even an extreme consumption could lead to poisoning.

In spite of this, there is a growing concern about negative health effects of high intakes of nutrients, mainly because the food supplement market is steadily increasing and new fortification regulations in EU will lead to more liberal fortification practices. In acknowledgement of the risk of excess intake levels of micronutrients due to micronutrient supplements and food fortification, both the Nordic countries and the US Food and Nutrition Board have published upper safe intake levels for micronutrients in recent years (24–26). The EU SCF and subsequently the European Food Safety Authority (EFSA) is working on the issue, and upper levels for a number of vitamins and minerals have been developed and are being published continuously (27).

A Nordic committee working with the same issue recently suggested dividing all vitamins and minerals into categories according to the safety margin they represent, *i.e.* the magnitude of the distance between recommended and safe upper intake levels (28):

CATEGORY A

Nutrients where the range between recommendations (or actual intake) and upper safe intake level is very narrow (≤ 5-fold), and great caution should be employed in *e.g.* regulatory contexts (vitamins A, D, nicotinic acid, folate and all the minerals).

CATEGORY B

Upper safe intake level 5–100 times the recommendation. Considerations should be taken regarding side effects or interactions with other components in the diet (vitamins B_6, B_{12}, C and E).

CATEGORY C

Upper safe intake range virtually impossible to set, as no adverse or toxic effects have been observed even at > 100 times the recommenda-

tions (thiamin and riboflavin) and interactive effects hitherto not observed.

This kind of categorisation has important implications when establishing guidelines for amounts of nutrients that may be added to foods and for maximum daily doses in dietary supplements.

REFERENCES

1. FAO/WHO. Preparation and use of food-based dietary guidelines. Report of a joint FAO/WHO consultation, Nicosia, Cyprus. Geneva: WHO, 1996.
2. Hay G, Sandstad B, Whitelaw A *et al.* Iron status in a group of Norwegian children aged 6–24 months. Acta Paediatr 2004; 93: 592–8.
3. Borch-Iohnsen B, Trygg K. Past and present: iron status and iron intake among adults in Norway. Scandinavian Journal of Nutrition 1997; 41: 125–8.
4. Osler M, Heitmann BL. Food patterns, flour fortification, and intakes of calcium and vitamin D: a longitudinal study of Danish adults. J Epidemiol Community Health 1998; 52: 161–5.
5. Holvik K, Meyer HE, Haug E *et al.* Prevalence and predictors of vitamin D deficiency in five immigrant groups living in Oslo, Norway: the Oslo Immigrant Health Study. Eur J Clin Nutr 2004.
6. Glerup H, Rytter L, Mortensen L *et al.* Vitamin D deficiency among immigrant children in Denmark. Eur J Pediatr 2004; 163: 272–3.
7. Erkkola M, Karppinen M, Jarvinen A *et al.* Folate, vitamin D, and iron intakes are low among pregnant Finnish women. Eur J Clin Nutr 1998; 52: 742–8.
8. WHO. Diet, nutrition and the prevention of chronic diseases. 916. Geneva: World Health Organization, 2003.
9. Food and Nutrition Board. Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington: National Academic Press, 2002.
10. Eurodiet Core report. Nutrition & diet for healthy lifestyles in Europe: Science and policy implications. Public Health Nutr 2001; 4: 265–74.
11. Gezondheidsraad. Dietary reference intakes. Energy, proteins, fats and digestible carbohydrates. Publication No. 2001/19E. The Hague, 2001.
12. van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, Fraser G, Goldbohm RA, Graham S, Kushi L, Marshall JR, Miller AB, Rohan T, Smith-Warner SA, Speizer FE, Willett WC, Wolk A, Hunter DJ. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol. 2000; 152: 514–27.
13. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev. 1993; 15: 36–47.
14. Clavel-Chapelon F, Gerber M. Reproductive factors and breast cancer risk. Do they differ according to age at diagnosis? Breast Cancer Res Treat. 2002 Mar; 72 (2): 107–15.
15. FAO. Handbook on nutritional requirements. FAO, editor. Rome: FAO, 1974.
16. Nielsen FH. Ultratrace elements of possible importance for human health: An update. Prog Clin Biol Res 1993; 380: 355–76.
17. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Pantothenic Acid, Biotin and Choline. Washington, DC: National Acedemy Press, 1998.
18. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium,

Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
19. Food and Nutrition Board, Institute of Medicine. Dietary reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington DC: National Academy Press, 1997.
20. Hill AB. The environment and disease: assocition or causal? Proc R Soc Med 1965; 58: 295–300.
21. Britton M. [Evidence-based medicine. Grading the scientific values and strength of conclusions in clinical trials]. Läkartidningen 2000; 97: 4414–5.
22. World Health Organization. Diet, nutrition and the prevention of chronic diseases. 916. Geneva, 2003.
23. World Health Organization. Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. Environmental Health Criteria 1994; volume 170.
24. Sandström B, Aro A, Becker W, Lyhne N, Pedersen JI, Þórsdóttir I. Nordiska näringsrekommendationer 1996. København: Nordisk Ministerråd, 1996.
25. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes: a risk assessment model for establishing upper intake levels for nutrients. Washington DC: National Academy Press, 1998.
26. Trumbo P, Yates AA, Schlicker S *et al.* Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 2001; 101: 294–301.
27. Scientific Committee on Food E. Tolerable upper intake levels for vitamins and minerals. http://europa.eu.int/comm/food/fs/sc/scf/index_en html 2002.
28. Meltzer HM, Aro A, Andersen NL *et al.* Risk analysis applied to food fortification. Public Health Nutr 2003; 6: 281–90.

CHAPTER 4

Use of NNR

NNR were originally established for planning purposes only. Today their application is much broader, and they are now used for the following main purposes:

– As guidelines for diet planning
– As a tool for evaluating dietary intake
– As a basis for food and nutrition policies
– As a basis for nutrition information and education
– As reference values in food production and marketing

Diet planning

The values for recommended intake (RI) presented in NNR are intended to be used mainly for planning diets for groups of healthy individuals. The aim is to compose a varied diet that meets the recommendations – not necessarily on a daily basis but as an average of a period, *e.g.* a week. The recommendations are 'net-intake' recommendations, *i.e.* they assume that losses of vitamins and minerals during peeling, cooking, keeping warm, and other handling procedures have been subtracted.

For groups of individuals who are reasonably homogeneous as to age, *e.g.* young schoolchildren, the diet should obviously be based on recommended intakes for that age group.

For heterogeneous groups, including for example young children, adult men and women and the elderly, the variation in nutrient requirements and energy intake is very large. A diet that is appropriate for these different subgroups therefore has to be planned in such a way that it contains sufficient amounts of all the nutrients per energy unit (MJ) to ensure that even subgroups with a low energy intake will obtain nutrient intakes at their recommended levels. A helpful tool in this context is the definition of the 'most demanding subject', for each nutrient. This is defined as the subgroup having the highest recommended intake of that nutrient per MJ (based on the reference energy intake). The 'most demanding subject' does not always refer to the same subgroup. For iron it is women of fertile age, whereas for calcium it is children.

A table of Recommended Nutrient Densities, aiming specifically at diet planning for heterogeneous groups, has been compiled (*Table 1.3*). It is based on the concept of the 'most demanding subject'. These recommendations are meant to be used as a general tool, with the exception of heterogeneous groups including infants, preschool children, pregnant and lactating women, and subjects with low energy intakes (< 8 MJ/d). A similar table can be made for a homogeneous group by dividing the recommended intake by the reference energy intake for that age or sex group. For example, for sedentary women aged 31–60 years the recommended intake of vitamin C is 75 mg/d and the reference energy intake is 9.2 MJ/day. The recommended density of vitamin C is then 8.2 mg/MJ for this group (8 mg/MJ when rounded up).

Assessment of dietary intake

Dietary reference values are also used for evaluating dietary intake. For vitamins and minerals (micronutrients) the main focus is usually on the adequacy of intake, *i.e.* whether intake is sufficiently high to meet requirements, although attention also has to be paid to high intakes. Here the average requirement (AR) is the primary reference value, but RI (recommended intake) and LI (lower intake level) can also be used as complementary values. For the energy-providing nutrients (macronutrients) the main concern is not adequacy of intake but inappropriate composition of the diet. In this chapter, intake is therefore evaluated separately for micronutrients and macronutrients.

Vitamins and minerals

Comparison of vitamin and mineral intakes with reference values is particularly useful for obtaining an overview as to which nutrients are present in ample amounts and which seem to be problematic and need further investigation, in the population as a whole or in a particular group.

It must be emphasised that comparison with reference values can never decide whether intake is adequate or not, it can only indicate the probability that it is. That is because nutrient intake data are not absolute true values, but calculated values based on food composition tables and reported intake, both of which have a considerable margin of error. Furthermore, calculated intake does not take variations in bioavailability and metabolism between individuals into account. Therefore, in order to find out whether intake of a particular nutrient is adequate, biochemical measurements of nutritional status for that nutrient are necessary (1).

Assessment of the adequacy of vitamin and mineral intake is based upon the distribution of individual requirements. The cumulative graph of this normal distribution is presented in *Figure 3.2* and shows the proportion of a group with a requirement above a given value. The graph also illustrates the risk that a random individual's requirement is not met at a given intake.

It can be shown that when intake equals average requirement (AR), the risk of inadequacy is 50%. Because 50% of individuals have a requirement larger than the average requirement, a random individual has a 50% probability of having a requirement larger than AR. When intake is 2 standard deviations (2 SD) below AR the risk of inadequacy is 98%, as 98% of the individuals have a higher requirement than this value. When intake is 2 standard deviations above AR the risk of inadequacy is 2%, as only 2% have a requirement that exceeds this value. These figures illustrate that intakes below the AR value imply a relatively high risk of being inadequate to meet requirements, the lower the intake the higher the risk. The opposite applies for intakes above the AR, where the risk gradually decreases from 50% to less than 2% at the recommended intake (RI).

The primary reference value when assessing the adequacy of vitamin and mineral intake is therefore the average requirement (AR), whereas the recommended intake (RI) and lower intake level (LI) are used as supplementary references.

Before comparing intake with reference values, it is crucial to check whether the intake data are suitable for assessing adequacy. The most important issues that need to be clarified before assessment are listed in *Table 4.1*.

Group intake assessment

It is a common misunderstanding that group intake, *by definition,* has to be adequate if the average intake of the group is equal to the recommended intake (RI). For vitamins and minerals that is not the case, because when evaluating intake the RI value is to be regarded as an aim for the intake of a reference individual, *i.e.* a value set to minimise the risk of a random individual (see *Chapter 3*). The key to an appropriate evaluation of adequacy at the group level is therefore to think in terms of a continuous risk-of-inadequacy scale, where risk increases as intake decreases, as shown in *Figure 3.1* in *Chapter 3*. Prevalence of inadequate intake may then be estimated by comparing the distribution of intake within the group with this risk scale. The average requirement (AR), where the risk of inadequacy is 50%, is a key reference value in this eval-

Table 4.1 Checklist for intake data

Questions that must be answered before assessment is started
a) How many days per individual are the intake data based on? Is the number of days sufficient to reflect 'usual intake'? Is the number of days sufficient to estimate the size of the risk group? (more than one day is needed) Is the number of days sufficient for assessment of a specific individual's intake?
b) Do the data include total intake from the diet? Food frequency questionnaires cover only the foods listed in the questionnaire, and no other foods are reported. The list may be more or less selective. Water, tea, coffee and other non-energy beverages are often excluded from the calculated intake, but they may be important sources of certain minerals and trace elements.
c) Is energy intake acceptable or is underreporting of 'usual intake' present? Underreporting of energy intake is very common in dietary surveys (3), and implies underreporting of most nutrients (including vitamins and minerals). *Therefore:* Check for underreporting in the group as a whole, and in subgroups, before assessment of nutrient intake. If a subgroup shows low intake of a micronutrient, check for underreporting of energy intake in that group. 1) Express energy intake as a ratio of estimated BMR (calculated from body weight). 2) Use published cut-off values for physiologically plausible EI/BMR values (3, 4) 3) Evaluate the presence, and extent, of underreporting. 4) Consider the potential implications for intake of the specific nutrients in question. Overreporting of energy intake is much less common than underreporting.
d) Do the data include nutrient supplements? Can this information be analysed separately? Is the information on nutrient content and dose specific enough for calculating intake from these sources?
e) Have losses of nutrients in cooking been taken into account in calculation of intake? This is particularly important for nutrients like ascorbic acid and folate, for which substantial losses occur in cooking.
f) Is the quality of the food composition database acceptable for all the nutrients calculated? For certain trace elements in particular the database may have many missing values, even for important foods, and this may result in substantial underestimation of intake. Database values for a specific nutrient may be based on an outdated analytical method that gives systematically higher or lower values than the method used as the standard today.

uation. Intakes below the AR, where the risk steadily increases above the 50% as intake decreases, are therefore associated with a considerable risk of not meeting the requirement according to the selected criterion.

Mean intake of the group is not sufficient information to evaluate adequacy of intake. Data on the distribution of intake within the group, in the form of percentiles and a histogram, are also necessary. Based on these data the following questions can be answered:

1) What proportion of the group has a relatively high risk of inadequate intake? If defined as a risk of above 50%, it means that this proportion has an intake below the average requirement, AR.
2) What proportion of the group has a very high risk of inadequate intake? *i.e.* an intake below the lower intake level, LI.
3) What proportion of the group has a minimal risk of inadequacy? If defined as a risk of less than 2% it means that this proportion has an intake above the recommended intake, RI.
4) What proportion of the group has a high risk of intake being too high? *i.e.* an intake above the upper limit, UL.

For a detailed description of this approach and the assumptions on which it is based, see (2).

This simple approach gives a rough estimate of the overall situation. It may then be elaborated on by also looking at the remaining part of the group with intakes between the reference points applied above, for example those between AR and RI. However, such an elaboration does not change the conclusions substantially (2).

An example of the simple assessment and its interpretation is shown below.

Example 1 Group intake assessment

The intake distribution of vitamin C for a group of women (18–74 yrs, n = 626) is presented below. The average requirement (AR) is 50 mg/d, the recommended intake (RI) is 75 mg/d, the upper limit (UL) is 1000 mg/d, and the lower intake level (LI) is 10 mg/d:

Percentiles of vitamin C intake, mg/d

P1	P5	P10	P25	P50	P75	P90	P95	P99
19	31	38	56	81	123	157	180	380

The table shows that less than 25% of the women had an intake below 50 mg/d (AR) and 50% had an intake above 80 mg/d (RI). This means that almost 25% of the women had a relatively high risk of not getting enough vitamin C (intake below AR, risk of inadequacy > 50%). About 50% had a minimal risk of inadequacy (intake above RI, risk of inadequacy < 2%). None of the women had an exceedingly high intake (all intakes << UL). In conclusion, the results indicate that vitamin C intake is not fully satisfactory, as a substantial proportion of the women, approximately ¼, had a relatively high risk of inadequacy, although no one had an intake below the lower intake level (LI).

If the assessment results cause concern, as do the results in Example 1, there are two things to do. First, the energy intake data in the risk group (intake below AR) must be scrutinised, as described in *Table 4.1* (point c). This is to find out whether the low intake of the nutrient in question may simply be ascribed to implausibly low reported energy intake, *i.e.* far too low to represent usual intake at weight maintenance. If that is

the case the focus of attention should become the energy intake in this subgroup and the validity of the intake data, rather than one specific nutrient. Second, if the low nutrient intake in the risk group cannot be explained by implausibly low reported energy intake the results indicate that the risk may be real. Biochemical measurements of nutritional status are necessary to clarify this and to find out whether there is an actual problem of insufficient intake of the nutrient in question.

Some population surveys only collect information on 1 day's intake per individual. However, dietary data based on information on only 1 day per individual (a 1 day food record or a single 24-hour recall) do not reflect the true distribution of intake in the group, but show a broader distribution with a higher proportion of high and low intakes. The size of a risk group with too small an intake may therefore be substantially overestimated when based on 1-day data. Such data are therefore unsuitable for assessment of dietary adequacy unless the intake distribution can be adjusted, based on several days' intake for a subgroup of the sample (see (2) and (9) for adjustment description). It is important to be aware of this aspect when assessing the adequacy of intake, for example in a population survey. Otherwise a serious misjudgement of the situation may be made.

Individual intake assessment

All judgements of the adequacy of intake at the individual level, based on intake data alone, must be regarded as rough estimates. That is because the error margin for the absolute intake of a specific individual is large, much larger than for group intake distribution. The demands on the quality of the dietary data are therefore much higher, in particular with respect to the length of the time period reflected by the intake data, and the accuracy of the quantitative information. However, even the best data are still based on calculations from a food table, thus introducing the inevitable errors included therein. As a consequence of the large error margin, all interpretations of adequacy assessment at the individual level must be very cautious and regarded as rough approximations. The only way to decide whether an individual meets their requirement for a certain nutrient is to measure their nutritional status with respect to this.

Bearing these reservations in mind, the first step in the assessment of an individual's intake is to characterise its risk of inadequacy by comparing intake with average requirement, AR. The LI and the RI values may then be used as secondary points of reference. An example of such as assessment is shown below:

Example 2 Individual intake assessment

In this example AR = 50 mg/d, RI = 75 mg/d, and the individual's intake = 55 mg/day. Intake is only slightly above AR, and that means that there is a considerable risk of inadequacy. If, however, intake was 75 mg/day instead, intake would be close to the NNR, and the risk would then be estimated as very small, although not minimal. In conclusion, an intake of 55 mg/d indicates that the person should be advised to improve their diet with respect to vitamin C intake.

When assessing the nutrient intake of an individual, it is always necessary to scrutinise their energy intake data for potential underreporting of intake (see *Table 4.1,* point c).

Comment

The approach described above for evaluating group and individual intake implies a well-defined relationship between requirement and recommended intake. This is not always the case, however, as when an average requirement value has not been defined for that nutrient the recommended intake has been based upon other evidence. Also, an extra safety margin may have been included in order to account for incomplete information. Before assessing the adequacy of intake by comparison with dietary reference values, the basis of these values should always be looked into by reading the chapter on the nutrient in question.

Energy-providing nutrients

As explained in *Chapter 3* the recommendations on macronutrients, that is the relative intake of fat, carbohydrate and protein, have a background completely different from the classical nutrient recommendations for vitamins and minerals. Furthermore, the NNR recommendations for macronutrients refer explicitly to the average and range of intake of a group.

The most relevant approach for assessing group intake of macronutrients is therefore to compare the mean intake with the recommendation. Median intake may be more relevant than the mean if the distribution of intake is skewed. This assessment of the group average may then be elaborated on somewhat by comparing the intake distribution with the desirable range, if such a range is indicated, and assessing how large a proportion of the group is within, above and below this range, respectively.

It would be a misinterpretation of the recommendation to use it as a goal for individual intake, and *e.g.* state that only x% of the group had an intake as low as the recommended 30 E% total fat, because the recom-

mendation explicitly refers to a group average. On the individual level it is highly relevant, however, to refer to the desirable range (such as a total fat intake between 25 and 35 E%). The situation is different for alcohol and refined sugars, as these recommendations refer to upper limits for an individual.

Before assessment of the macronutrient composition of the diet it is always necessary to check the energy intake data (see *Table 4.1 c*), as underreporting may be nutrient-specific and thus may be reflected in the macronutrient composition of the diet (5).

Food and nutrition policy

Recommended nutrient intakes and other reference values are an important basis for food and nutrition policy formulation and decisions. In particular the recommended composition of the diet as to the relative amounts of fat, carbohydrates and protein has been a key element when setting national goals for dietary intake in Western countries, including the Nordic countries, during recent decades.

In nutrition and public health policy, where health promotion through better dietary habits and increased physical activity is now becoming an integral part, recommended intakes serve as an important yardstick when the need for change and action is to be documented. The recommendations are also used as reference points in evaluation of programmes, interventions and other initiatives.

Food and nutrition policies may include *food-based* dietary guidelines as well as nutrient recommendations. For example, a large number of countries now have recommendations on fruit and vegetable intake ('5-a-day' etc.). Both sets of recommendations may be relevant in the contexts discussed above. For planning food supply at the national level and for evaluating long-term trends in intake based on national food supply statistics, the food-based dietary guidelines are particularly useful. Such food supply data have been used extensively for several decades, also in the Nordic countries, in spite of all the shortcomings of that type of data.

Two aspects of food and nutrition policy specifically deal with vitamins and minerals, namely addition of nutrients to foods and use of dietary supplements.

Addition of nutrients to foods

Addition of a nutrient to selected foods may be used in nutrition policy as a means to increase intake of that specific nutrient in the popula-

tion. Addition of iodine to salt as a means to increase iodine intake is used all over the world and is one of the classical examples. This type of addition is often called 'fortification'. In the Nordic countries fortification of selected foods began as early as the 1930s, with the most common being fortification of salt, flour and margarine. Two Nordic reports give an overview of the development and status of fortification policy in the different Nordic countries (6, 7).

Before the authorities decide to introduce fortification with a given nutrient, the following five questions need to be answered:

i) Is there a documented need for increasing the intake of this nutrient in this population?
ii) Is fortification an efficient way to increase the intake of the target group?
iii) Are there other possibilities for increasing the intake of the target group?
iv) Are there any potential adverse effects of the fortification?
v) How can the effect of the fortification be evaluated?

Dietary reference values serve several purposes in this context. First, when the need for increasing intake is assessed, dietary reference values are used for evaluating the adequacy of current intake. Intake data are not sufficient, however, and nutritional status information must also be available. Second, when planning how much should be added in order to obtain a relevant effect on nutrient intake in the target group, the dietary reference values are used as a reference frame. Third, when the risk at a high level of intake is assessed, the upper limit reference value is used as a yardstick. For the first two purposes the average requirement (AR) is the primary reference value to use, with the lower intake level (LI) and the recommended intake (RI) as supplementary measures. The use of these values for assessing intake is discussed earlier in this chapter.

Some of the recent fortification programmes within the Nordic countries have published the rationale behind their decisions, including their calculations of how much the fortification is expected to improve the adequacy of intake (8). A fortification programme always has to be monitored, and its effect evaluated, by measurements of nutritional status as well as intake before and after the start of the programme.

Addition of nutrients to foods is used on a much larger scale than simply the fortification programmes initiated by the authorities, based on documented nutritional relevance. In the food industry nutrient addition is widely used, not primarily because of a significant nutri-

tional impact but rather as a parameter of enhanced quality that can be used in the marketing of the product.

Dietary supplements

Intake of a specific nutrient can be increased by using a dietary supplement. In nutrition and public health policy, supplement use may be recommended for a specific target group that has a requirement that is too high to be met through diet alone. For example, the physiological need of some women for iron cannot be satisfied during the last two thirds of pregnancy with foods only and supplemental iron is needed.

NNR values are used for assessing the adequacy of the diet and thus the need for substantially increasing the intake of a specific nutrient (see above). However, intake data alone are not enough for this assessment. Information on nutritional status with respect to the nutrient in question, measured by biochemical measurements, must therefore be obtained (1).

In the Nordic countries a varied diet that meets the recommendations on macronutrient composition will usually contain adequate amounts of most vitamins and minerals. Therefore there is not a general need for dietary supplements in the healthy part of the population. If energy intake is very low, however, as when energy expenditure is low, more demand is made on the nutritional quality and density of the diet (see *Table 4.1*) and adequate levels may be difficult to achieve through diet alone unless special attention is paid to the selection of foods. For energy intakes below 6.5 MJ/d, adequate nutrient intakes are difficult to achieve without special effort. For some nutrients the general intake level in the Nordic countries is relatively low compared to the recommendations (vitamin D, iron intake in young women). In order to obtain adequate intake of these nutrients special attention has to be paid to including foods rich in these nutrients in the diet if requirements are to be met through diet alone.

The NNR aim is that the nutritional requirements are met through a varied diet rather than dietary supplements, as that gives the best opportunity for achieving a balanced intake of the whole spectrum of nutrients, including trace elements and other bioactive compounds.

There are certain circumstances, however, where the use of dietary supplement is, or may be, relevant:

Newborn infants should receive a vitamin K supplement (immediately) after birth, in order to stimulate normal coagulation of blood and thus reduce the risk of intra-cranial bleeding.

Infants should receive a vitamin D supplement from 4 weeks of age. This is often combined with vitamin A, although the nutritional rationale behind this is not well documented. Infants who do not receive food products fortified with iron or iron supplements have a considerable risk of developing iron deficiency.

Preschool and School children. For the majority of children of this age, who have a normal dietary intake and who are healthy and physically active, there is no nutritional reason to routinely give a dietary supplement. An exception to this is children who have long-term problems with food intake, while children who on a long-term basis eat a special diet such as a vegan diet may need to take a dietary supplement such as a multivitamin/mineral product. Exclusively vegetarian diets need vitamin B_{12} supplementation.

Pregnant and lactating women. NNR includes recommendations on increased intake during pregnancy and lactation. If the diet is varied and well balanced the increased nutrient requirement can usually be met through that diet. However, the iron requirement of some pregnant women cannot be met during the last two thirds of pregnancy through diet alone so iron supplement is necessary. Folic acid supplement is sometimes recommended to all young women planning to become pregnant, as the preventive effect of folic acid against neural tube defect is needed at a very early stage in the pregnancy. Mothers who only consume a vegan diet need a vitamin B_{12} supplement.

Elderly. An elderly person who consumes a reasonable amount of a varied and well balanced diet does not need a vitamin/mineral supplement because of age *per se*. A vitamin D supplement (10 µg/d) is recommended for elderly subjects over 60 years of age with little or no sun exposure. A multivitamin/mineral tablet is more relevant than vitamin D alone if energy intake and energy expenditure are very low (< 6.5 MJ/d).

Specific groups and circumstances. Attention should be paid to the potential need for dietary supplementation in connection with food allergies, slimming, vegan diets and some types of vegetarian diets. In general, individuals with a very low energy intake often have problems achieving adequate intakes of all micronutrients and a multivitamin/mineral supplement may therefore be relevant. Some immigrant groups are particularly vulnerable to specific deficiencies such as vitamin D deficiency, due to food and cultural habits, and supplements may therefore be relevant. There is no evidence to support vitamin and mineral supplementation among athletes, with the possible exception of iron in some cases.

Disease and drugs. A number of diseases make use of dietary supplements necessary, but these are not dealt with here as that is outside the scope of NNR (see *Chapter 3*). However, attention should be paid to the fact that numerous common drugs interfere with the absorption and metabolism of vitamins and minerals.

Advice to individuals on whether to use a dietary supplement should be given by a medical doctor or a dietician. The advice should be based on thorough dietary assessment and, if possible, on biochemical measurements, in particular if a specific supplement is to be used. Special attention must be paid to the potential harmful effects of high doses, as the content and sometimes the bioavailability of the supplement is generally much higher than when the nutrient comes from foods (see *Chapter 3*).

Nutrition information and teaching

Dietary information and advice

In order to make NNR a useful instrument for dietary advice, they have to be translated into practical advice on food selection and meal composition. Each country must then adjust these in order to make them fit with their own foods and traditions. It is important to point out that there are many ways to compose a diet that fulfils the recommendation. However, some parts of the advice are more important than others if the recommendations are to be met. Particularly important is the advice on reduction of fat intake and increase in carbohydrate and dietary fibre intake.

Compared to the present dietary habits in the Nordic countries, a diet that complies with the NNR recommendations on macronutrient composition involves considerable changes in food selection and meal composition. The dietary information work should therefore concentrate on the changes that are most important for the particular target group in question. It should be emphasised that a healthy diet is not synonymous with a puritanical and dull diet. The approach should be positive and emphasise the good choices. The examples should be realistic for the target group and include advice on choice of raw materials, food preparation and meal composition. The advice must take into account the target group's situation and food culture, including the practical possibilities of implementing the suggested changes. The differences in food preferences and culture between men and women must also be taken into account if the message is to be communicated efficiently to men as well as women.

It is the everyday diet that counts most in the overall picture. The dietary information should therefore focus on the everyday aspects.

Teaching

NNR is an important basis in the teaching of nutrition and food science. At university level, the NNR report can be used directly as teaching material. Food composition tables and data on dietary habits are relevant as supplementary material in that context.

Two aspects of the NNR should be stressed in the teaching at all levels. First, the main emphasis should be placed on the dietary composition as regards macronutrients, the fat and the carbohydrate content in particular, and how recommendations on these can be met (see previous section on Nutrition information). Second, it should be underlined that the recommendations do not have to be met every single day, although they are expressed as amount per day, but that they refer to an average intake of several days, or approximately one week. Some days an individual may obtain more of a certain nutrient, other days less, depending upon the foods consumed.

In teaching, as in nutrition information, the nutrient recommendations should be linked to foods and food-based dietary guidelines.

Food production and marketing

The recommended intake values may be used as benchmarks when defining the desirable nutrient content of a food product. They also serve as reference points for nutritional labelling. Obviously no single food, dish or meal is expected to contain the recommended daily amount of all nutrients, unless it is a special product such as infant formula or a dietetic product used in clinical nutrition. The nutritional content of a food product is usually expressed as percentage of a recommended daily reference intake. It may also be compared with recommended energy distribution of macronutrients. Complete meals may be evaluated by comparison with the recommended macronutrient composition of the diet. In the European Union the regulations on nutritional labelling include a set of specific reference values for certain vitamins and minerals, which have to be used in labelling. These may differ from national recommended intakes.

REFERENCES

1. van den Berg H, Heseker H, Lamand M, Sandström B, Thurnham D. FLAIR Concerted Action No. 10 Status Papers. Introduction, Conclusions and Recommendations. Int J Vit Nutr Res 1993; 63: 247–316
2. Institute of Medicine. Dietary reference intakes. Applications in dietary assessment. Washington DC: National Academy Press 2000.
3. Black A. Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metoabolic rate. A practical guide to its calculation, use and limitations. Int J Obesity 2000; 24: 1119–1130.
4. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM. Critical evaluation of energy intake data using fundamental principles of energy physiology. 1. Derivation of cut-off values to identify underrecording. Eur J Clin Nutr 1991; 45: 569–581
5. Heitmann B, Lissner L. Diet underreporting by obese individuals. Is it specific or non-specific? Br Med J 1995; 311: 986–989.
6. Nordisk Ministerråd. Tilsætning af vitaminer og mineraler til levnedsmidler. PNUN rapport 1989:4. København: Nordisk Ministerråd, 1989.
7. Nordic Council of Ministers. Addition of nutrient to foods – principles and practices. TemaNord 1995:643. Copenhagen. Nordic Council of Ministers, 1995.
8. Levnedsmiddelstyrelsen. Jod, er der behov for berigelse af kosten? Levnedsmiddelstyrelsen, Sundhedsministeriet, Publ. Nr. 230, Søborg: Levnedsmiddelstyrelsen, 1995.
9. Hoffmann K, Boeing H, Dufour A, Volatier J-L, Telman J, Virtanen M, Becker W, de Henauw S – for the EFCOSUM Group. Estimating the distribution of usual dietary intake by short-term measurements. Eur J Clin Nutr 2002; 56 (Suppl. 2): S53–S62.

CHAPTER 5

Breastfeeding

The benefits of breastfeeding are well documented. Breast milk gives the newborn essential nutrients in efficiently absorbable combination, and protects against infection and probably several other diseases as well. Breastfeeding as such may also play a role in the infant's emotional development. More recently, the possible long-term benefits of breastfeeding on adult health have been extensively discussed.

It is recommended that infants are breast fed exclusively for about 6 months, *i.e.* given no food other than breast milk. Vitamin D supplements are recommended in the Nordic countries. If breastfeeding is for some reason not possible, infant formula formulated to serve as the only food for infants should be given. Beyond this reference, recommendations for single nutrients are not given in NNR 2004 for the age 0–6 months.

Health benefits of breastfeeding

Energy yielding nutrients in human milk have multiple functions. Breast milk protein provides amino acids in adequate amounts and proportions to support growth and maintenance of muscle tissue in breast fed infants, and facilitates optimal development of important physiological functions in newborns (1).

Anti-infective properties of breastfeeding

Scientific evidence shows that breastfeeding effectively protects against several infections, especially gastroenteritis (diarrhoea) and otitis media (2). The immunological protection against infections lasts for some years after cessation of breastfeeding. Development of immunological tolerance may also play a role in protection against autoimmune diseases (3). Breastfeeding is associated with lower risk of hospitalisation for respiratory disease in infancy (4), and also protects against allergic rhinitis in children (5). Thus, breast milk contains many protective factors that may exert long-term health effects through the child's immune system.

Breastfeeding and atopy and asthma

Protection against atopy and asthma is more controversial. Exclusive breastfeeding during the first months of life is associated with lower asthma rates during childhood. This effect, which could be caused by immunomodulatory qualities of breast milk, avoidance of allergens, or a combination of these and other factors, strengthens the advantage of breastfeeding, especially if a family history of atopy is present (6). A study that compared children breast fed longer than four weeks to those who received breast milk for shorter time periods did not find that breastfeeding protects against atopy and asthma (7). However, a more recent multidisciplinary review concluded that breastfeeding protects against the development of atopic disease (8).

Breastfeeding and neurological development

Many studies indicate an effect of breastfeeding on healthy neurological development of the infant. This favourable effect may be caused by the high content of docosahexaenic acid (22:6, n-3) in breast milk, as the fatty acid is present in high amounts in nerve cell membranes. A meta-analysis by Andersson *et al.* (9) indicated that breastfeeding was associated with higher cognitive development scores than formula feeding. However, other authors conclude that the question of whether breastfeeding and formula feeding have differential effects on cognitive development has not yet been comprehensively answered (10).

Iron status and breastfeeding

An earlier study found iron status to be negatively affected by exclusive breastfeeding for nine months (11). In a recent epidemiological Nordic study, a shorter duration of breastfeeding was found for iron-deficient one-year-old children, who had been breast fed for five months on average, compared to non-deficient children, who were breast fed for eight months on average (12). However, effects of complementary feeding including high-protein and low-iron foods could not be excluded in this study, but another study found that there is no need to give iron supplementation to infants breast fed longer than half a year (13).

Long-term health benefits of breastfeeding

Increasing numbers of studies also indicate a long-term beneficial effect of breastfeeding for health. Breastfeeding may diminish the risk for development of type 1 diabetes (14, 15, 16, 17, 18, 19), although the results are contradictory. This possible protective effect of breast milk may depend on the different proteins in breast milk and infant formula.

Breastfeeding also seems to reduce the risk of obesity in children (20, 21, 22). However, findings from a recent Brazilian study were not conclusive and indicated that for the population studied, breastfeeding had no marked protective effect against adolescent adiposity (23). The mechanisms for the proposed beneficial effect of breastfeeding against overweight and obesity is unknown, but one hypothesis is that obesity is an inflammatory condition and that the interaction between long chain polyunsaturated fatty acids, brain growth and development, pro-inflammatory cytokines, neurotransmitters and bone morphogenic proteins (BMPs) may explain the relationship (24). Another hypothesis is related to the fact that breast fed infants tend to grow more slowly in late infancy than those not breast fed. Programming of high serum leptin concentrations relative to fat mass through formula feeding and faster growth in infancy has been suggested as one mechanism linking early nutrition with later obesity (25). Childhood overweight has been related to rapid growth in infancy (26) and to catch-up growth from birth to two years (27). Rapid weight gain in infancy is also associated not only with childhood obesity but also with obesity in young adulthood (28). The discrepancy in growth rates between those breast fed and those not breast fed becomes prominent during the latter half of the first year of life (29, 30). The reason for the difference may be better self-regulation of energy intake among breast fed infants (31), which again has been linked to the lower metabolic rate and lower weight gain of breast fed infants compared to formula fed infants (32, 33). In 1995, Dewey and co-workers pointed out that infants breast fed for 12 months grew more rapidly during the first two months but more slowly from 3 to 12 months than their formula fed peers, and that longer duration of breastfeeding was associated with a greater decline in weight for age and weight for length but not in length for age (34). On the basis of this, a new international growth reference for young children is being developed (35). Breastfeeding has recently been shown to be related to lower BMI at the age of 6 years, which was also associated with a slightly slower growth in the latter half of infancy (36, 37). In light of recent studies, the solely positive implications of fast growth during infancy, as suggested earlier, have been seriously questioned. It is not exactly known how effectively breastfeeding can protect from overweight and obesity, and other feeding practices in childhood may also influence later risk of obesity.

Breastfeeding has also been associated with lower blood pressure in childhood and adolescence (38). Some studies further suggest that breastfeeding protects against inflammatory bowel disease (39) and malignant lymphoma (40), while other studies have failed to confirm

these results (41, 39). The prevalence of breast cancer is lower in women who have breast fed than in women who have not (42).

Recommendation for breastfeeding

In NNR 2004, exclusive breastfeeding is recommended until the infant is about six months. This is in accordance with the latest recommendation from the World Health Assembly, WHA, (43). The breast fed baby only needs to be supplemented with vitamin D, in the form of drops or as cod liver oil. From the age of six months, other foods should be gradually introduced into the child's diet as recommended in each country's dietary guidelines for infants. Breast milk as part of the diet is recommended throughout the child's first year, and partial breastfeeding can be continued for as long as it suits mother and child. Breastfeeding in the second and third year has been shown to decrease infections in developing countries, but health effects in industrialized countries have not been properly investigated.

Breastfeeding has strong health implications and is therefore an important part of general dietary guidelines. Special guidance should be given to groups of families with low frequency of breastfeeding, typically young mothers with limited education (44). Information should be given through primary healthcare maternity and child programmes. Routines and support in maternity wards have been shown to be important for the establishment of breastfeeding, and have provided the background for the UNICEF and WHO 'Baby Friendly Hospital Initiative'. Reports from Sweden show an increased frequency of breastfeeding after the routines in maternity wards were improved (45). All birth clinics in Sweden have 'Baby Friendly Hospital Initiative' status and now there is a trial to include a breastfeeding support chain into the maternity and child healthcare system. The 'Baby Friendly Hospital Initiative' was introduced in Norway in 1993 and by 1996 about 75% of all infants were born in designated hospitals (46). In a regional Norwegian study, increased rates of both exclusive and total breastfeeding (the sum of exclusive and partial breastfeeding) were observed from 1992 to 1997, and it was found that this improvement could be due to the 'Baby Friendly Hospital Initiative' (47).

If for some reason breastfeeding is not possible during the first 6 months of life, commercial infant formula formulated to serve as the only food for infants should be given. Parents should be given guidance on how to prepare and feed formula to their babies. The composition of the formula should comply with EU guidelines (48). The EU Scientific Committee on Food has recently made a thorough review of the litera-

ture to provide a basis for the composition of infant formula (49) and as a result of this review it is expected that the EU directive will be updated. According to the Infant Formulae Directive, 'infant formula' means a foodstuff intended for particular nutritional use by infants during the first four to six months of life. This should satisfy all the nutritional requirements of this category of persons, whereas 'follow-on formula' means a foodstuff intended for particular nutritional use by infants aged over four months and young children and constituting the principal liquid element in a progressively diversified diet of this category of persons (48). Some infants will need complementary feeding before 6 months of age. Experts agree that solid food should not be introduced before the age of 4 months. From 6 months of age, gradual introduction of a diversified diet is recommended and should be given under the supervision of the national child healthcare system. Recommendations on how to introduce complementary feeding differ slightly between the Nordic countries, *i.e.* type of grain, fruit and vegetables.

Prevalence of breastfeeding in the Nordic countries

Prevalence of breastfeeding in the Nordic countries is high. Most mothers start to breastfeed for a period immediately after parturition, and national statistics show that the prevalence of breastfeeding has increased during recent decades.

Figure 5.1 shows breastfeeding rates in the individual Nordic countries as given in national reports.

With the exception of the Swedish data, exclusive breastfeeding is defined as no food except breast milk. However, vitamin drops (AD drops) or cod liver oil could have been given. The prevalence of exclusive breastfeeding at 4 months of age was 50% in Denmark (44, data from 1994); 15% in Finland (50, data from 2000); 46% in Iceland (30, data from 1995–1997); and 44% in Norway (51, data from 1998). The cross-sectional Icelandic study from 1995–1997 gave similar results to a recent report including data from 1999–2000 (52). The Swedish prevalence value for exclusive breastfeeding at 4 months was 68%, but that figure also included infants given food in small portions to taste, *e.g.* porridge, fruits, etc. (53, data from 2000–2001).

Breastfeeding rates are increasing. In Finland during a 5-year period up to 2000, exclusive and total breastfeeding at 4 months of age increased from 10% and 61% to 15% and 66%, while total breastfeeding at six months increased from 45% to 51% during the same period (50).

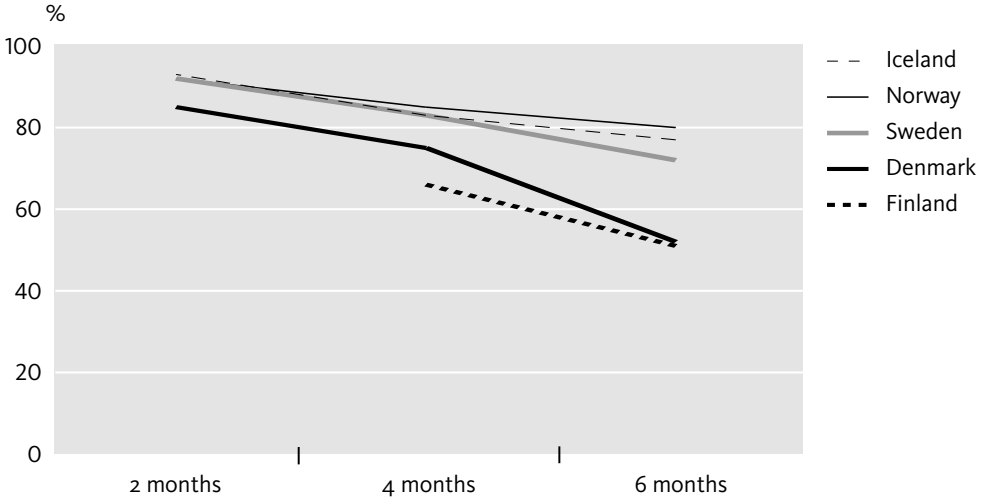

Figure 5.1 Breastfeeding of Nordic infants. National data on breastfeeding (total: exclusive and partial), were taken from: Denmark 1994 (44), Norway 1998 (51), Finland 2000 (50), Sweden 2000–2001 (53), Iceland 1996–1997 (30)

REFERENCES

1. Lönnerdal B. Nutritional and physiological significance of human milk proteins. Am J Clin Nutr 2003;77: 1537S–43S.
2. Standing Committee on Nutrition of the British Paediatric Association. Is breastfeeding beneficial for infants in the UK? Arch Dis Child 1994; 71: 376–80.
3. Hanson LÅ, Korotkova M, Håversen L, Mattsby-Baltzer I, Hahn-Zoric M, Silfverdal SE *et al*. Breastfeeding, a complex support system for the offspring. Pediatrics International 2002; 44: 347–52.
4. Bachrach VR, Schwarz E, Bachrach LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. Arch Pediatr Adolesc Med 2003; 157: 237–43.
5. Mimouni Bloch A, Mimouni D, Mimouni M, Gdalevich M. Does breastfeeding protect against allergic rhinitis during childhood? A meta-analysis of prospective studies. Acta Paediatr 2002; 91: 275–9.
6. Gdalevich M, Mimouni D, Mimouni M. Breastfeeding and the risk of bronchial asthma in childhood: A systematic review with meta-analysis of prospective studies. J Pediatr 2001; 139: 261–6.
7. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO *et al*. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: A longitudinal study. Lancet 2002; 360: 901–7.
8. van Odijk J, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA *et al*. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966–2001). Allergy 2003; 58: 833–43.
9. Andersson JW, Johnstone BM, Temley DT. Breastfeeding and cognitive development: A meta-analysis. Am J Clin Nutr 1999; 70: 525–35.
10. Drane DL, Logemann JA. A critical evaluation of the evidence on the association between type of infant

feeding and cognitive development. Paediatr Perinat Epidemiol 2000; 14: 349–56.
11. Pizarro F, Yip R, Dallman PR, Olivares M, Hertrampf E, Walter T. Iron status with different infant feeding regimens: Relevance to screening and prevention of iron deficiency. J Pediatr. 1991; 118: 687–92.
12. Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G. Iron status at 12 months of age – effects of body size, growth and diet in a population with high birth weight. Eur J Clin Nutr 2003; 57: 505–13.
13. Domellof M, Lonnerdal B, Abrams SA, Hernell O. Iron absorption in breast fed infants: Effects of age, iron status, iron supplements, and complementary foods. Am J Clin Nutr 2002; 76: 198–204.
14. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, Christy M, Zachau-Christiansen B, Kastrup K, Nerup J. Relation between breastfeeding and incidences rates of insulin dependent diabetes mellitus. A hypothesis. Lancet 1984; 2: 1083–6.
15. Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ. Reduced risk of IDDM among breast fed children. Diabetes 1988; 37: 1625–32.
16. Virtanen SM, Rasanen L, Aro A, Lindström J, Sippola H, Lounamaa R, *et al.* Infant feeding in Finnish children < 7 yr of age with newly diagnosed IDDM. Diabetes Care 1991; 14: 415–7.
17. Kostraba JN, Cruickshanks KJ, Lawler-Heavner J, Jobim LF, Rewers MJ, Gay EC, *et al.* Early exposure to cow's milk and solid foods in infancy, genetic predisposition and risk of IDDM. Diabetes 1993; 42: 288–95.
18. Gerstein HC. Cow's milk exposure and type I diabetes mellitus. A critical overview of the clinical literature. Diabetes Care 1994; 17: 13–9.
19. Virtanen SM, Knip M. Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at young age. Am J Clin Nutr 2003; 78: 1053–67.
20. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breastfeeding and obesity: Cross sectional study. BMJ 1999; 19: 147–50.
21. Gillman MW, Rifas-Shiman SL, Camargo CA, Berkey CS, Frazier AL, Rockett HR *et al.* Risk of overweight among adolescents who were breast fed as infants. JAMA 2001; 285: 2461–7
22. Dewey KG. Is breastfeeding protective against child obesity? J Hum Lact 2003; 19: 9–18.
23. Victora CG, Barros FC, Lima RC, Horta BL, Wells J. Anthropometry and body composition of 18 year old men according to duration of breastfeeding: Birth cohort study from Brazil. BMJ 2003; 327: 901–4.
24. Das UN. Is obesity an inflammatory condition? Nutrition 2001; 17: 953–66.
25. Singhal A, Faarooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations in later life. Am J Clin Nutr 2002; 75: 993–9.
26. Stettler N, Bovet P, Shamlaye H, Zemel BS, Stallings VA, Paccaud F. Prevalence and risk factors for overweight and obesity in children from Seychelles, a country in rapid transition: The importance of early growth. Int J Obesity 2002; 26: 214–9.
27. Ong KK, Ahmed ML, Emmet PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: Prospective cohort study. BMJ 2000; 320: 967–71.
28. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am J Clin Nutr 2003; 77: 1374–8.
29. Nielsen GA, Thomsen BL, Michaelsen KF. Influence of breastfeeding and complementary food on growth between 5 and 10 months. Acta Paediatr 1998; 87: 911–17.
30. Atladottir H, Thorsdottir I. Energy intake and growth of infants in Iceland – a population with high frequency of breastfeeding and high birth weight. Eur J Clin Nutr 2000; 54: 695–701.

31. Dewey KG. Growth characteristics of breast fed compared to formula-fed infants. Biol Neon 1998; 74: 94–105.
32. Butte NF, Wong WW, Ferlic L, Smith EO, Klein PD, Garza C. Energy expenditure and deposition of breast fed and formula fed infants during early infancy. Pediatr Res 1990; 28: 631–40.
33. Davies PS. Energy requirements and energy expenditure in infancy. Eur J Clin Nutr 1992; 46; S29–S35.
34. Dewey KG, Peerson JM, Brown KH, Krebs NF, Michaelsen KF, Person LA *et al*. Growth of breast fed infants deviates from current reference data: A pooled analysis of US, Canadian, and European data sets. WHO Working Group on Infant Growth. Pediatrics 1995; 96: 495–503.
35. Garza C & De Onis M. A new international growth reference for young children. Am J Clin Nutr 1999; 70: 169S–72S.
36. Thorsdottir I. Gunnarsdottir I, Palsson G. Association of birth weight and breastfeeding to coronary heart disease risk factors at the age of 6 years. Nutr Metab Cardiovasc Dis 2003; 13: 267–72.
37. Gunnarsdottir I, Thorsdottir I. Relationship between growth and feeding in infancy and body mass index at the age of 6 years. Int J Obes Relat Metab Disord 2003; 27: 1523–7.
38. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: Two cohorts after randomised trials. Lancet 2001;357: 413–9.
39. Davis MK. Breastfeeding and chronic disease in childhood and adolescence. Pediatr Clin North Am. 2001; 48: 125–41.
40. Bener A, Denic S, Galadari S. Longer breastfeeding and protection against childhood leukaemia and lymphomas. Eur J Cancer. 2001; 37: 234–8.
41. Hardell L, Dreifaldt AC. Breastfeeding duration and the risk of malignant diseases in childhood in Sweden. Eur J Clin Nutr. 2001; 55: 179–85.
42. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: Collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries. Lancet. 2002; 360: 187–95.
43. World Health Assembly (WHA). Infant and young child nutrition. Geneva: World Health Organization, 2001. (Resolution No WHA 54.2.)
44. Michaelsen KF, Larsen PS, Thausen BL, Samuelson G. The Copenhagen Cohort Study on Infant Nutrition and Growth: Duration of breastfeeding and influencing factors. Acta Paediatrica 1994; 83: 565–71.
45. Socialstyrelsen. Amning av barn födda 1993. Statistik, Hälsa och Sjukdomar 1996: 2. Stockholm: Socialstyrelsen.
46. Norwegian Board of Health. Mother-Baby-Friendly-Initiative in Norway, Project report 1993–96, IK-2572 (in Norwegian). Oslo: The Norwegian Board of Health; 1997.
47. Andreassen M, Bale M, Kaaresen PI, Dahl LB. Breastfeeding in Tromsø before and after 'the Baby Friendly Hospital Initiative' (in Norwegian). Tidsskr Nor Laegeforening 2001; 121: 3154–8.
48. Commission Directive 1991/321/EEC OF 14th May 1991 on infant formula and follow-on formula.
49. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae (April 2003).
50. Hasunen K. Infant feeding in Finland 2000. ISBN 952-00-1067-X, ISSN 1236-2115.
51. Lande B, Andersen LF, Bærug A, Trygg KU, Lund-Larsen K, Veierød MB, Bjørneboe GEA. Infant feeding practices and associated factors in the first six months of life: The Norwegian infant nutrition survey. Acta Pædiatr 2003; 92: 152–61.
52. Gunnlaugsson G. Breastfeeding in Iceland: Children born in 1999–2000. Report series. Reykjavik: Center for Child Health Services 2002; 1–18.
53. Socialstyrelsen, Epidemiologiskt centrum. Amning av barn födda 2000. Statistik, Hälsa och sjukdomar: Socialstyrelsen, 2002: 7.

CHAPTER 6

Food-based dietary guidelines

The Nordic Nutrition Recommendations give values for the intake of, and balance between, individual nutrients which, based on current knowledge, are adequate for development and function as well as contributing to the risk-reduction of certain diet-associated diseases. These values are mainly to be used in planning diets for various population groups. The practical implementation of such recommendations into foods, dishes and meals is dependent on a variety of factors such as market supply and cultural and culinary traditions. The concept *food-based dietary guidelines,* originally used by FAO, was defined as advice expressed at the food level aimed at the general population or specific population groups (1). Food-based dietary guidelines represent a 'translation' of the recommendations on energy and nutrients to foods and may include recommendations or advice on food choice, amounts, frequencies and eating pattern.

The dietary habits in the Nordic countries show several common features. These include an ample supply of milk and milk products, moderate to high consumption of meat and a moderate consumption of vegetables and fruit. Consumption of fish is moderate to high, but lower in Denmark. Potatoes and cereal products constitute major staple foods in most of the countries. The food consumption development during the last two or three decades also shows some common trends, *e.g.* increased consumption of low-fat milks and spreads, decreased total consumption of edible fats, and increased consumption of cheese, fruit and vegetables (2). However, the cultural and culinary traditions, *e.g.* concerning eating pattern, food choices and traditional dishes, differ. This has influenced the development and formulation of national food-based dietary guidelines.

An inventory of national guidelines in the Nordic countries used in the last decade showed that most countries have food-based guidelines aimed at improving the nutritional composition of the diet. These are intended for both the general population and health professionals. In some countries the guidelines are used as policy documents for use in

advocacy as a basis for political decisions. The choice of target group influences the formulation and detail of the guidelines. The focus of the guidelines also varies among the countries. Quantitative recommendations on *e.g.* fruit and vegetable consumption have been developed in most Nordic countries, although they are expressed in different ways, *e.g.* frequencies, portions or amounts per day (3–6).

Experiences from national implementations of the 3rd edition of NNR (1996) have shown that certain general changes in the diet are essential, *e.g.* to fulfil the recommendations regarding the balance between energy-yielding nutrients, dietary fibre and intakes of several micronutrients for a typical reference person (3, 7). These include an increased consumption of fruit and vegetables, bread and cereals, especially wholegrain fibre-rich products, and fish. For milk and meat, choice of lean alternatives is preferred and the consumption of edible fats should be moderate and soft and/or fluid varieties, low in saturated and trans fatty acids, chosen. The consumption of energy-dense and/or low nutrient dense foods such as soft drinks, sweets, snacks and sweet bakery products needs to be limited compared to the current situation. The intake of alcoholic beverages should also be limited.

Thus, the guidelines in each country support these general dietary changes. However, more detailed guidelines on types of foods, frequencies and portions, as well as eating pattern, have to be adapted to fit into the national situation. In NNR, no common dietary guidelines are given for these reasons.

In addition to food-based guidelines developed on nutritional considerations, there are guidelines on consumption of specific foods based on occurrence of environmental or other contaminants, *e.g.* dioxins and heavy metals. In many cases contamination is related to defined geographical areas and specific foods. The guidelines give advice on amounts or frequencies that can be consumed on a regular basis without exceeding the upper limit or guidance level of the substance in question. Such guidelines have to be considered in parallel with dietary guidelines based on nutritional considerations where applicable.

Dietary change and disease risk

Several controlled intervention studies have shown that a shift to a diet in line with the NNR recommendations (about 30 E% fat, less than 10 E% saturated fatty acids, and rich in fruit, vegetables and wholegrain cereal products) in combination with regular physical activity can reduce the risk of diabetes and cardiovascular disease (8–10). In two controlled

intervention studies, such changes resulted in an almost 60% reduced risk of developing diabetes compared to a control group (8, 9). Two controlled intervention studies showed that a shift from a habitual to a recommended diet reduced blood pressure in spite of weight stability (11, 12). Several studies have shown beneficial effects of dietary changes on risk factor levels in healthy individuals (13–15) and reduced risk for coronary heart disease in subjects with a previous myocardial infarction (16–18). Of the ten major risk factors for non-communicable disease in Europe listed in the WHO World Health Report for 2002, six were directly related to dietary factors (19). In 2003, WHO also published population nutrient intake goals (20), which are in line with the current NNR.

Fruit and vegetables

Fruit and vegetables are important sources of several nutrients in the Nordic countries, *e.g.* vitamin C, folate, potassium and other constituents such as dietary fibre, carotenoids and flavonoids. The nutrient density of these foods is generally very high, while the energy density is low.

A large body of epidemiological evidence supports the hypothesis that vegetables and fruit are protective in the aetiology of chronic diseases and risk factors including cancer, *e.g.* lung, gastrointestinal and hormone-related cancers (21–24), blood pressure (11, 12, 25), LDL-levels (26), cardiovascular disease (27–32), and type 2 diabetes (33). A positive link between vegetable and fruit consumption and bone health has also been suggested (34, 35). The protective role of fruit and vegetables for other diseases has also been studied, with promising results, but the evidence is still insufficient.

A whole variety of mechanisms have been postulated as potential disease-preventive mechanisms of vegetables and fruit. Antioxidant activity, modulation of detoxifying enzymes, stimulation of the immune system, decrease in platelet aggregation, alteration in cholesterol metabolism, modulation of steroid hormone metabolism, blood pressure reduction and even antibacterial and antiviral activity have been hypothesized as mechanisms (36). Vegetables and fruit are known to have high contents of vitamins (*e.g.* folate and vitamin C) minerals (*e.g.* potassium and magnesium) and fibre, but also a wide variety of other phytochemicals (*e.g.* carotenoids, flavonoids, phytosterols, isothiocyanates, etc.) compared to their energy content. In addition to the contribution of these foods to nutrient intake, they may play a role in improving the dietary pattern by replacing other, less favourable foods in the diet. It has been shown in many countries that a diet high in vegetables and

fruit contains less fat and more fibre than a diet low in vegetables and fruit (see *e.g.* 37). These foods may also serve as indicators of healthy food habits, as they may be connected with other healthy food choices or more widely with a healthy lifestyle of the consumer.

It has been estimated that an increase in vegetable and fruit consumption to at least 400 g/d would reduce the cancer incidence by 20% in Sweden (38). Similar estimations have been made for Denmark (39) and Norway (40). An increase in the vegetable and fruit consumption level to 600 g/d has been estimated to be associated with a 10–20% reduction in cardiovascular disease incidence (5). The results of such calculations depend on the current and targeted average consumption levels and the disease pattern of the target population, among other factors (22, 38). Smoking has also been suggested to be an important factor determining the potential benefits associated with fruit and vegetable consumption. Data from the 25-year follow-up of men in the Seven Countries Study showed that fruit intake was associated with reduced risk of lung cancer, but the beneficial effect was limited to heavy smokers (41).

A high and varied consumption of fruit and vegetables is desirable. In addition to providing a range of nutrients, ample consumption of fruit and vegetables may confer additional health benefits.

Potatoes

Potatoes are a staple food in the Nordic countries. Potatoes contribute starch and add to the daily intake of several nutrients, *e.g.* potassium, vitamin B_6, vitamin C and fibre. Boiled potatoes generally have a high glycaemic index (42), but also a high satiating index (43, 44). Based on the glycaemic properties of potatoes and the lack of data of a protective effect of potato consumption on chronic disease risk, a restricted consumption of potatoes has been recommended by some researchers (45, 46). Relatively few studies have investigated the association between potato consumption as such and disease risk. In some epidemiological studies, dietary patterns including a high consumption of potatoes in various preparation forms have been associated with increased risk of *e.g.* diabetes type 2 (47, 48) coronary heart disease (49) and weight gain (50). However, in many of these studies potatoes were reported as fried (*e.g.* chips) and were generally associated with a dietary pattern characterized by a high consumption of meat and high-fat and high-sugar foods and a low consumption of fruit, vegetables and fibre-rich cereals (47, 48, 50) and with a less favourable nutrient profile, *e.g.* more fat and less dietary fibre (48, 50). In other epidemiological studies using the dietary pattern approach, consumption of *e.g.* baked potatoes and sweet

potatoes was more frequent in healthy eating patterns (characterized by *e.g.* a high consumption of fruit and vegetables, low-fat dairy products and whole grains) associated with a lower mortality (51). Epidemiological or case-control studies on cancer in which potato consumption is analysed are few, and the results are conflicting (52–58). Potato consumption has not been associated with stroke risk (32).

In the 8-month trial by Sandström *et al.* (13), the intervention diet included potatoes in amounts exceeding 100 g per 10 MJ. This diet, which also included an increase in fruit and vegetables and fish, together with a decrease in meat and cheese, resulted in an improved serum lipid profile and reduced blood pressure and other risk markers of ischaemic heart disease (13, 14). In contrast to what is generally assumed, the traditional Mediterranean Cretan diet contained amounts of potatoes (150–200 g/d) comparable to those consumed in many Nordic countries today (59). In summary, due their contribution to the daily intake of several nutrients and to their traditional use, potatoes have a place in a diet based on NNR.

Cereals

Cereals are a major source of carbohydrate and dietary fibre in the Nordic diet. Cereals, especially wholegrain products, also provide a number of nutrients including potassium, magnesium, vitamin E, folate, and other biologically active constituents (*e.g.* phenolic acids, lignans and phytosterols).

The potential protective effect of cereal consumption against chronic diseases has been mainly connected with the consumption of wholegrain cereals. Several observational studies support the beneficial role of wholegrain consumption or cereal fibre in reducing the risk of CVD (60–62) and total mortality (63). High wholegrain consumption has also been associated with a reduced risk of developing insulin resistance and diabetes (64–67), hypertension (68) and some types of cancer (69–71).

The phytochemicals of wholegrain cereals may serve as both antioxidants and phytoestrogens (72). The potential beneficial effects of wholegrain food include lowering of serum total and LDL-cholesterol, in some cases also hypotriglyceridaemic effects, antioxidant properties and possibly also antithrombotic and decreased platelet-aggregating effects (14, 73). In addition, the effects of wholegrain foods on insulin resistance and the beneficial insulinaemic response to wholegrain foods is of great potential importance in reducing the risk of CHD and type 2 diabetes.

An increased consumption of wholegrain cereals is desirable. Apart from providing dietary fibre and other nutrients, wholegrain consumption may confer additional health benefits.

Fish

Fish and seafood provide a range of nutrients and contribute especially to the intake of vitamin D, iodine and selenium. Fish, especially fatty fish, is also a major source of long-chain n-3 fatty acids.

There is a body of evidence suggesting beneficial effects of fish consumption on health (74–79). Available evidence strongly indicates that eating fish or n-3 fatty acids from fish reduces the risk of fatal coronary heart disease (CHD), especially in high-risk populations. In observational studies it has been shown that in the general population, the consumption of moderate amounts of fish or n-3 fatty acids from fish is associated with a lower risk of fatal coronary heart disease and in particular sudden cardiac death (80–85). A few secondary prevention trials showed that prescription of fish or n-3 fatty acids to patients with prior myocardial infarction is effective in preventing mortality due to CHD (86–88).

The long chain n-3 fatty acids may modify the key risk factors for cardiovascular disease in several ways. The most plausible hypothesis today is that n-3 fatty acids reduce the risk of CHD mortality via anti-arrhythmic effects (78, 81). It is also possible that the protective effect of fish intake against CHD and related diseases is attributable to constituents other than n-3 fatty acids, for example fish proteins (89–91).

High intakes of n-3 fatty acids in the form of fish oil (median 4 g/d) have been associated with reduced blood pressure (92), but the effects of doses below 0.5 g/d are uncertain.

The evidence for a protective role of fish consumption against major cancers is less clear, partly due to lack of properly designed studies (93). Available epidemiological studies show somewhat inconsistent results, but overall there seems to be either no association or an inverse association between fish consumption and risk of breast, colorectal and prostate cancer.

Regular consumption of fish, both fatty and lean varieties, contributes to the intake of iodine, selenium, vitamin D and n-3 fatty acids in particular, and is recommended as part of a balanced diet.

Milk and milk products

Milk, fermented milks and cheese are traditional foods in the Nordic diet. Milk provides several nutrients, *e.g.* calcium, potassium, riboflavin and selenium. Cheese contains largely similar amounts of nutrients on an energy basis, except for *e.g.* lactose and potassium, which are concentrated in the whey. Milk fat is rich in saturated fatty acids, which is the main reason for recommending choice of low-fat varieties. Intervention studies show that diets containing moderate to high amounts of

low-fat dairy products and conforming to *e.g.* Nordic recommendations have been associated with favourable changes in risk factor profile for coronary heart disease, *e.g.* on serum cholesterol (13, 14), blood pressure (12, 13) and diabetes (8). Consumption of milk or conventional fermented milk products has in some earlier studies been shown to have less pronounced effects on serum lipids than could be expected from their fat content (94, 95). Many of the studies included very large amounts of milk, which could have affected the general dietary composition. More recent intervention studies in humans including mainly fermented milk products containing different bacteria strains have shown varying results (96–100).

In cross-sectional and epidemiological studies, consumption of milk and milk products has been associated with both increased and decreased risk of cardiovascular disease (101–106). The observed risk was in some studies related to the fat content (102–104), while this aspect was not considered in other studies (105, 106). Dietary milk fat or specific fatty acids have also been associated with some favourable metabolic features in some cross-sectional and epidemiological studies, but any causal relationship has yet to be identified (107, 108). Some of the relationships observed may be due to incomplete and selective dietary reporting (109).

In epidemiological studies, consumption of milk (and calcium intake) has been associated with reduced risk of colorectal cancer (110, 111), while case-control studies are inconclusive. No or weak associations with breast cancer have been reported (112, 113). Some case-control studies have reported an increased risk of prostate cancer (114), while findings from cohort studies show no or weak associations (115–119). The interpretation of the studies should be made with caution since data did not always allow a differentiation of type of milk or milk product, *e.g.* with respect to fat content. In studies in which this aspect was analysed, different risk estimates were found (111, 120).

Due to their high content of calcium, milk and milk products have been promoted for adequate bone formation during childhood and the prevention of post-menopausal osteoporosis. Adequate or high intakes of calcium seem to increase bone density in adolescence and supplementation with calcium or milk has similar effects in older women. Some epidemiological studies showed no protective effect of milk consumption or high calcium intake on osteoporotic hip fractures in postmenopausal women (121). An evaluation of available studies (122) concluded, however, that high consumption of milk and dairy products seems to have beneficial effects on bone development in adolescence and to postpone pre-menopausal as well as postmenopausal bone loss.

A low consumption of milk and dairy products seems to increase the risk of osteoporotic fractures. In addition, no evidence has been found to support the hypothesis that milk and dairy products have negative effects on bone mineral content or on the risk of osteoporotic fractures.

Regular consumption of milk and milk products, mainly lean varieties, contributes *e.g.* calcium, riboflavin and other nutrients, and is recommended as a part of a balanced diet.

Meat

Meat and meat products are traditional foods in the Nordic diet and contribute protein, readily available iron, selenium, zinc and a range of B-vitamins. Meat also provides fat, especially saturated fatty acids, and cholesterol. Processed meat products usually contain relatively high amounts of sodium (as salt). A relatively high, but variable, proportion of women of childbearing age in the Nordic countries is iron deficient. Inclusion of lean meat into the diet improves iron availability and status. Due to the relatively high proportion of saturated fatty acids, high consumption of fatty meat and meat products can contribute to increased LDL-cholesterol levels and thereby an increased risk of cardiovascular disease (123). High consumption of meat, especially red and processed meat (*e.g.* beef, pork, sausages) has also been regarded as a risk for colorectal cancer. A report by the World Cancer Research Fund (21) contains the following statement: 'if eaten at all, consumption of red meat should be less than 80 g per day'. The statement was largely based on epidemiological and animal evidence (124). In more recent years this statement has been re-evaluated. Case-control and cohort studies have shown that relationship between meat intake and colon cancer incidence is dependent on the amount eaten and proportion of protective agents in the diet. Two large cohort studies from North America (125, 126) showed that only the highest intake group (more than 140 g red meat per day) had an excess risk of colorectal cancer. In a large prospective cohort (n = 265 000) study from Japan an inverse relationship between meat intake and colorectal cancer was found in the group consuming green-yellow vegetables daily. For those who never ate vegetables there was a positive correlation between meat intake and cancer of the colon and rectum (127). The current evidence suggests that meat consumption is not carcinogenic *per se*. However, meat may contain harmful components that are formed during cooking (heterocyclic amines and polycyclic aromatic hydrocarbons), processing (nitrates and nitrites) or during intestinal metabolism (N-nitroso compounds). The carcinogenic potential of these compounds may be diminished by avoiding exposure of meat surfaces to flames and high temperatures and also

by increasing dietary intake of protective constituents derived from plant foods. The balance of promoting and protecting factors within the diet is important for the protection against cancer (128).

Meat and meat products provide a range of nutrients. Consumption of moderate amounts of meat, preferably lean varieties, is recommended as part of a balanced and varied diet.

Edible fats

Edible fats, *e.g.* butter, margarines and vegetable oils, are the major sources of fat in the Nordic diet. The recommendations on choice of fats are mainly based on the evidence regarding fat composition and risk of coronary heart disease, which emphasises a limitation of saturated and trans fatty acids, and an increase in n-3 fatty acids, while maintaining the n-6 fatty acid intake (see *Chapter 11* on fat).

In practice, to achieve the recommendation on fat composition, soft or fluid vegetable fats, low in saturated and trans fatty acids, should primarily be chosen. An increase in the intake of n-3 fatty acids can be achieved by choosing suitable edible fats, *e.g.* rapeseed oil and rapeseed oil based fats, in addition to other good sources such as fish and seafood.

Energy-dense and sugar-rich foods

Foods rich in fat and/or refined sugars, such as soft drinks, sweets, snacks and sweet bakery products, mainly contribute 'empty calories'. Frequent consumption of such foods decreases the nutrient density and increases the risk of nutritional imbalance and inadequacy and also dental caries. Some studies (129–130), but not all (131), indicate that a high intake of refined sugars in fluid form, *e.g.* soft drinks, may increase the risk of overweight (see *e.g.* chapter on carbohydrates). In order to fulfil the NNR, consumption of such foods needs to be limited.

Salt

The recommendation on sodium (salt) is mainly based on effects on blood pressure (see *Chapter 32*). A major factor in order to achieve the recommended salt intake is a reduction in the sodium levels in many processed foods. In addition, household and individual use of table salt needs to be moderated.

REFERENCES

1. FAO/WHO. Preparation and use of food-based dietary guidelines. Geneva, WHO, 1998.
2. Becker W, Nordhamn K, Berge S, Kortesmaa T, Nielsen B, Thorgeirsdóttir H, Wikberger C. Livsmedelskonsumtionen i Norden 1965–1998.

Nationell, årlig *per capita* statistik (Food consumption in the Nordic countries 1965–1998. National, annual food balance sheet data). TemaNord 2001:527. Nordic Council of Ministers.
3. Icelandic Nutrition Council (2001). Hollustukarfa Manneldisráðs Íslands. www.manneldi.is/ht/b/hollustukarfa.htm. (Accessed 20 April 2004.)
4. Becker W, Hagman U. Mer frukt och grönt bra för hälsan (More fruit and vegetables is beneficial for health). Vår Föda 1999; 51 (6): 24–28.
5. Ovesen L, Lyhne Andersen N, Dragsted L, Godtfredsen J, Haraldsdottir J, Stender S, Sølling K, Tjønneland A, Trolle E. Frugt, grønt og helbred. Opdatering af vidensgrundlaget (Fruit, vegetables and health. An update of the scientific evidence). Fødevarerapport 2002: 22, Fødevaredirektoratet, Copenhagen: 2002.
6. Blomhoff R, Lande G, Ose T. Nye anbefalinger for inntak av frukt og grønnsaker (New recommendations on fruit and vegetable consumption), Statens Ernæringsråd, Oslo: 1995.
7. Enghardt Barbieri H, Lindvall C. Svenska Näringsrekommendationer översatta till livsmedel. Underlag till generella råd på livsmedelsnivå. (Swedish Nutrition Recommendations translated into foods). Livsmedelsverket, Rapport 1-2003.
8. Tuomilehto J *et al.* The Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001; 344: 1343–50.
9. Knowler WC *et al.* Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346: 393–403.
10. Mensink M, Blaak EE, Corpeleijn E, Saris WH, de Bruin TW, Feskens EJ. Lifestyle intervention according to general recommendations improves glucose tolerance. Obes Res. 2003; 11: 1588–96.
11. Appell LJ *et al.* A clinical trial on the effects of dietary patterns on blood pressure, N Engl J Med 1997; 336: 1117–24.
12. Sacks FM *et al.* Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. N Engl J Med 2001; 344: 3–10.
13. Sandström B, Marckmann P, Bindslev N. An eight-month controlled study of a low-fat high-fibre diet: effects on blood-lipids and blood pressure in healthy young subjects. Eur J Clin Nutr 1992; 46: 95–109.
14. Marckmann P, Sandström B, Jespersen J. Low-fat, high-fiber diet favorably affects several independent risk markers of ischemic heart disease: Observations on blood lipids, coagulation, and fibrinolysis from a trial of middle-aged Danes. Am J Clin Nutr 1994; 59: 935–9.
15. van Dokkum W, van der Beek E, de Pee S, Schafsmaa G, Wesstra A, Wedel M. Dutch dietary guidelines: Impact on blood lipids, blood pressure, body composition and urinary mineral excretion of Dutch middle-aged men. Eur J Clin Nutr 1991; 45: 431–39.
16. Johnson LK, Hjermann I, Tonstad S. Diet and secondary prevention of coronary heart disease – are our recommendations good enough? Tidsskr Nor Laegeforen 2001; 121: 1092–8.
17. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon Diet Heart Study. Circulation 1999; 99: 779–85.
18. Singh RB *et al.* Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomized single-blind trial. Lancet 2002; 360: 1455–61.
19. WHO. The World Health Report 2002. Reducing risks, promoting healthy life, Geneva, 2002.
20. WHO. Diet, nutrition and the prevention of chronic diseases. Report of the

WHO/FAO Joint expert consultation. WHOTechn Rep Ser 916, Geneva: 2003.
21. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: A global perspective. Washington, DC: American Institute for Cancer Research, 1997.
22. van't Veer P, Jansen MC, Klerk M, Kok FJ. Fruits and vegetables in the prevention of cancer and cardiovascular disease. Public Health Nutr 2000; 3: 103–7.
23. Feskanich D, Ziegler RG, Michaud DS, Giovannucci EL, Speizer FE, Willett WC, Colditz GA. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000; 92: 1812–23.
24. Voorrips LE, Goldbohm RA, Verhoeven DT, van Poppel GA, Sturmans F, Hermus RJ, van den Brandt PA. Vegetable and fruit consumption and lung cancer risk in the Netherlands. Cohort Study on diet and cancer. Cancer Causes Control 2000; 11: 101–15.
25. John JH *et al.* Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: A randomised controlled trial. Lancet 2002; 359: 1969–74.
26. Djousse L, Arnett DK, Coon H, Province MA, Moore LL, Ellison RC. Fruit and vegetable consumption and LDL cholesterol: The National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr. 2004; 79: 213–7.
27. Klerk M, Jansen MCJF, van't Veer P, Kok FJ. Fruits and vegetables in chronic disease prevention. Wageningen: Wageningen Agricultural University, 1998.
28. Singh RB, Niaz MA, Ghosh S, Singh R, Rastogi SS. Effect on mortality and reinfarction of adding fruit and vegetables to a prudent diet in the Indian experiment of infarct survival (IEIS). Am J Coll Nutr 1993: 12: 255–61.
29. Ness A, Powles JW. Fruit and vegetables, and cardiovascular disease: A review. Int J Epidemiol 1997;26: 1–13.
30. Law MR, Morris JK. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? Eur J Clin Nutr 1998; 52: 549–56.
31. Gillman MW, Cuppies LD, Gagnon D, Millen Posner B, Ellison RC, Castelli WP, Wolf PA. Protective effect of fruit and vegetables on development of stroke in men. JAMA 1995; 273: 1113–17.
32. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Hennekens CH, Spiegelman D, Willett WC. Fruit and vegetable intake in relation to risk of ischemic stroke. JAMA 1999; 282: 1233–39.
33. Williams DE, Wareham NJ, Cox BD, Byrne CD, Hales CN, Day NE. Frequent salad vegetable consumption is associated with a reduction in the risk of diabetes mellitus. J Clin Epidemiol 1999; 52: 329–35.
34. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 2000; 71: 142–51.
35. Tylavsky FA, Holliday K, Danish R, Womack C, Norwood J, Carbone L. Fruit and vegetable intakes are an independent predictor of bone size in early pubertal children. Am J Clin Nutr. 2004; 79: 311–7.
36. Lampe JW. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. Am J Clin Nutr 1999; 70: 475S–490S.
37. Valsta LM. Food-based dietary guidelines for Finland – a staged approach. Br J Nutr 1999; 81 (Suppl. 2): S49–55.
38. Hoffmann K, Boeing H, Volatier J-L, Becker W. Evaluating the potential health gain of the WHO recommendation concerning vegetable and fruit consumption. Publ Health Nutr 2003; 6: 765–72.
39. Gundgaard J, Nielsen JN, Olsen J, Sørensen J. Increased intake of fruit and vegetables: Estimation of impact in terms of life expectancy and healthcare costs. Public Health Nutr. 2003; 6: 25–30.

40. Blomhoff R. Fruit and vegetables in the prevention of cancer (in Norwegian). Tidsskr Nor Lægeforen 1998; 118: 3462–5.
41. Jansen MC, Bueno-de-Mesquita HB, Rasanen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ, Kromhout D. Cohort analysis of fruit and vegetable consumption and lung cancer mortality in European men. Int J Cancer 2001; 92: 913–18.
42. Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr 2002; 76: 5–56.
43. Holt SH, Miller JC, Petocz P, Farmakalidis E. A satiety index of common foods. Eur J Clin Nutr. 1995; 49: 675–90.
44. Kaplan RJ, Greenwood CE. Influence of dietary carbohydrates and glycaemic response on subjective appetite and food intake in healthy elderly persons. Int J Food Sci Nutr. 2002; 53: 305–16.
45. Willett WC, Stampfer MJ. Rebuilding the food pyramid. Sci Am. 2003; 288 (1): 64–71.
46. McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, Spiegelman D, Hunter DJ, Colditz GA, Willett WC. Diet quality and major chronic disease risk in men and women: Moving toward improved dietary guidance. Am J Clin Nutr. 2002; 76: 1261–71.
47. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA. 1997; 277: 472–7.
48. van Dam RM, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Dietary patterns and risk for type 2 diabetes mellitus in US men. Ann Intern Med. 2002; 136: 201–9.
49. Fung TT, Willett WC, Stampfer MJ, Manson JE, Hu FB. Dietary patterns and the risk of coronary heart disease in women. Arch Intern Med. 2001; 161: 1857–62.
50. Newby PK, Muller D, Hallfrisch J, Andres R, Tucker KL. Dietary patterns and changes in body mass index and waist circumference in adults. Am J Clin Nutr. 2003; 77: 1417–25.
51. Kant AK, Graubard BI, Schatzkin A. Dietary patterns predict mortality in a national cohort: The National Health Interview Surveys, 1987 and 1992. J Nutr. 2004; 134: 1793–9.
52. Hu J, Mao Y, Dryer D, White K; Canadian Cancer Registries Epidemiology Research Group. Risk factors for lung cancer among Canadian women who have never smoked. Cancer Detect Prev. 2002; 26: 129–38.
53. Jansen MC, Bueno-de-Mesquita HB, Buzina R, Fidanza F, Menotti A, Blackburn H, Nissinen AM, Kok FJ, Kromhout D. Dietary fiber and plant foods in relation to colorectal cancer mortality: The Seven Countries Study. Int J Cancer. 1999; 81: 174–9.
54. Franceschi S, Favero A, La Vecchia C, Negri E, Conti E, Montella M, Giacosa A, Nanni O, Decarli A. Food groups and risk of colorectal cancer in Italy. Int J Cancer. 1997; 72: 56–61.
55. Steinmetz KA, Potter JD. Food-group consumption and colon cancer in the Adelaide Case-Control Study. I. Vegetables and fruit. Int J Cancer. 1993; 53: 711–9.
56. Ngoan LT, Mizoue T, Fujino Y, Tokui N, Yoshimura T. Dietary factors and stomach cancer mortality. Br J Cancer. 2002; 87: 37–42.
57. Jansen MC, Bueno-de-Mesquita HB, Rasanen L, Fidanza F, Menotti A, Nissinen A, Feskens EJ, Kok FJ, Kromhout D. Consumption of plant foods and stomach cancer mortality in the seven countries study. Is grain consumption a risk factor? Seven Countries Study Research Group. Nutr Cancer. 1999; 34: 49–55.
58. De Stefani E, Correa P, Boffetta P, Ronco A, Brennan P, Deneo-Pellegrini H, Mendilaharsu M. Plant foods and risk of gastric cancer: A case-control study in Uruguay. Eur J Cancer Prev. 2001; 10: 357–64.
59. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, *et al.* Food consumption pat-

terns in the 1960s in seven countries. Am J Clin Nutr. 1989; 49: 889–94.
60. Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr 1999; 70: 412–19.
61. Liu S, Manson JE, Stampfer MJ, Rexrode KM, Hu FB, Rimm EB, Willett WC. Whole grain consumption and risk of ischemic stroke in women: A prospective study. JAMA. 2000; 284: 1534–40.
62. Mozaffarian D, Kumanyika SK, Lemaitre RN, Olson JL, Burke GL, Siscovick DS. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. JAMA. 2003; 289: 1659–66.
63. Jacobs DR Jr, Meyer HE, Solvoll K. Reduced mortality among whole grain bread eaters in men and women in the Norwegian County Study. Eur J Clin Nutr. 2001; 55: 137–43.
64. Salmerón J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 1997; 20: 545–550.
65. Salmerón J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA 1997; 277: 472–77.
66. Liese AD, Roach AK, Sparks KC, Marquart L, D'Agostino RB Jr, Mayer-Davis EJ. Whole-grain intake and insulin sensitivity: The Insulin Resistance Atherosclerosis Study. Am J Clin Nutr. 2003; 78: 965–71.
67. Montonen J, Knekt P, Jarvinen R, Aromaa A, Reunanen A. Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr. 2003; 77: 622–9.
68. Ascherio A, Rimm EB, Giovannucci EL, Colditz GA, Rosner B, Willett WC, Sacks F, Stampfer MJ. A prospective study of nutritional factors and hypertension among US men. Circulation 1992; 86: 1475–84.
69. Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997; 29: 95–120.
70. Bingham SA *et al.* Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003; 361: 1496–501.
71. Slattery ML, Curtin KP, Edwards SL, Schaffer DM. Plant foods, fiber, and rectal cancer. Am J Clin Nutr. 2004; 79: 274–81.
72. Slavin JL. Mechanisms for the impact of whole grain foods on cancer risk. J Am Coll Nutr 2000; 19, 300S–307S.
73. Anderson JW. Dietary fibre, complex carbohydrate and coronary artery disease. Can J Cardiol 1995; 11 (Suppl. G), 55G–62G.
74. Fødevaredirektoratet. General view of fish and fish products (in Danish with English summary). FødevareRapport 2003: 17. Danish Food and Veterinary Administration, Copenhagen.
75. Scientific Advisory Committee on Nutrition/Committee of Toxicity. Advice on fish consumption: benefits & risks. London: TSO, 2004.
76. Kris-Etherton PM, Harris WS, Appel LJ *et al.* AHA Scientific Statement. Fish Consumption, Fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002; 106: 2747–57.
77. Steingrímsdóttir L. Positive health effects of seafood intake – epidemiological evidence. In Fish as food II. Report from a Nordic seminar. TemaNord 2001:532, Copenhagen: Nordic Council of Ministers.
78. Jones PJ, Lau VW. Effect of n-3 polyunsaturated fatty acids on risk reduction of sudden death. Nutr Rev 2002; 60, 407–09.
79. de Deckere EA, Korver O, Verschuren PM, Katan MB. Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. Eur J Clin Nutr. 1998; 52: 749–53.
80. Marckmann P, Grønbæk M. Fish consumption and coronary heart disease mortality. A systematic review of

prospective cohort studies. Eur J Clin Nutr. 1999; 53: 585–90.
81. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE. Fish consumption and risk of sudden cardiac death. JAMA 1998; 279, 23–28.
82. Yuan JM, Ross RK, Gao YT, Yu MC. Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai, China. Am J Epidemiol 2001; 154, 809–16.
83. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: A meta-analysis of randomized controlled trials. Am J Med 2002; 112: 298–304.
84. Erkkilä AT, Lehto S, Pyörälä K, Uusitupa MI. n-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. Am J Clin Nutr 2003; 78, 65–71.
85. Nordøy A, Marchioli R, Arnesen H, Videbaek J. n-3 polyunsaturated fatty acids and cardiovascular diseases. Lipids 2001; 36 Suppl.: S127–9.
86. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and reinfarction trial (DART). Lancet 1989; 2: 757–61.
87. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V, Moshiri M. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: The Indian experiment of infarct survival-4. Cardiovasc Drugs Ther 1997; 11: 485–91.
88. Anonymous. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: Results of the GISSI-Prevenzione trial. Lancet 1999; 354, 447–55.
89. Lavigne C, Tremblay F, Asselin G, Jacques H, Marette A. Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. Am J Physiol Endocrinol Metab 2001; 281: E62–E71.
90. Tremblay F, Lavigne C, Jacques H, Marette A. Dietary cod protein restores insulin-induced activation of phosphatidylinosol 3-kinase/Akt and GLUT4 translocation to the T-tubles in skeletal muscle of high-fat-fed obese rats. Diabetes 2003; 52: 29–37.
91. Yahia DA, Madanni S, Prost E, Prost J, Bouchenak M, Belleville J. Tissue antioxidant status differs in spontaneously hypertensive rats fed fish protein or casein. J Nutr 2003; 133: 479–482.
92. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: Metaregression analysis of randomized trials. J Hypertens. 2002; 20: 1493–9.
93. Hjartåker A. Fish consumption and risk of breast, colorectal and prostate cancer: A critical evaluation of epidemiological studies. Scand J Nutr 2003; 47: 111–22.
94. Bosaeus I. Milk and cholesterol (in Swedish with English summary). Vår Föda 1991; 43: 98–101.
95. Eichholzer M, Stahelin H. Is there a hypocholesterolemic factor in milk and milk products? Int J Vitam Nutr Res. 1993; 63: 159–67.
96. St-Onge MP, Farnworth ER, Jones PJ. Consumption of fermented and non-fermented dairy products: effects on cholesterol concentrations and metabolism. Am J Clin Nutr. 2000; 71: 674–81.
97. Anderson JW, Gilliland SE. Effect of fermented milk (yogurt) containing Lactobacillus acidophilus L1 on serum cholesterol in hypercholesterolemic humans. J Am Coll Nutr. 1999; 18: 43–50.
98. de Roos NM, Schouten G, Katan MB. Yoghurt enriched with Lactobacillus acidophilus does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. Eur J Clin Nutr. 1999; 53: 277–80.
99. Kiessling G, Schneider J, Jahreis G. Long-term consumption of fermented dairy products over 6 months

increases HDL cholesterol. Eur J Clin Nutr. 2002; 56: 843–9.
100. Agerholm-Larsen L, Raben A, Haulrik N, Hansen AS, Manders M, Astrup A. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur J Clin Nutr. 2000; 54: 288–97.
101. Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A. Food intake patterns and 25-year mortality from coronary heart disease: Cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. Eur J Epidemiol. 1999; 15: 507–15.
102. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr. 1999; 70: 1001–8.
103. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr. 2000; 72: 912–21.
104. Kabagambe EK, Baylin A, Siles X, Campos H. Individual saturated fatty acids and nonfatal acute myocardial infarction in Costa Rica. Eur J Clin Nutr. 2003; 57: 1447–57.
105. Elwood PC, Pickering JE, Fehily AM, Hughes J, Ness AR. Milk drinking, ischaemic heart disease and ischaemic stroke I. Evidence from the Caerphilly cohort. Eur J Clin Nutr. 2004; 58: 711–7.
106. Elwood PC, Pickering JE, Hughes J, Fehily AM, Ness AR. Milk drinking, ischaemic heart disease and ischaemic stroke II. Evidence from cohort studies. Eur J Clin Nutr. 2004; 58: 718–24.
107. Sjögren P, Rosell M, Skoglund-Andersson C, Zdravkovic S, Vessby B, de Faire U, Hamsten A, Hellenius ML, Fisher RM. Milk-derived fatty acids are associated with a more favorable LDL particle size distribution in healthy men. J Nutr. 2004; 134: 1729–35.
108. Warensjö E, Jansson JH, Berglund L, Boman K, Ahren B, Weinehall L, Lindahl B, Hallmans G, Vessby B. Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study. Br J Nutr. 2004; 91: 635–42.
109. Rosell MS, Hellenius ML, de Faire UH, Johansson GK. Associations between diet and the metabolic syndrome vary with the validity of dietary intake data. Am J Clin Nutr. 2003; 78: 84–90.
110. Cho E, Smith–Warner SA, Spiegelman D, Beeson WL, van den Brandt PA, Colditz GA, *et al.* Dairy foods, calcium, and colorectal cancer: A pooled analysis of 10 cohort studies. J Natl Cancer Inst. 2004; 96: 1015–22.
111. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. Eur J Clin Nutr. 2003;57: 1–17.
112. Missmer SA, Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, van den Brandt PA, *et al.* Meat and dairy food consumption and breast cancer: A pooled analysis of cohort studies. Int J Epidemiol. 2002; 31: 78–85.
113. Moorman PG, Terry PD. Consumption of dairy products and the risk of breast cancer: A review of the literature. Am J Clin Nutr. 2004; 80: 5–14.
114. Qin LQ, Xu JY, Wang PY, Kaneko T, Hoshi K, Sato A. Milk consumption is a risk factor for prostate cancer: Meta-analysis of case-control studies. Nutr Cancer. 2004; 48: 22–7.
115. Chan JM, Pietinen P, Virtanen M, Malila N, Tangrea J, Albanes D, Virtamo J. Diet and prostate cancer risk in a cohort of smokers, with a specific focus on calcium and phosphorus (Finland). Cancer Causes Control. 2000; 11: 859–67.

116. Michaud DS, Augustsson K, Rimm EB, Stampfer MJ, Willet WC, Giovannucci E. A prospective study on intake of animal products and risk of prostate cancer. Cancer Causes Control. 2001; 12: 557–67.
117. Chan JM, Stampfer MJ, Ma J, Gann PH, Gaziano JM, Giovannucci EL. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. Am J Clin Nutr. 2001; 74: 549–54.
118. Rodriguez C, McCullough ML, Mondul AM, Jacobs EJ, Fakhrabadi-Shokoohi D, Giovannucci EL, Thun MJ, Calle EE. Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. Cancer Epidemiol Biomarkers Prev. 2003; 12: 597–603.
119. Berndt SI, Carter HB, Landis PK, Tucker KL, Hsieh LJ, Metter EJ, Platz EA; Baltimore Longitudinal Study of Aging. Calcium intake and prostate cancer risk in a long-term aging study: The Baltimore Longitudinal Study of Aging. Urology. 2002; 60: 1118–23.
120. Goodman MT, Wu AH, Tung KH, McDuffie K, Kolonel LN, Nomura AM, Terada K, Wilkens LR, Murphy S, Hankin JH. Association of dairy products, lactose, and calcium with the risk of ovarian cancer. Am J Epidemiol. 2002; 156: 148–57.
121. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: A prospective study among postmenopausal women. Am J Clin Nutr. 2003; 77: 504–11.
122. Mortensen LS, Charles P, Beck Jensen J-E, Sørensen OH. The importance of milk as a calcium source and in the development of osteoporosis (Betydningen af mælk som calciumkilde og for udvikling af osteoporose). Publ. 32, 2003, Ernæringsrådet, København.
123. Ovesen L. Kødindtaget i Danmark og dets betydning for ernæring og sundhed (Meat intake in Denmark and its importance for nutrition and health). Fødevarerapport 2002: 20, Fødevaredirektoratet, Avd for Ernæring, Søborg.
124. Potter JD, Slattery ML, Bostick RM, Gapstur SM. Colon cancer: A review of the epidemiology. Epidemiol Rev 1993; 15: 499–545.
125. Willet WC, Stampfer MJ, Colditz GA *et al.* Relation of meat, fat and fiber intake to the risk of colon cancer in a prospective study among women. New Engl J Med 1990; 323: 1664–1672.
126. Giovannucci E, Rimm EB, Stampfer MJ *et al.* Intake of fat, meat and fiber in relation to risk of colon cancer in men. Cancer Res 1994; 54: 2390–2397.
127. Hirayama T. Life style and mortality. In article: Hill M. Meat, cancer and dietary advice to the public. Eur J Clin Nutr 2002; 56; S36–S41.
128. Potter, JD. Colorectal cancer: Molecules and populations (a review). JNCI; 1999; 91: 916–932.
129. Ludwig DS, Peterson KE, Gortmarker SL. Relation between consumption of sugar-sweeted drinks and childhood obesity: a prospective observational analysis. Lancet 2001; 357: 505–508.
130. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, Hu FB. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. JAMA. 2004; 292: 927–34.
131. Newby PK, Peterson KE, Berkey CS, Leppert J, Willett WC, Colditz GA. Beverage consumption is not associated with changes in weight and body mass index among low-income preschool children in North Dakota. J Am Diet Assoc. 2004; 104: 1086–94.

CHAPTER 7

Eating pattern

Nutritional status, health, mood and performance may be influenced not only by *what* you eat, but also by *when* and *how often.* Daily eating frequency and the size (energy content) and composition (nutrients) of the meals are linked to metabolic and behavioural processes such as hunger and satiation, habits and societal conditions. Furthermore, metabolism after a meal – postprandial metabolism – varies during the 24-hour day due to circadian variations in digestion, absorption and metabolism.

Eating pattern, here defined as frequency, periodicity and distribution of food intake over the day, is determined by social, psychological and biological demands and needs (1–3). Environmental factors in the 24-hour Western society seem to support an unhealthy lifestyle characterised by over-eating energy, physical inactivity, mental stress, irregular work hours and sleep deficiency. Foods and take-away meals are on offer in abundance around the clock. In many societies, it is no longer considered inappropriate to eat alone, to eat outside the home or to eat outside 'traditional' meal times (4, 5). Taken together, changes in attitudes, behaviour and environment challenge the biological capacity of individuals to regulate energy intake. Because of this complexity, guidelines on appropriate eating patterns are a pre-requisite for planning meals for individuals, families and groups, *e.g.* in schools and workplaces (6).

Studying eating patterns: methodological aspects

It is difficult to evaluate and describe eating patterns from observational studies due to a number of methodological problems (7, 8). Most studies lack objective and reliable criteria for eating event classification. Eating events are subjectively grouped into categories such as 'meal', 'snack', 'main meal' or 'lunch'. The basis for classification varies, *i.e.* it relates to the hour of the day, to the chronological order of the eating events or to the perceived nutritional quality of the foods consumed. Neither types of foods nor nutrients are reflected in the terminology used. Furthermore, both the subjects themselves and the investigators may ignore intakes not perceived as 'proper meals'. Concepts for classi-

fication of eating events have been developed and applied by relatively few researchers (9, 10). Only one concept is strictly food based, *i.e.* qualitative (11). Due to methodological pitfalls, *i.e.* underreporting of dietary intake, much of the cross-sectional epidemiological data on eating periodicity and nutritional status, *e.g.* hypercholesterolaemia, CHD, glucose tolerance and BMI (12–14), must also be questioned (3, 5).

Either experimental or intervention studies are required to study links between eating periodicity, postprandial response and nutritional status, *i.e.* serum lipids, lipoproteins and energy metabolism. A number of well-controlled studies are available (15–22) but must be interpreted with caution, since they included relatively few subjects and had a short duration. For example, the intervention *per se* may affect habitual eating pattern, food choice and other lifestyle factors of importance for the outcome. Intervention studies often compare nibbling/grazing (6–17 daily meals) and gorging (1–2 meals). Thus, many of the studies reflect an exceptional eating frequency.

Physiology and metabolism

The biological clock

The eating behaviour of animals and humans is strongly related to the daylight cycle. Oscillators in the central nervous system (CNS), which are synchronised by the changes in light and darkness, determine the temporal organisation of eating to a large extent. Human beings are genetically pre-disposed to be day-active (diurnal). The body clock is situated in the paired hypothalamic suprachiasmatic nuclei (SCN) in the brain (23, 24). The endogenous circadian ('about 24-hour') rhythm (25) is disturbed by external factors, such as time zone transitions, light, darkness and behaviour (eating, sleeping, working). The role of the body clock is not only to increase body activity during daytime, but also to decrease it, and so to conserve energy and promote restitution, during nocturnal sleep (1, 26–28). Hormonal changes that might affect appetite, metabolism and body weight are to some extent related to seasonal variations in daylight, *e.g.* an increased intake of energy when daily photoperiod decreases during autumn (29, 30). After a time zone transition, during night work or weekends with prolonged morning sleep, the normal synchronicity between lifestyle and body clock is temporarily lost. As a consequence, the ability of individuals to sleep, metabolise nutrients and perform physical and mental work, as well as their desire to eat and sleep, no longer matches their habitual lifestyle and they suffer from 'jet-lag' of various degrees (31).

Circadian rhythms in metabolism

Postprandial response to a meal varies as a function of time of day. Gastric emptying rate is lowered during evening and night (32) while glucose tolerance is decreased in the evening (33, 34). There is a circadian variation in postprandial lipaemia (35). Plasma triacylglycerol (TAG) circulating after a meal appears to be influenced by both the circadian clock and sleep time, with higher levels during biological night defined as the time between the onset and offset of melatonin secretion (36). During normal conditions (where sleep is nocturnal) energy is stored during daytime, when lipogenesis takes place in the adipose tissue and glycogenesis in the liver. From an energy balance perspective, net lipolysis and glycogenolysis occur during the dark, resting and fasting phase (37) when appetite also decreases as a function of circadian variations in energy-regulating hormones (28, 38). There is a profound circadian variation in carbohydrate metabolism (39). During nocturnal sleep and fasting, metabolism is designed to mobilise endogenous blood glucose for the brain cells and to save blood glucose by a decreased uptake in muscle cells. This phenomenon is called insulin resistance. Therefore, nocturnal eating might create an added risk for type 2 diabetes in persons already at risk. Eating at night in combination with long-term sleep deficiency also impairs carbohydrate metabolism in healthy subjects and increases the risk for development of type 2 diabetes and the metabolic syndrome (40). Thus, from a metabolic point of view, it is clear that metabolism is in favour of eating before and after overnight fasting and avoiding nocturnal eating.

It is possible that the time of eating and circadian rhythm phase might determine the energy intake in accordance with a postprandial response that leads to meal termination. Evening meals seem to have a less satiating effect than morning meals (41). There is one very consistent finding that obese people – children, adolescents, adults – tend to eat less in the morning than lean controls, and to eat more in the afternoon and evening (5).

Morning

Eating within a couple of hours after over-night fasting is appropriate with respect to circadian variations in (carbohydrate) metabolism. At this time of day, glycogen stores in the liver are depleted and body metabolism is in favour of carbohydrate oxidation after food intake rather than oxidation of fatty acids from adipose tissue. Glucose tolerance is high in the morning compared to the evening and night and therefore eating carbohydrates seems to be favourable in the morning (34).

Breakfast eating is associated with more adequate dietary intake in children (42), adolescents and adults (43).

Eating or omitting breakfast seems to affect macronutrient composition in the diet and substrate utilisation over the day. Observational studies of fasting during the Holy Month of Ramadan have shown that diurnal fasting, characterised by lack of a morning meal and condensed eating during late evening, affects the rhythms of melatonin and cortisol. Subjects also decrease their spontaneous intake of carbohydrates in the daytime, but not their total energy intake. Diurnal fasting affects nutrient utilisation, carbohydrate oxidation decreases in the morning and fat oxidation increases during the day (44–46). An intervention study has shown that the amount of energy eaten at breakfast influences nutrient utilisation during the rest of the day. A high energy breakfast (2,900 kJ), corresponding to 25% of the daily energy intake) led to a strong inhibition of fat oxidation throughout the day compared to a low energy breakfast (420 kJ) of the same composition (47).

Evening and night

Glucose tolerance decreases from morning to night. The insulin secretion is lower and the glucose level is higher after a meal eaten in the evening compared to an equal meal eaten in the morning (39).

During (nocturnal) sleep, endogenous blood glucose is mobilised through glycogenolysis in the liver and gluconeogenesis from lactate, glycerol or amino acids. Growth hormone (GH, sleep dependent) and cortisol (circadian rhythm dependent) are key hormones to stimulate the nocturnal, endogenous mobilisation of blood glucose (34). The nocturnal peak of GH diminishes with increasing age and disappears at approximately 60 years of age, which may affect nocturnal glucose mobilisation and sleep quality. Cortisol rhythm is robust and persists, although de-synchronised, across work shift schedules (36, 48).

Insulin has a great impact on the control of postprandial lipid metabolism. Due to circadian variations in insulin response (34), eating late or during the night could be unfavourable with respect to lipid and carbohydrate metabolism. This association might explain findings of impaired metabolism in shift workers (49–55).

Individual and social factors

Eating patterns defined as preferred hours for eating and eating frequency vary between and within individuals due to genetic factors (chronotype), sleeping habits, social circumstances and age (31, 56, 57). The need for many or few meals a day depends on the energy density of the diet and the individual's energy expenditure. Subjects eating low

energy, fibre-rich foods require larger portions and more frequent meals than subjects having a more energy-dense diet.

Appetite is a psychological desire to eat related to pleasant sensations, while hunger is a more painful feeling signalling a biological need for food (39, 58, 59). Besides nutritional and psychological aspects, eating offers an opportunity to take a break, rest, recover and socialise.

New-born babies have an on-demand feeding rhythm of about 4 hours. The sleep hormone melatonin in the mother's breast milk probably helps the baby to adjust its rhythm to nocturnal sleep and diurnal activity. It takes approximately six months for the cells in the SCN to mature and run rhythms of eating and sleep (60).

Puberty is an anabolic period of intense growth associated with morning tiredness. A follow-up study of Swedish school children from 13 to 15 years of age showed that the prevalence of evening-orientated subjects increased from 34% to 45%, while the prevalence of morning-orientated subjects decreased from 13 to 7% (57). Studies of adults indicate that the proportion of morning- and evening-orientated subjects is more even, 10–15% and 15–20%, respectively. With increasing age, circadian rhythms in almost all humans move towards a morning-orientated pattern (24, 61).

Chronotype is a genetic setting that may be modified, but not completely changed, by habits or social demands. The internal clock in morning-active subjects keeps close to the 24-hour astronomic day, while evening-orientated subjects have an internal drive to establish a day >24-hour under free-living conditions, *i.e.* isolated from environmental time-cues setting the internal clock. Evening types are more flexible in sleeping habits and tolerate shift work better. After 45 years of age, the tolerance for shift work and varying sleeping hours decreases in many subjects (24, 62). Lack of appetite in the morning might reflect awakening in the 'wrong' circadian phase, when the body is not prepared for activity.

Experimental studies show that chronic sleep deprivation in healthy subjects increases appetite, impairs carbohydrate metabolism and increases the risk for type 2 diabetes. Variations in glucose tolerance occur during sleep and the quality of sleep markedly influences the nocturnal utilisation of glucose. Chronic sleep disturbances, in for instance elderly people, night workers and obese subjects with sleep apnoea, may be associated with disturbed glucose regulation (40).

Shift work, eating pattern and health

Only two studies seem to have been designed to assess the effect of work schedules on the temporal distribution of eating events. Energy

intake seems to be reduced during 8-hour night shifts (35% of 24-hour intake) compared to morning (47%) and afternoon shifts (51%) (63). A lower intake during nocturnal shifts is probably related to circadian rhythms in appetite (38). The 24-hour temporal distribution of eating events and energy intake seems to differ markedly between work shift days, while the total 24-hour dietary intake seem to be almost unaffected by work schedule (64, 65).

Observational studies of shift workers show that nocturnal eating is associated with an impaired regulation of blood glucose (increased fasting levels), serum cholesterol (increased LDL, decreased HDL) and TAG metabolism (51, 52). Epidemiological studies show that shift work is an established risk factor for cardiovascular disease and the metabolic syndrome, *i.e.* impaired lipid and glucose metabolism (49, 50, 54, 55). However, metabolic disorders in shift workers might also be linked to other factors, *e.g.* irregular and/or nocturnal eating, sleep debt and circadian rhythm factors (31, 40).

Experimental studies of eating around the clock show that postprandial endocrine response and gastrointestinal activity vary (36, 66–68). Insulin, pancreatic polypeptide (PP), thyroid stimulating hormone, free thyroxine, cortisol and leptin responses differ with the hour of the day. The observed decreased evening and nocturnal cortisol and PP responses indicate that nocturnal eating might have health implications (67). However, data show that the human body seems to buffer small variations in meal size and timing during a 24-hour period, provided that energy balance is maintained. Comparison of 4 isoenergetic daytime meals followed by nocturnal fasting (while awake), and 6 isoenergetic meals eaten every 4-hour around the clock showed that energy expenditure, blood glucose, TAG, insulin and glucagon concentrations were lower and fatty acid concentrations were higher during the nocturnal fasting than during nocturnal eating. However, no 24-hour differences in average metabolic responses between the two dietary regimes were apparent (69).

Eating pattern and energy balance

The cumulative energy cost to digest foods and absorb nutrients is around 10% of the total energy intake (58). The thermic effect of feeding (TEF), also referred to as diet-induced thermogenesis (DIT), is primarily influenced by the quantity and quality of the energy ingested. Short-term studies show no significant difference in total TEF between nibbling and gorging (70–73). Studies using whole body calorimetry and

testing 1–2 meals against 3–7 meals also failed to demonstrate any effects of meal pattern on total energy expenditure (20–22, 69, 74, 75).

A central problem in short-term studies is whether the postprandial measurements capture the entire thermogenic peak. Many studies are restricted to a 3-hour duration due to difficulties in keeping subjects completely still for longer periods during calorimetric measurements. The increase in metabolic rate after a meal lasts for at least 5 hours, while the thermogenic peak may take much longer to subside, particularly after a very large meal.

In early studies, a nibbling pattern has been associated with favourable effects on body weight and metabolism (12, 13). However, both observational and epidemiological studies are inconclusive due to design problems and confounding factors (3, 7, 11, 70, 76). Intervention studies of several weeks of restricted energy intake show that eating frequency does not affect the total energy expenditure and the rate of weight loss (5, 14, 74).

Several studies show that obese subjects have an abnormal distribution of their dietary intake, *i.e.* lack of appetite in the morning (anorexia) and evening over-eating (hyperphagia) (9, 77, 78). One can speculate whether this abnormal and displaced eating behaviour is related to a dysfunction in the endogenous, circadian rhythm system. Rat studies show that dysfunction or lesions in the feeding area involved in the circadian control of food choice in the hypothalamus make rats lose control of intake and timing of eating (28). In summary, body weight is related to total energy intake and there is no support in the available data for a major role of eating frequency in the development of overweight. However, an increase in the daily number of eating occasions must be accompanied by a reduction in portion size and vice versa (5, 59), to maintain body weight. There may be indirect relationships between eating frequency and body weight, although there is no current evidence of such. For instance, frequent eating might prevent some individuals from over-eating due to strong hunger sensations following long intervals between meals. On the other hand, for other subjects every eating occasion might bear a risk for over-eating and losing control of food intake.

Eating pattern and carbohydrate metabolism

Cross-over studies have been carried out with normal subjects and type 2 diabetics to investigate the effect of nibbling (8–17 eating events per day) and gorging (3 daily eating events) on carbohydrate metabolism. In the normal subjects, glucose concentration was similar during both

regimes, but mean serum insulin levels were decreased significantly by eating 17 times compared to 3 times daily (16). In type 2 diabetics, no potential benefits (fasting plasma glucose and insulin concentration) of increased eating frequency were observed (79, 80).

Studies on acute effects (< 24 hours) of eating frequency have also been carried out. In healthy subjects there was no difference between eating a meal every 4 hours over a 24-hour period and eating 4 meals between 8 a.m. and 8 p.m., regarding total response of blood glucose or insulin. However, postprandial response differed between night and day (69). In type 2 diabetics, a flattened pattern was observed in glucose and insulin response to more frequent eating (81) but meal frequency still did not affect the mean concentration of plasma glucose (82).

Eating pattern and serum lipids

In normal subjects, postprandial lipaemia peaks 3–4 hours after a meal and subsides to a baseline concentration after 5–6 hours. Two or more postprandial peaks in TAG usually occur during daytime, whereas postprandial peaks after an overnight fast are usually monophasic (58). Postprandial lipid, lipoprotein and apolipoprotein concentrations are affected by circadian rhythm factors (35). The complex interactions between factors determining serum lipids and lipoproteins explain why epidemiological studies on meal frequency and lipid metabolism are biased due to the data collection method and the presence of several confounders (83). Meal composition in laboratory studies very often has an unrealistically high content of fat (84).

There is evidence from epidemiological and experimental cross-over studies that increased meal frequency may be associated with a favourable lipoprotein profile likely to be linked to a reduced risk of cardiovascular disease. There is evidence for plausible mechanisms by which increased meal frequency might reduce levels of total and LDL-cholesterol (83).

Eating pattern and cognitive function

In a long-term perspective, deficient intake of a number of vitamins and minerals impairs brain function and behaviour (85). Short-term alteration in nutritional intake might have an effect on functions such as alertness, learning, memory, information processing and also mood. Tests have been constructed that are monotonous and robust to bias (*e.g.* learning or intelligence) and that can be used to study the effects of eating or omitting a meal on cognitive variables. Examples of such tests

are : simple reaction time test, vigilance test, matching figure test, memory tasks (86). Almost no research has been done to examine the effects of meals or food composition on a complicated task such as car driving, a task that requires a number of cognitive functions (87). Studies on the effect of food intake on performance are difficult to design and interpret, since there are a number of other aspects interacting with the nutritional effects (88).

Dieting usually results in a performance decrement, *i.e.* poor vigilance and a higher vulnerability to stress (89).

Breakfast

Psychological tests have shown that eating breakfast improves performance related to memory (90), sustained attention and improves other functions sensitive to sleepiness, *e.g.* vigilance (91). A review of studies on school children suggests that omitting breakfast interferes with cognition and learning, and that the effect is more pronounced in children at nutritional risk than in well-nourished children (92).

Lunch/mid-day meal

In a realistic setting, a reduction in alertness and efficiency is generally observed shortly after lunch compared with morning or late afternoon hours. This decline in mental function is referred to as the 'post-lunch dip'. It has been suggested that this simply reflects endogenous alertness rhythm, with a decline in alertness 12 hours after the circadian nadir (24). Results from different studies are divergent, but taken together, there is no consensus as to what extent a 'post-lunch dip' is affected by food intake.

Over-day fasting

Over-day fasting is associated with cognitive slowing and longer reaction times. Performance related to memory, numerical performance and coping with time stress is not impaired. The feeling of sleepiness and fatigue increases during afternoon in a fasting state (93). Morning fast followed by doubled intakes at lunch and dinner has no effects on performance or night sleep onset (91).

Work at night

Eating activities *per se*, rather than energy intake or types of nutrients consumed, seem to improve wakefulness for a short while in drowsy subjects. Laboratory studies show that glucose intake does not improve wakefulness more than fructose or water (94). Furthermore, a large intake of energy (1,000 kcal) from solid foods does not improve wakeful-

ness more than a small intake (100 kcal) of the same food (95). A field study of truck drivers shows that neither energy content (840/440 kcal) nor balance between macronutrients in four test meals eaten at breakfast or lunch (day drivers) or as dinner and between meals (night drivers) had any effect on self-estimated wakefulness (96). Shift workers have a spontaneously reduced intake during night shifts (63) and satiation occurs with less food during the night (38).

In conclusion, eating breakfast and avoiding over-day fasting may improve mental performance. However, hunger and the circadian rhythm phase can influence mental performance and wakefulness even more than eating does.

Eating patterns in the Nordic countries

The concept 'eating pattern' involves many dimensions: hour, sequence, food combinations, nutrient intake. It is difficult to compare results from different studies due to lack of standardised meal characterisation.

Some dimensions of the 'eating system', *i.e.* when eating occurred, what was eaten, what the social context was like, where and with whom the meal took place, were compared in the Nordic countries (8). The data show cultural differences between the countries with respect to 'peak hours' for eating and the daily number of meals. A hot lunch is eaten in Finland and Sweden in contrast to Denmark and Norway, where sandwiches constitute a midday meal. A hot dinner is usually eaten between 4 p.m. and 8 p.m. in all countries, and it is served earliest in Norway, followed by Finland and Sweden. Five eating occasions a day is common in Denmark and Finland, while Swedes seem to eat a little less frequently, 4–5 times, according to this study. The lowest frequency, 4 times a day, was observed in Norway due to no intermediate meal between lunch and dinner.

The daily frequency of eating has been evaluated in six Swedish population groups using a Food Based Method for Classification of Eating (11, 97–102). The mean frequency of daily eating events in adults ranged from 4.4 times a day in retired people to 6.5 times per day in male shift workers, while it was 5.4 times a day in pre-school children.

Guidelines

Available data do not allow specification of a universal, optimal eating pattern in terms of number of meals and temporal distribution. Results from observation studies show, however, that most subjects in the

Nordic countries and elsewhere eat 4–6 times daily, out of which 2–3 are main eating occasions (8, 103).

In general, the daily eating frequency does not affect body weight unless the energy intake exceeds energy expenditure. The higher the energy expenditure, the greater the need for food. Frequent meals are associated with favourable serum lipid profiles. Metabolism is in favour of a meal after overnight fasting, namely breakfast. Eating breakfast is associated with the positive effects of better dietary quality and cognitive function, and a lower risk of developing obesity, although a direct causality has not been established. Nocturnal eating and eating late at night should be avoided.

The daily food and energy intake could be distributed as follows

Morning meal (breakfast)	20–25%
Midday (lunch)	25–35%
Evening (dinner)	25–35%
1–3 intermediate meals of good nutritional quality	5–30% in total

A morning meal, preferably with carbohydrate-rich foods, is recommended within 1–2 hours after overnight fasting. A midday meal is recommended before the metabolism peak in the afternoon. An evening meal is recommended no later than 1–2 hours before sleep.

A regular eating pattern is recommended in order to avoid frequent snacking.

Physical activity of at least recommended duration and intensity facilitates good eating habits by allowing for a greater intake. Sleep deprivation and mental stress impair regulation of food intake and involve a risk for over-eating.

The advice to eat a relatively greater part of the daily intake as dinner than as breakfast is based on observations that hot meals are often the main contributors of energy and macro-nutrients within Nordic populations.

Children

Children do not have the same capacity as adults to eat large portions or to mobilise stored energy. Two to three intermediate meals are recommended. The need for an evening intermediate meal depends on bedtime. As childhood is a period of intense growth, children leaving daycare or pre-school late in the afternoon might need an intermediate meal before going home. Children having dinner early might need an extra meal before going to bed.

Example of daily meal distribution for pre-school children

Breakfast	20%
Intermediate meal	5%
Noon/midday/lunch meal	25%
Intermediate meal	15–20%
Afternoon/evening/dinner meal	25%
Evening 'intermediate' meal	5–10%

Adolescents

Puberty is a pronounced phase of growth. About 25% of an individual's height is achieved during adolescence. To meet the increased need for energy and nutrients, as many as three intermediate meals, or maybe even more, may be needed in addition to main meals, particularly in physically active persons.

Adolescents in general are less morning-orientated, have difficulty waking up, and are more evening-orientated, being alert during late hours. This is due to puberty-related variations in circadian rhythms. When appetite for breakfast is lacking, a minor breakfast at home may be complemented by breakfast eaten at school.

Elderly

The capacity to absorb, digest, store and metabolise nutrients may be slower in elderly people. Many also have a diminished ability to mobilise blood glucose during postprandial fasting and night sleep, which is due to weaker circadian hormonal control (61). Overnight fasting should not last longer than 11 hours. Elderly people in general become more morning-orientated and might need their first meal early in the morning.

Night and shift work

Shift-workers are recommended to eat at approximately the same hours every day during the work shift cycle, irrespective of working hours. The following eating pattern is advised.

Morning meal/breakfast: after night work, before day-sleep
Midday meal/lunch: after day sleep
Evening meal/dinner: at work or outside working hours
At night:
– avoid eating at all or eat reduced portions, as the metabolism changes
– avoid sugar-rich beverages and sweets, as sugar (glucose, sucrose) does not improve wakefulness and vigilance

REFERENCES

1. Armstrong S. A Chronometric Approach to the Study of Feeding Behaviour. Neurosci. Behav. Physiol. 1980; 4: 27–53.
2. Woods S, Strubbe J. The Psychobiology of Meals. Psychol Bull Rev 1994; 1: 141–155.
3. Gibney M, Wolever T. Periodicity of eating and human health: present perspective and future dimensions. British Journal of Nutrition 1997; 77 (Suppl. 1): S3–5.
4. Chiva M. Cultural aspects of meals and meal frequency. British Journal of Nutrition 1997; 77 (Suppl. 1): S21–S28.
5. Bellisle F. Impact of the daily meal pattern om energy balance. Scandinavian Journal of Nutrition 2004; 28 (3): 114–118.
6. Kearney J, Gibney M. Eating patterns – temporal distribution, converging and diverging foods, meals eaten inside and outside of the home – implications for developing FBDG. Public Health Nutrition 2001; 4 (2B): 693–8.
7. Gatenby SJ. Eating frequency: methodological and dietary aspects. British Journal of Nutrition 1997; 77 (Suppl. Number 1): 7–20.
8. Kjaernes U. (editor). Eating Patterns – a Day in the Lives of Nordic People. Oslo: Statens Institutt for forbrukerforskning; 2001.
9. Bertéus Forslund H, Lindroos A, Sjöström L, Lissner L. Meal patterns and obesity in Swedish women – a simple instrument describing usual meal types, frequency and temporal distribution. European Journal of Clinical Nutrition 2002; 56: 740–747.
10. Carlgren G, Rossander L. Bearbetning och evaluering av kostvanor registrerade under 4 dagar. Näringsforskning 1976; 20: 201–209.
11. Lennernäs M, Andersson I. Food Based Classification of Eating Episodes (FBCE). Appetite 1999; 32: 53–65.
12. Fabry P, Fodor J, Hejl Z, *et al.* The frequency of meals: its relation to overweight, hypercholesterolemia, and decreased glucose-tolerance. Lancet 1964; ii: 614–15.
13. Fabry P, Fodor J, Hejl Z. Meal frequency and ischemic heart disease. Lancet 1968; ii: 190–91.
14. Garrow JS, Durrant M, Blaza S, Wilkins D, Royston P, Sunkin S. The effect of meal frequency and protein concentration on the composition of the weight lost by obese subjects. British Journal of Nutrition 1981; 45: 5–15.
15. Murphy MC, Chapman C, Lovegrove JA, *et al.* Meal frequency; does it determine postprandial lipaemia? European Journal of Clinical Nutrition 1996; 50: 491–7.
16. Jenkins DJ, Wolever TM, Vuksan V, *et al.* Nibbling *versus* gorging: metabolic advantages of increased meal frequency. New England Journal of Medicine 1989; 321 (14): 929–34.
17. Jenkins D, Ocana A, Jenkins A, Wolever T, Vuksan V, Katzman L, *et al.* Metabolic advantages of spreading the nutrient load: effects of increased meal frequency in non-insulin-dependent diabetes. American Journal of Clinical Nutrition 1992; 55 (2): 461–467.
18. Jones PJ, Leitch CA, A PR. Meal frequency effects on plasma hormone concentrations and cholesterol synthesis in humans. American Journal of Clinical Nutrition 1993; 1993 (57): 868–74.
19. Jordan J, Novascone M. Effect of altered feeding patterns on serum lipids and lipoproteins in adult males. Journal of Human Nutrition and Dietetics 1989; (2): 19–24.
20. Verboeket – van de Venne WPHG, Westerterp KR. Influence of feeding frequency on nutrient utilization in man: Consequences for energy metabolism. European Journal of Clinical Nutrition 1991; 45: 161–169.
21. Verboeket – van de Venne WPHG, Westerterp KR, Kester ADM. Effect of pattern of food intake on human

energy metabolism. British Journal of Nutrition 1993; 70: 103–115.
22. Wolfram G, Kirchbegner M, Müller H, Hollomey S. Thermogenesis in human subjects with differing meal frequencies. Annuals of Nutrition and Metabolism 1987; 31: 88–97.
23. Lewin L, Larhammar D. Biologiska klockor. In: Har du tid? Naturvetenskapliga Forskningsrådets årsbok 2000. Uppsala: Ord & Vetande; 2000.
24. Åkerstedt T, Waterhouse J. The Body Synchronic. In: 1998 Medical and Health Annual. London: Encyclopaedia Brittanica, Inc.; 1998.
25. Czeisler C. Stability, precision and Near-24-Hour period of the Human Circadian Pacemakter. Science 1999; 284: 2177–2180.
26. Strubbe JH. Circadian rhythms of food intake. In: Westerterp-Plantenga MS, Fredrix EWHM, Steffens AB, editors. Food Intake and Energy Expenditure. Boca Raton: CRC Press; 1994. p. 155–174.
27. Strubbe JH. Regulation of food intake. In: Westerterp-Plantenga MS, Fredrix EWHM, Steffens AB, editors. Food Intake and Energy Expenditure. Boca Raton: CRC Press; 1994. p. 141–154.
28. Strubbe JH. Circadian organization of feeding behaviour. In: Westerterp-Plantenga MS, Steffens AB, Tremblay A, editors. Regulation of food intake and energy expenditure: EDRA; 1999.
29. de Castro JM. Seasonal Rhythms of Human Nutrient Intake and Meal Pattern. Physiology & Behaviour 1991; 50: 243–248.
30. van Staveren WA, Deurenberg P, Burema J, Groot L, Hautvast JGAJ. Seasonal variation in food intake, pattern of physical activity and change of body weight in a group of young adult Dutch women consuming self selected diets. International Journal of Obesity 1986; 10: 133–145.
31. Waterhouse J, Minors D, Atkinson G, Benton D. Chronobiology and meal times: internal and external factors. British Journal of Nutrition 1997; 77 (Suppl. 1): S29–S38.
32. Goo R, Moore J, Greenberg E, Alazraki N. Circadian variation in gastric emptying of meals in humans. Gastroenterology 1987; 93: 515–518.
33. van Cauter E, Blackman JD, Roland D, Spire J-P, Refetoff S, Polonsky KS. Modulation of Glucose Regulation and Insulin Secretion by Circadian Rhythmicity and Sleep. J. Clin.Invest. 1991; 88: 934–942.
34. van Cauter E, Polonsky E, Scheen A. Roles of Circadian Rhythmicity and Sleep in Human Glucose Regulation. Endocrine Reviews 1997; 18 (5): 716–38.
35. Romon M, LeFur C, Lebel P, Edme J, Fruchart J, Dallongeville J. Circadian variation of postprandial lipemia. American Journal of Clinical Nutrition 1997; 65 (4): 934–940.
36. Morgan L, Hampton S, Gibbs M, Arendt J. Circadian aspects of postprandial metabolism. Chronobiol Int. 2003; 20 (5): 795–808.
37. Strubbe JH, van Dijk G. The temporal organization of ingestive behaviour and its interaction with regulation of energy balance. Neuroscience and Biobehavioral Reviews 2002; 26: 485–498.
38. Lowden A, Holmbäck U, Åkerstedt T, Forslund A, Forslund J, Lennernäs M. Time of day type of food – relation to mood and hunger during 24 hours of constant conditions. Journal of Human Ergology 2001; 30: 381–386.
39. van Cauter E, Shapiro T, Tillil H, Polonsky KS. Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. Am. J. Physiol. 262 (Endocrinol. Metab. 25) 1992: E467–E475.
40. Speigel K, Leproult R, Van Cauter E. Impact on feep sleep dept on metabolic and endocrine function. The Lancet 1999; 353: 1435–39.
41. de Castro JM. The time of Day of Food Intake Influences Overall Intake in Humans. J Nutr 2004; 134: 104–111.
42. Nicklas T, Bao W, Webber LS, Berenson GS. Breakfast consumption affects adequacy of total daily intake

in children. J Am Diet Assoc 1993; 93 (8): 886–891.
43. Keski-Rahkonen A, Kaprio J, Rissanen A, Virkkunen M, Rose R. Breakfast skipping health-compromising behaviours in adolescents and adults. Eur J Clin Nutr 2003; 57: 842–853.
44. Ati J, Beji C, Danguir J. Increased fat oxidation during Ramadan fasting in healthy women: an adaptive mechanism for body weight maintenance. Am J Clin Nutr 1995; 62: 302–307.
45. Bogdan A, Bouchareb B, Touitou Y. Ramadan fasting alters endocrine and neuroendocrine circadian patterns. Meal-time as a synchronizer in humans? Life Sciences 2001; 68: 1607–1615.
46. Kassab S, Abdul–Ghaffar T, Nagalla D, Sachdeva U, Nayar U. Serum leptin and insulin levels during chronic diurnal fasting. Asia Pac J Clin Nutri 2003; 12 (4): 483–7.
47. Martin A, Normand S, Southier M, Peyrat J, Louche-Pelissier C, Laville M. Is advice for breakfast consumption justified? Results from a short-term dietary and metabolic experiment in young healthy men. Br J Nutr 2000; 84 (3): 337–344.
48. Waterhouse J, Åkerstedt T, Lennernäs M, Arendt J. Chronobiology and nutrition: Internal and external factors. Can J Diab C 1999; 23: 82–88.
49. Boggild H. Shift work and heart disease, Epidemiological and risk factor aspects [Ph.D.]. Aarhus: University of Aarhus; 2000.
50. Knutsson A, Åkerstedt T, Jonsson B, Orth-Gomer K. Increased risk of ischaemic heart disease in shift workers. Lancet 1986; 12; 2: 89–92.
51. Lennernäs M, Åkerstedt T, Hambreus L. Nocturnal eating and serum cholesterol of three-shift workers. Scandinavian Journal of Work Environment and Health 1994; 20: 401–406.
52. Romon M, Nuttens M-C, Fievet C, Pot P, Bard JM, Furon D, *et al.* Increased Triglyceride Levels in Shift Workers. The American Journal of Medicine 1992; 93: 259–262.
53. Sopowski MJ, Hampton SM, Ribiero DCO, Morgan L, Arendt J. Postprandial Triacylglycerol Responses in Simulated Night and Day Shift: Gender Differences. Journal of Biological Rhythms 2001; 16 (3): 272–276.
54. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and havning metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med 2001; 58: 747–752.
55. Karlsson B, Knutsson A, Lindahl B, Alfredsson L. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. Int Arch Occup Environ Health 2003; 76: 424–430.
56. Lennernäs M. Dygnsrytm, matlust och udda matvanor. Scandinavian Journal of Nutrition/Näringsforskning 2000; 44: 118–20.
57. Nordlund G, Norberg H, Lennernäs M, Gillberg M, Pernler H. Dygnsrytm och skolarbete. Umeå universitet: Pedagogiska institutionen; 2004. Report No.: 74.
58. Goran M, Astrup A. Energy Metabolism. In: Gibney M, Vorster H, Kok F, editors. Introduction to Human Nutrition. Oxford: Blackwell Science Ltd.; 2002. p. 30–45.
59. Prentice A, Jebb S. Energy Intake/ Physical Acitivity Interactions in the Homeostasis of Body Weight Regulation. Nutrition Reviews 2004; 62 (7): 98–104.
60. Honnebier M, Swaab D, Mirmiran M. Diurnal rhythmicity during early human development. In: Reppert S, editor. Development of Circadian Rhythmicity and Photoperiodism in Mammals. New York: Perinatology Press; 1989. p. 221–244.
61. Copinschi G, Van Reeth O, Can Cauter E. Biological rhythms. Effect of aging on the desynchronization of endogenous rhythmicity and environmental conditions. Presse Med. 1999; 28: 942–6.
62. Åkerstedt T. Vaken på udda tider. Rådet för Arbetslivsforskning; 1996.
63. Lennernäs M, Åkerstedt T, Hambraeus L. Work shift related dietary intake in day- and shiftworkers. Appetite 1995; 25: 253–65.

64. Lennernäs M, Abrahamsson L, Hambraeus L, Åkerstedt T. Nutrition and 3-shift work. The 24-hour intake of energy and nutrients. Ecology of Food and Nutrition 1994; 32: 157–165.
65. Lennernäs A, Abrahamsson L, Hambraeus L. Kostvanor bland skiftesarbetare vid ett västsvenskt pappersbruk – en pilotstudie. Näringsforskning 1985; 29: 74–80.
66. Hampton S, Morgan L, Lawrence N, Anastasiadou T, Norris F, Deacon S, *et al.* Postprandial hormone and metabolic responses in simulated shift work. Journal of Endochrinology 1996; 151: 259–267.
67. Holmbäck U, Forslund A, Lowden A, Forslund J, Åkerstedt T, Lennernäs M, *et al.* Endocrine responses to nocturnal eating – possible implications for night work. European Journal of Clinical Nutrition 2003; 42: 75–83.
68. Holmbäck U, Forslund A, Forslund J, Hambraeus L, Lennernäs M, Lowden A, *et al.* Metabolic responses to nocturnal eating in men are affected by sources of dietary energy. Journal of Nutrition 2002; 132 (7): 1892–1899.
69. Holmbäck U, Lowden A, Åkerfeldt T, Lennernäs M, Hambraeus L, Forslund J, *et al.* The Human Body May Buffer Small Differences in Meal Size and Timing during a 24-hour wake Period Provided Energy Balance Is Maintained. Journal of Nutrition 2003; 133: 2748–2755.
70. Bellisle F, McDevitt R, Prentice AM. Meal frequency and energy balance. British Journal of Nutrition 1997; 77 (Suppl. 1): S57–S70.
71. Kinabo JLD, Durnin JVGA. Effect of meal frequency on the thermic effect of food in women. European Journal of Clinical Nutrition 1990: 44: 389–395.
72. Molnar D. The effect of meal frequency on postprandial thermogenesis in obese children. Pädiatrie und Pädologie 1992; 27: 177–181.
73. LeBlanc J, Mercier I, Nadau A. Components of postprandial thermogenesis in relation to meal frequency in humans. Canadian Journal of Physiology and Pharmacology 1993; 71: 879–883.
74. Verboeket-Van DeVenne WPHG, Westerterp KR. Frequency of feeding, weight reduction and energy metabolism. International Journal of Obesity 1993; 17: 31–36.
75. Dallosso HM, Murgatroyd PR, James WPT. Feeding frequency and energy balance in adult males. Human Nutrition: Clinical Nutrition 1982; 36C: 25–39.
76. Crawley H, Summerbell C. Feeding frequency and BMI among teenagers aged 16–17 years. International Journal of Obesity Related Metabolic Disorders 1997; 21 (2): 159–161.
77. Ortega RM, Requejo AM, Lopez-Sobaler AM, Quintas ME, Andres P, Redondo MR *et al.* Difference in the breakfast habits of overweight/obese and normal weight schoolchildren. Int J Vitam Nutr 1998; 68 (2): 125–132.
78. Andersson I. Eating habits, weight reduction and long-term treatment results in obese men. The 'Gustaf' study. Stockholm, Sweden; 1997.
79. Arnold L, Mann JI, Ball MJ. Metabolic effects of alterations in meal frequency in type 2 diabetes. Diabetes Care 1997; 20 (11): 1651–4.
80. Thomsen C, Christiansen C, Rasmussen OW, Hermansen K. Comparisons of the effects of two weeks' intervention with different meal frequencies on glucose metabolism, insulin sensitivity and lipid levels in non-insulin dependent diabetic patients. Ann Nutr Metab 1997; 41: 173–80.
81. Bertelsen J, Christiansen C, Thomsen C, Poulsen PL, Vestergaard S, Steinov A, *et al.* Effect of Meal Frequency on Blood Glucose, Insulin, and Fatty Acids in NIDDM Subjects. Diabetes Care 1993; 16 (1): 4–7.
82. Segura A, Josse R, Wolever T. Acute metabolic effects of increased meal frequency in type II diabetics: three vs six, nine and twelve meals. Diabetes Nutrition and Metabolism 1995; 8: 331–338.

83. Mann J. Meal frequency and plasma lipids and lipoproteins. British Journal of Nutrition 1997; 77 (Suppl. 1): S83–90.
84. Gibney M. Periodicity of eating – an overview of the research challenge. Lecture in seminar: Tid för Måltid; 2004; Stockholm: Kungliga Vetenskapsakademiens Nationalkommitté för näringslära, SNF Swedish Nutrition Foundation; 2004.
85. Karanek R, Marks–Kaufman R. Nutrition and Behaviour: New Perspectives. New Yord: Van Nostrand Reinhold; 1991.
86. Gamberale F, Iregren A, Kjellberg A. SPES: the computorized Swedish performance evaluation system. In. Solna: National Institute of Occupational Health, Arbete och Hälsa 6; 1989.
87. Dye L, Blundell J. Functional foods: psychological and behavioral functions. British Journal of Nutrition 2002; 88 (Suppl. 2): S187–S211.
88. Karanek R. Psychological effects of snacks and altered meal frequency. British Journal of Nutrition 1997; 77: S105–S120.
89. Wardle J, Steptoe A, Oliver G, Lipsey Z. Stress, dietary restraint and food intake. J Psychosom Res. 2000; 48 (2): 195–202.
90. Benton D, Parker P. Breakfast, blood glucose and cognition. Am J Clin Nutr 1998; 67 (4 Suppl.): 772S–778S.
91. Lowden A, Vestergren P, Lennernäs M, Åkerstedt T. Food deprivation and the effects on functional ability and recovery sleep. J Sleep Reserach 2002; 2002 (Suppl. 1): 140.
92. Pollitt E, Mathews R. Breakfast and cognition: an integrative summary. Am J Clin Nutr 1998; 67 (4): 804s–813s.
93. Lowden A, Holmbäck U, Åkerstedt T, Forslund J, Lennernäs M, Forslund A. Performance and sleepiness during a 24 h wake in constant conditions are affected by diet. Biological Psychology 2004; 65: 251–263.
94. Landström U, Knutsson A, Lennernäs M, Söderberg L. Laboratory studies of the effects of carbohydrate consumption on wakefulness. Nutrition and Health 2000; 13: 213–225.
95. Landström U, Knutsson A, Lennernäs M, Stenudd A. Onset of drowsiness and satiation after meals with different energy contents. Nutrition and Health 2001; 15: 87–95.
96. Landström U, Knutsson A, Lennernäs M. Field studies on the effects of food content on wakefulness. Nutrition and Health 2000; 14: 195–204.
97. Andersson I, Lennernäs M, Rössner S. Meal pattern and risk evaluation in one year completers of a weight reduction program for obese men – the 'Gustaf' study. Journal of Internal Medicine 2000; 347: 30–38.
98. Byström M, Landström U, Lennernäs M. Måltider, sömn, sömnighet och dygnsrytm bland högskolestuderande. Umeå: Arbetslivsinstitutet; 2001. Report No.: 10.
99. Lennernäs M, Hambraeus L, Åkerstedt T. Nutrition and shift work. The use of meal classification as a new tool for qualitative/quantitative evaluation of dietary intake in shift workers. Ergonomics 1993; 36: 247–254.
100. Sidenvall B, Lennernäs M, Ek A. Elderly patient's meal pattern – a retrospective study. Journal of Human Nutrition 1996; 9: 263–72.
101. Wissing U, Lennernäs M, Ek A, Unosson M. Monitoring of dietary quality in outpatients by qualitative meal classification method. Journal of Human Nutrition and Dietetics 1998; 11: 125–33.
102. Sepp H, Lennernäs M, Pettersson R, Abrahamsson L. Children's nutrient intake at pre-school and at home. Acta Paediatrica 2001; 90: 483–491.
103. Becker W, Enghardt H, Robertson A-K. Kostundersökningar i Sverige 1950–1990. Uppsala: Livsmedelsverket; 1994.

CHAPTER 8

Fluid and water balance

Water is the largest single component of the human body. It comprises about 60% of body weight in young adults, and the proportion declines with age and increasing obesity. Total body water is divided into two compartments: about 40% is intracellular water, whereas the extracellular water is found mainly in plasma represents about 20% of body weight (1).

Physiology and metabolism

The regulation of fluid balance is closely bound to the regulation of electrolyte balance (balance between sodium, potassium and chloride). In kidneys, the excretion of water and electrolytes is regulated by hormones. With excess water in the body, diluted urine is excreted. If there is too high a concentration of electrolytes in body fluids, the thirst centre in the brain is stimulated, which leads to a feeling of thirst and reduced excretion of water by the kidneys.

Foods contain on average 1,000 to 1,500 ml water per day. Oxidation of fat, carbohydrates and protein yields an additional 300 to 350 ml water per day. Loss occurs by four routes: urinary output and the water in stools, and by evaporation from the respiratory tract and the skin. The daily urinary output exceeds 600 ml in healthy adults and is normally between 1,000 and 2,500 ml. The water content of stools is generally 100 to 200 ml per day but may be increased considerably by diarrhoea. The daily insensible losses by evaporation are on average 300 to 500 ml per m^2 body surface in a temperate climate. Losses by sweating are generally small but they may increase to several litres per day in a warm and humid environment with exercise or exposure to the sun.

During total parenteral nutrition, the daily requirement for total water is generally considered to be 30 ml per kg body weight, corresponding to 2,250 ml for a person with a body weight of 75 kg.

Requirement and recommended intake

In the recent US recommendations (2) it was concluded that the evidence is insufficient to establish water intake recommendations as a means to reduce the risk of chronic diseases, and that normal hydration status for all adults, as measured by serum osmolality, can be achieved with a wide range of water intakes. Therefore, an adequate intake (AI) for total water (*i.e.* from drinking water, beverages and food) was set at 3.7 litres per day for men and at 2.7 litres per day for women based on the median intakes reported in the NHANES III survey. The AI for total water was 1.3 litres per day for children 1–3 years and 1.7 litres per day for children 4–8 years of age, 2.4 and 2.1 litres per day for 9–13 year old boys and girls respectively, and 3.3 and 2.3 litres per day for 14–18 year old boys and girls, respectively.

Guiding values for total water intake in relation to energy intake are about 0.25 ml/kJ (≅1 ml/kcal) for adults. For healthy adults with a sedentary lifestyle in a temperate climate, the average daily water balance is estimated to be approximately 2,500 ml, including 1,000–1,200 ml from drinking fluids (3). Thus, in the conditions of the Nordic countries the guiding value for daily intake of drinking fluids for adults is about 1 litre in addition to that derived from foods. For elderly people, whose capacity to concentrate the urine is limited and who often have impaired feeling of thirst, a broader safety margin is needed, and the guiding value for intake is 1.5 litres per day (4).

Few data exist on daily water turnover and water intake in healthy children. In relation to body weight, a total water intake of approximately 65–70 ml per kg body weight for 2–3-year old children, falling to 40 ml per kg body weight for 15-year-olds, seems to be adequate (5). In relation to energy intake, a total water intake of approximately 0.25 ml/kJ seems to be adequate for children older than 3 years (6, 7).

In the conditions of the Nordic countries, the guiding value for daily intake of drinking fluids for children is 1 litre in addition to that derived from foods.

Lactating women increase their fluid intake in relation to the volume of breast milk. A volume of 750 ml per day of breast milk during the first six months increases the requirement for fluid by about 600–700 ml per day. This is generally compensated for by a self-regulatory increase in fluid intake of 12–16% (8).

Lower and upper limits of intake

Mild dehydration defined as a 1% to 2% loss of body weight caused by fluid losses results in headache, fatigue, loss of appetite and vertigo, while dehydration in excess of 3% to 5% of body weight decreases endurance and strength and is the primary cause of heat exhaustion (9). Dehydration of 15% to 25% body weight lost as water is fatal (10).

Excessive voluntary intake of water or mineral water has been recommended by 'health experts' for the enhancement of beauty or vitality, but this is not supported by scientific evidence. Acute water toxicity has been reported (11) due to rapid consumption of large quantities of fluids that greatly exceed the kidney's maximal excretion rate of 0.7 to 1.0 litre per hour. Excessive ingestion of water may increase the risk of water intoxication and hyponatraemia during pregnancy (8).

Dietary intake

The usual volume of ingested water and other fluids amounts to 1,000–2,000 ml per day in the Nordic countries. This brings the total amount of available water to 2,000–3,500 ml per day, which is about 10% of total body water.

Hydration status in relation to coffee and alcohol

Coffee is reported to increase 24-hour urine excretion in subjects with no habitual intake (12), while hydration status seemed unaffected in habitual coffee drinkers (13). Strong tea seems to have a light diuretic effect (14). As the main diuretic compound in coffee and tea is caffeine, it seems as if caffeine tolerance develops after habitual consumption.

Strong alcohol has a diuretic effect by inhibiting the vasopressin secretion. Moderate amounts of less strong alcohol such as beer and wine seem to have little or no effect on hydration status (4, 15, 16).

Health effects of coffee and tea

In general, studies examining the relationship between fluid intake/small urine volumes and specific disease are conflicting and far from conclusive (9, 17). For the health effects of other beverages like milk, soft drinks and alcohol, see *Chapters 11, 12* and *14.*

Epidemiological studies have shown that high consumption of coffee is associated with osteoporosis. Several early studies suggested an association between high consumption of coffee and incidence of coro-

nary heart disease. However, the statistical association disappeared when the results were adjusted for smoking. Regular consumption of coffee was associated with a slight increase of systolic and diastolic blood pressure in a meta-analysis of 11 trials (18). The cancer risk effect of coffee is considered minimal. High consumption of coffee has been associated with reduced risk of type 2 diabetes in prospective studies from several countries including Finland and Sweden (19, 20). Caffeine may reduce insulin sensitivity, and it is assumed that the putative antidiabetogenic effect of coffee depends on constituents other than caffeine. Epidemiological evidence linking the consumption of tea with coronary heart disease is conflicting but, in a recent study, regular tea consumption after myocardial infarction was associated with reduced mortality (21).

In the Tromsø study, it was found that individuals with high consumption of coffee had higher serum cholesterol concentrations than coffee abstainers (22). Subsequent epidemiological studies in Norway (23), Finland (24) and Sweden (25) showed that the connection between coffee and cholesterol was caused by higher cholesterol levels in those who consumed traditionally boiled coffee.

Boiled coffee that has not been filtered through paper increases the concentrations of LDL-cholesterol and apoprotein B in serum, whereas filtered coffee and instant coffee have little or no effect on serum lipids (26). The effect of boiled coffee is caused by the diterpenoid cafestol (27). It may influence lipid metabolism in the liver, but the mechanism is so far unknown. The consumption of boiled coffee has declined gradually in all Nordic countries, and its effects in the population are diminishing. Individuals with hypercholesterolaemia and those who have higher than average consumption of coffee are advised to use filtered coffee instead of boiled coffee.

A number of epidemiological studies have investigated the possible relation between caffeine intake, *e.g.* as coffee, and fertility, pregnancy outcome and behavioural or cognitive effects in children (28). Whereas the total information gave no indications of a significant correlation for most of the outcomes, a link between high caffeine intake during pregnancy and foetal growth retardation cannot be ruled out and a link between caffeine intake and spontaneous abortion is indicated. Although the available epidemiological studies in this field are partly contradictory and not easy to interpret, depending mainly on problems with confounding, an increased risk associated with high coffee consumption during pregnancy and early spontaneous abortion is currently supported by some recent, well-designed and well-performed studies (*e.g.* 29). Those who consumed 500 mg caffeine or more per day had an

increased risk for spontaneous abortion. In addition, a recent prospective follow-up study among 18,000 pregnant Danish women found coffee drinking associated with an increased risk of stillbirth but not with infant death. Compared with women who did not drink any coffee during pregnancy, the adjusted risk of stillbirth was lower among women who drank 1–3 cups per day, slightly increased among women who drank 4–7 cups per day, and more than doubled among women who drank 8 or more cups per day (30).

Tea and chocolate contain certain flavonoids and coffee contains phenolic acids. These have been suggested to mediate beneficial health effects, but these are so far not supported by data from clinical trials. Coffee and tea contain tannins that may reduce the absorption of non-haeme iron. Therefore, persons with iron deficiency may benefit from avoiding drinking coffee and tea with their main meals.

REFERENCES

1. Iversen PO, Nicolaysen G. Vann – for livet. Tidskr Nor Laegeforen 2003; 123: 3402–3405.
2. The National Academy of Sciences. Dietary reference intakes for water, potassium, sodium, chloride, and sulfate (2004). www.nap.edu/openbook/0309091691/html/73.html
3. Valtin H. 'Drink at least eight glasses of water a day'. Really? Is there scientific evidence for '8×8'? Am J Physol Regul Integr Comp Physiol 2002; R993–R1004.
4. Ingerslev J, Beck AM, Bjørnsbo KS, Hessov I, Hyldstrup L, Pedersen AN. Ernæring og Aldring. Ernæringsrådet, 2002.
5. Fusch C, Hungerland E, Scharrer B, Moeller H. Water turnover of healthy children measured by deuterated water elimination. Eur J Pediatr 1993; 152: 110–114.
6. Ballauff A, Kersting M, Manz F. Do children have an adequate fluid intake? Water balance studies carried out at home. Ann Nutr Metab 1988; 32: 332–339.
7. Manz F, Wentz A, Sichert–Hellert W. The most essential nutrient: Defining the adequate intake of water. J Pediatr 2002; 141: 587–592.
8. Zetterström R. Voluntary and therapeutic causes of water intoxication and hypertonic dehydration: perinatal risks in mother and offspring. Scand J Nutr 2003; 47: 108–110.
9. Kleiner SM. Water: An essential but overlooked nutrient. J Am Diet Assoc 1999; 99: 200–206.
10. Saltmarsh M. Thirst: Or why do people drink? Nutr Bull 2001; 26: 53–58.
11. Farrel DJ, Bower L. Fatal water intoxication. J Clin Pathol 2003; 56: 803–804.
12. Neuhauser-Berthold, Beine S, Verwied SC, Luhrmann PM. Coffee consumption and total body water homeostasis as measured by fluid balance and bioelectrical impedance analysis. Ann Nutr Metab 1997; 41: 29–36.
13. Grandjean AC, Reimers KJ, Bannick KE, Havens MC. The effect of caffeinated, non-caffeinated, caloric and non-caloric beverages on hydration. J Am Coll Nutr 2000; 19: 591–600.
14. Højgaard L, Arffmann S, Jørgensen M, Krag E. Tedrikker syndom. Ugeskr Laeger 1981; 143: 2893–2895.
15. McDonald JT, Margen S. Wine *versus* ethanol in human nutrition II. Fluid,

sodium, and potassium balance. Am J Clin Nutr 1979; 32: 817–822.
16. Shirreffs SM, Maughan RJ. Restoration of fluid balance after exercise-induced dehydration: Effects of alcohol consumption. J Appl Physiol 1997; 83: 1152–1158.
17. Grandjean AC, Reimers KJ, Buyckx ME. Hydration: Issues for the 21st century. Nutr Rev 2003; 61: 261–271.
18. Jee SH, He, J, Whelton PK, Klag MJ. The effect of chronic coffee drinking on blood pressure. A meta-analysis of controlled clinical trials. Hypertension 1999; 33: 647–652.
19. Rosengren A, Dotevall A, Wilhelmasen L, Thelle D, Johansson S. Coffee and incidence of diabetes in Swedish women: A prospective 18-year follow-up study. J Intern Med 2004; 255: 89–95.
20. Tuomilehto J, Hu G, Bidel S, Lindström J, Jousilahti P. Coffee consumption and risk of type 2 diabetes mellitus among middle-aged Finnish men and women. JAMA. 2004; 291: 1213–1219.
21. Mukamal KJ, Maclure M, Muller JE, Sherwood JB, Mittleman MA. Tea consumption and mortality after acute myocardial infarction. Circulation 2002; 105: 2476–2481.
22. Thelle DS, Arnesen E, Førde OH. The Tromsø Heart Study: Does coffee raise serum cholesterol? N Engl J Med 1983; 308: 1454–1457.
23. Bønaa K, Arnesen E, Thelle D, Førde OH. Coffee and cholesterol: Is it all in the brewing? Br Med J 1988; 297: 1103–1104.
24. Pietinen P, Aro A, Tuomilehto J, Uusitalo U, Korhonen H. Consumption of boiled coffee is correlated with serum cholesterol in Finland. Int J Epidemiol 1990; 19: 586–590.
25. Lindahl B, Johansson I, Huhtasaari F, Hallmans G, Asplund K. Coffee drinking and blood cholesterol – effects of brewing method, food intake and life style. J Intern Med 1991; 230: 299–305.
26. Jee SH, He J, Appel LJ, Whelton PK, Suh I, Klag MJ. Coffee consumption and serum lipids: a meta-analysis of randomized controlled clinical trials. Am J Epidemiol 2001; 153: 353–362.
27. Weusten-van der Vouw MPME, Katan MB, Viani R, *et al.* Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. J Lipid Res 1994; 35: 721–733.
28. Andersson HC, Hallström H, Kihlman BA. Intake of caffeine and other methylxanthines during pregnancy and risk for adverse effects in pregnant women and their foetuses. Nordic Council of Ministers, TemaNord 2004:565. In press.
29. Cnattingius S, Signorello LB, Annerén G, Clausson B, Ekbom A, Ljunger E *et al.* Caffeine intake and the risk of first-trimester spontaneous abortion. New Engl J Med 2000; 343: 1839–45.
30. Wisborg K, Kesmodel U, Bech BH, Hedegaard M, Henriksen TB. Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: Prospective study. Brit Med J 2003; 326: 420–423.

CHAPTER 9

Energy

Components of daily energy expenditure

Definitions

The basic principle for energy requirement reference values is energy balance, *i.e.* a physiological state in which daily energy intake equals energy expenditure (EE), and both body weight and energy content (defined by body composition) are unchanged. For some people, namely those who are markedly over- or underweight, the recommended energy intake may be smaller or larger than energy expenditure for a prescribed time period, but long-term energy balance is the ultimate goal even in treatment of undernourishment and obesity. The NNR therefore define *energy requirement in adults as the energy intake needed to cover energy expenditure in individuals with body weight, body composition and physical activity compatible with good health.* However, because body energy stores are very large (at least 30 times daily energy expenditure), there is no need for energy intake and EE to be equal over short periods, such as 1–4 days (1).

Energy requirements are based on data of daily energy expenditure. The doubly labelled water (DLW) technique is now the 'gold standard' method for determining energy expenditure in a way that is both non-invasive and does not constrain normal activity pattern (2). The technique uses two stable isotopes of water (^{2}H and ^{18}O) given orally, which are measured in urine over a period of days (2). Other methods, such as the factorial (diary) method, heart rate monitors, the accelerometer and the ventilated hood method (indirect calorimetry) are needed to describe components of daily energy expenditure.

The daily energy expenditure can be divided into the following components:

- Resting (or basal) energy expenditure (REE or BEE)
- Diet-induced thermogenesis (DIT)
- Energy expenditure caused by physical activity (PAEE)

The basic unit of energy expenditure is kJ (or MJ = 1,000 kJ) per time unit (usually MJ/d). 1 kJ equals 0.24 kcal (or 1 kcal = 4,184 kJ), a unit which is still often used in the literature.

In individuals with approximately equal physical activity level, daily EE is strongly related to body mass and particularly to fat free mass (FFM = body mass – fat mass) (3). Fat mass (FM) also shows a positive correlation with daily EE, but the slope (increase in EE per kg FM) is less steep than for FFM (3). However there is a variation in BEE, explained by the heterogeneous composition of the FFM compartment. Thus, the slope of BEE against FFM is lower at high FFM and, conversely, higher at low FFM (4). Organ metabolic rate is much higher than skeletal muscle and fat metabolic rate because of higher oxidative capacity in organ mass. In adults 70–80% of BEE is derived from organs that comprise 5% of the body weight (5).

Daily EE is also higher in men than in women, but the difference disappears after adjustment for the sex difference in body composition. Very cold or hot environments, genetic differences, hormonal status (*e.g.* concentrations of thyroid and growth hormone), sympathetic activity, psychological state, pharmacological agents and several disease states may increase or decrease daily EE, mainly by affecting REE (6, 7).

Basal energy expenditure

Basal energy expenditure (BEE) or basal metabolic rate (BMR) is defined as the energy expenditure of an individual lying at physical and mental rest in a thermoneutral environment, about 12 hours after the previous meal. Resting energy expenditure (REE) is measured in less rigorous conditions than BEE. Therefore, REE is considered to be approximately 5% higher than BEE. The mean EE during sleep is slightly lower than during waking hours (3). Therefore, sleeping energy expenditure (SEE) is about 10% lower than BEE. However, despite small systematic differences SEE, BEE and REE are very strongly inter-correlated and they are often used interchangeably.

The strongest determinant of BEE is FFM, which explains up to 80% of the inter-individual variation (3,7). The relationship between these parameters seems to be linear, at least within a normal range of adult FFM (40–100 kg). The inter-individual variation at a given FFM is about 2.1 MJ, indicating the possible magnitude of difference in REE between two individuals with a similar FFM. Genetic variation, body composition, variation in hormone concentrations, energy balance and physical fitness are factors that have been found to explain the variation in BEE after adjustment for FFM (6–9).

Because of the technical constraints of BEE measurements, determi-

nations of energy requirements are usually based on predicted BEE. *Table 9.1* shows prediction equations for BEE, as given by Schofield *et al.* (10), WHO/FAO/UNU (11) and the Commission of the European Communities (12). However, these equations are not applicable in obese subjects and when applied in populations where overweight is common they tend to produce biased estimates of BMR (13, 14).

Table 9.1 Equations for calculating average basal energy expenditure (MJ/d) for men and women based on body weight (W, kg) and for children and adolescents based on W and height (H, m)

Age year	BEE MJ/d on weight	BEE MJ/d on weight and height
Girls		
< 3	0.244 W – 0.13	0.068 W + 4.28 H – 1.73
4–10	0.085 W + 2.03	0.071 W + 0.68 H + 1.55
11–18	0.056 W + 2.90	0.035 W + 1.95 H + 0.84
Women		
19–30	0.0615 W + 2.08	
31–60	0.0364 W + 3.47	
61–75	0.0386 W + 2.88	
> 75	0.0410 W + 2.61	
Boys		
< 3	0.249 W – 0.13	0.0007 W + 6.35 H – 2.58
4–10	0.095 W + 2.11	0.082 W + 0.55 H + 1.74
11–18	0.074 W + 2.75	0.068 W + 0.57 H + 2.16
Men		
19–30	0.064 W + 2.84	
31–60	0.0485 W + 3.67	
61–75	0.0499 W + 2.93	
> 75	0.035 W + 3.43	

Diet-induced thermogenesis

Diet-induced thermogenesis (DIT) or diet-induced EE can be defined as the increase in EE above BEE divided by the energy content of the food ingested (15). The postprandial rise in EE lasts for several hours, but about 90% of DIT is observed within 4 hours after the meal. DIT is assumed to be 10% of daily EE in individuals in energy balance consuming an average mixed diet. However, DIT of fats is only 5% of their energy content, whereas DIT of proteins is approximately 20% of energy content. DIT of carbohydrates is normally around 10% of energy content, but may be up to 20% if carbohydrates are directly converted to fat (*de novo* lipogenesis), a process that normally only occurs to a minor degree in individuals consuming diets typical for the Nordic countries (16).

Physical activity

Physical activity is defined as any body movement achieved by contraction of skeletal muscles that increases EE above resting levels (17). Exercise is a subcategory of physical activity: exercise is voluntary, deliberate physical activity performed because of anticipated positive consequences on physical, psychological and/or social well-being. The daily physical activity level (PAL) is defined as total EE divided by BEE (or REE). This way of quantifying physical activity is based on the assumption that the variation in daily EE is based on physical activity and body size.

Daily physical activity (and physical activity induced EE) can be divided into occupational and leisure activity. The latter can be further divided into exercise and non-exercise activity, with different grades of intensity. Inactivity means a state with EE close to REE, which in everyday life means sitting or lying while awake. Spontaneous physical activity corresponds to small, involuntary muscle movements, such as fidgeting, and may be related to weight control (18), but the data are limited. The associations between physical activity and health are described in detail in *Chapter 10*.

Energy balance and health

Body mass index and health

Body mass index (BMI), defined as body weight (kg) divided by height (m) squared, has a U or J shaped association with total mortality and morbidity. However, these relationships are difficult to interpret, because pre-existing illnesses may affect body weight rather than vice versa. Generally in adults, the BMI that is compatible with the lowest mortality (and morbidity) is approximately 22–23. According to the WHO definition (19), the normal (or recommended) BMI is between 18.5 and 24.9 (*Table 9.2*). A BMI of 30 or more is considered to be obesity. The reference intervals of *Table 9.2* are applicable in all Nordic countries. The prevalence of obesity in the Nordic countries is shown in *Table 9.3*.

Table 9.2 Body mass index, definitions of underweight, overweight and obesity and health risks for adults (18–64 years)

Body mass index	Definition	Morbidity and mortality
< 18.5	Underweight	Slightly increased
18.5–24.9	Normal weight	Low
25.0–29.9	Overweight	Slightly increased
30.0–34.9	Grade I obesity	Increased
35.0–39.9	Grade II obesity	Much increased
> 40.0	Grade III obesity	Very much increased

Obesity in children and adolescents may be defined using BMI, but the cut-off points differ significantly from those presented in *Table 9.2.* Cole *et al.* (20) have published international age- and sex-specific BMI cut-off points for overweight (85th percentile) and obesity (95th percentile) for children and adolescents between 2 and 18 years.

Table 9.3 Prevalence (%) of adult (25–64 y) obesity (BMI ≥ 30.0) in the Nordic countries, assessed from self-reported body weight

	Women	Men	Reference
Denmark	9.8	10.9	(21)
Finland	13.8	13.5	(22)
Iceland	13.5	13.4	(23)
Norway	8.6	10.0	(24)
Sweden	10.2	12.2	(25)

The BMI-related risk for some diseases (*e.g.* type 2 diabetes and some cancer forms) increases more linearly than *e.g.* total mortality. However, it should be kept in mind that BMI may represent different levels of fatness and body fat distribution depending on age, sex and ethnicity or race (26, 27). Therefore, BMI on individual level should be used with great caution. Other simple measures, such as waist circumference (see section on Obesity) may assist in assessment of obesity-related health risks.

Studies have found that ageing is associated with decreasing height, weight and body mass index (BMI) (28, 29) and with loss of muscle mass and gain of body fat (30). These changes imply that optimal BMI may be different in old people compared to younger. Several studies have found the nadir (BMI associated with lowest relative mortality) to be higher in old people compared to recommendations for younger populations (31–35). The suggested cut-off limits range from 24–33 but the exact level is still unknown (36). Unfortunately the data are inadequate to make any precise recommendations for optimal BMI among the elderly.

Studies that relate BMI to functional ability have found both a high and a low BMI to be related to disability (37, 38). However, marked obesity is clearly associated with physical disability and difficulties in performing the activities of daily living (39, 40).

Weight stability and health

According to epidemiological studies, stable weight is related to the lowest total mortality, whereas weight gain is clearly related to increased mortality (41). Many epidemiological studies indicate that weight loss is also associated with increased mortality (*e.g.* 41–44). However, these

data should be interpreted with caution, because of difficulties in separating voluntary and involuntary (due to pre-existing disease) weight reduction. Moreover, epidemiological studies do not separate different techniques or rates of weight reduction, or composition of lost body weight (45). In contrast, even a modest (5–10% of body weight) weight reduction in high-risk individuals improves health (19). Weight cycling (weight reduction, then increase to previous levels) may have adverse effects on mortality and morbidity (46, 47), but the data do not provide compelling evidence (48). A 25-year study in Gothenburg, Sweden, has found an age-related decrease in body weight from the age of 70 to 95 of approximately 0.5–1.0 kg body weight for every 5 years, more pronounced in the highest quintiles of body weight (29).

Obesity

Definitions: Obesity and abdominal obesity

Obesity is defined as a state with adverse health effects related to the amount or anatomical distribution of body fat. According to the WHO (19), obesity is defined as a BMI above 30 (see *Table 9.2*). In addition, abdominal fat distribution, being an indicator of intra-abdominal fat mass, may also be used as an indicator of obesity (49). *Table 9.4* presents cut-off points for waist circumference measurement, as suggested by the National Institute of Health (50) and WHO (19). Intra-abdominal fat mass, or abdominal fat distribution, may even be more strongly associated with metabolic disturbances than the amount of total body fat. The cut-off points are probably higher for elderly subjects (51, 52).

Table 9.4 Waist circumference (cm) and the risk of metabolic complications associated with abdominal obesity in adults (18–64 years)

Risk level	Women	Men
Low	≤ 79	≤ 93
Increased	80–87	94–101
High	≥ 88	≥ 102

Determinants of obesity and weight control

Weight gain is caused by positive energy balance. Several retrospective and prospective population-based studies have evaluated factors related to obesity or weight gain. Many comparisons between countries and short-term laboratory experiments have identified high fat intake as an important dietary predictor of obesity (53). However, observational

(cohort) studies have yielded inconclusive evidence regarding fat as a causal factor for obesity and weight gain (54, 55). An explanation may be underreported fat intake (56) weakening the association between fat intake and obesity. Fat quality, total intake of sucrose, moderate alcohol consumption and meal frequency have not been shown to be associated with obesity or weight gain (57). Moreover, variations in carbohydrate quality (indicated by dietary glycaemic index) have not been conclusively shown to predict weight change in intervention studies (58). However, an abundant intake of sucrose in beverages may induce weight gain, at least in individuals with obesity (59). A decrease in fat intake leads to short-term (about 6 months) weight loss, but the long-term (> 1 y) consequences are not equally clear (53, 60).

Low levels of physical activity are associated with obesity and more age-related weight gain. Most studies have examined leisure activity, and data on occupational energy expenditure adjusted to *e.g.* socioeconomic factors in relation to obesity are lacking. High levels of physical activity are also associated with less weight being regained after weight reduction (61). However, most of the above findings are observational and retrospective. The studies are still inconclusive on whether physical activity could be regarded as a single predictor of weight control. Obesity is also associated with education level and socioeconomic status. The general trend is that higher social classes have a lower prevalence of obesity compared with lower social classes (61, 62).

Secular trends in obesity, dietary intake and physical activity

The prevalence of obesity has increased in all Western countries during the past 20 years, and a rapid increase is also seen in developing countries undergoing an economic transition to market economy (19). The trend for increasing obesity has also been observed in all the Nordic countries. Comparisons of national data are difficult because of methodological and sampling differences, but the available results suggest that the prevalence of obesity in adults is highest in Finland (about 13–14% of adults with self-reported BMI >30) (see *Table 9.3*).

The prevalence of overweight and obesity has increased steadily in Finland since 1980 (63) but in recent years it seems to be levelling off in Finnish women, at least when overweight (BMI >25) is used as the criterion. In Norway, measured weight and height from health surveys in 8 counties show that the prevalence of obesity (BMI > 30) in 40–42 year old men increased from 10% in 1994–96 to 14% three years later (64), while the corresponding figures for women were an increase from 9% in 1996–99 to 12% three years later. The prevalence of obesity has also risen in Denmark, at least since 1987 (21).

The change in eating habits and patterns has been fairly rapid during recent decades. Secular trends in the Nordic countries show an increase in *e.g.* fruit, vegetable, meat, vegetable fat and alcohol consumption, and consumption of more sweets, fast food and eating out in general. At the same time, the intake of saturated and total fat has decreased. The change in fat quality has been particularly prominent in Finland. In Norway the dietary content of fat decreased from 40 to 35 E% during 1975–1990, followed by a modest decrease to 34 E% in the subsequent ten years (65). Despite large changes in consumption of food items, energy and fat intake show much less secular variation (66).

Traditions of measuring and evaluating food consumption on a population level are well established in many Nordic countries. In contrast, the methods to assess physical activity are often crude. The available data on the prevalence of vigorous physical activity suggest little change during the past 20 years (66). Occupations with high energy expenditure seem to have decreased drastically over time. The data on leisure non-exercise activity are very scarce, partly due to the severe difficulties of measuring activity, and partly to the fact that the importance of non-exercise activity on our health has only recently been recognised. Nevertheless, proxy measures, such as the time of TV-watching and the number of cars per household, strongly suggest that the non-exercise leisure energy expenditure may have decreased considerably (67). Moreover, Finnish data show that commuting to and from work by walking or bicycling decreased between 1982 and 1992 (66).

Reference values for energy requirements in children and adolescents

Part of the energy intake of children and adolescents is used for growth, and the energy requirement per kg body weight is therefore higher for these age groups than for adults. During the first four months of life, approximately 27% of the energy intake is used for growth. At the end of the first year of life, this fraction decreases to approximately 5%; at age 1–3 years it represents approximately 3% and is thereafter less than 2% (68).

The energy intake of healthy, thriving infants and children, as measured by dietary surveys, was previously used as the basis for energy reference. Intake studies were used as it was not possible to measure energy expenditure and specify with confidence the allowance for a desirable physical activity level (PAL) in infants and young children (69). The doubly labelled water (DLW) technique is now the preferred method for determining energy expenditure even in children. However,

when studying children, the energy cost of growth must be taken into consideration. This can be divided into two parts : first the energy cost of producing and laying down new tissue, which is included in the measurement of total energy expenditure (TEE) and second, the energy deposited in new tissue, which is not a part of TEE (70). This can be roughly calculated using energy deposition rates consistent with good health and added to TEE to provide an estimate of energy requirements in children. Studies using the DLW technique to measure the energy expenditure in infants and children have accumulated and are used as a basis for new reference values for infants and children, 0–5 years, in the current NNR. However, fewer studies using DLW have been performed for older children and adolescents. The new reference values are therefore based on total energy expenditure using estimations of basal metabolic rate (BMR) and physical activity level (PAL) (71), with values for different activity levels from 10 years and up.

Reference values for energy requirements of children and adolescents should be based on their energy expenditure and requirements for growth as well as their habitual physical activity. Energy expenditure should be consistent with the attainment and maintenance of long-term good health, including recommended physical activity (71).

Age 0–23 months

Previous reference values, which were based on the energy intake of healthy infants fed energy-dense formula, are too high since measurements of energy expenditure using DLW have consistently given lower values (11, 69–73) and are also supported by recent studies on energy intake (74). The differences are still apparent when adjustments are made for the energy cost of energy deposition in order to produce better comparisons of expenditure and intake (70). The reference values for energy requirements for children 0–12 months were lowered in NNR 1996 from NNR 1989 and are now further decreased by 3–13% for the age group 0–23 months (*Table 9.5*). Accumulation of available data and increased knowledge about the use of the DLW technique for young children, in addition to more accurate calculations, support the above findings (69, 75, 76). The estimated energy requirement for one-month-old infants is based upon a review of studies using DLW by Butte and coworkers (75), while the EER for older infants is based on recent data from studies on healthy infants, also using DLW (69, 76). New reference values do not necessarily mean that infants and children should lower their energy intake, but that science and scientific methods have progressed towards giving a more realistic picture of how much energy infants and children really need.

Table 9.5 Estimated average daily energy requirements (per kg body weight) for children 0–23 months

Age months	Average daily energy requirements kJ/kg BW per day
1	390
3	365
6	355
12	355
18	355

Some studies have shown that breast fed infants have a lower intake of energy-yielding nutrients than formula fed infants (77–79), especially infants breast fed for more than seven months, which results in less body weight gain from six to 10 months than in infants breast fed for a shorter period (74, 80). The effect of infant feed source on energy requirements was found to persist throughout the second year of life in one of the studies used as a basis for the estimated energy requirement (69). This was primarily because of the higher REE in the formula fed infants than in the breast fed (6, 9), although varying digestibility might also play a role (81). However, the differences between feeding groups never exceeded 20 kJ/kg, and current NNR give one energy requirement valid for both breast fed and formula fed infants. An explanation for the lower reference values today is therefore also an increase in the proportion of breast fed babies compared to formula fed babies.

Age 2–5 years

The reference values for this age group have been changed from the reference values in NNR 1996 and are now based on a review by Torun and co-workers (71) of studies compiling data on energy expenditure using DLW. The values have been adjusted by adding 2% for the energy cost of new tissue. Earlier reference values were based on adjusted reference values from FAO/WHO (11). NNR 1996 recognized that newer studies indicated that the reference value for energy intake in the 2–5-year age group might still be too high (82), but concluded that there was still not enough evidence to change the reference values. However, more studies using the DLW technique now support the view that the previous reference values were too high for this group (73, 83), and they have been lowered by 4–16% (*Table 9.6*), compared with NNR 1996.

Age 6–9 years

The reference values have primarily been decreased slightly for this age group, compared with NNR 1996. The new reference values are based

on a review by Torun and co-workers (70), using Schofield's equation as before to calculate BMR (10), but also a new evaluation of moderate PAL, using studies on physical activity from many locations, particularly Europe and the USA (*Table 9.6*). The evaluations are based on studies assessing physical activity using DLW, heart rate monitoring and activity-time allocation studies (71). The Schofield equation has been found to be in good agreement (± 8%) with measured values in 5 to 15-year-old children and adolescents (84). Fewer studies using DLW have been performed in this age group compared with younger children. The new reference values, however, coincide reasonably well with the data from DLW studies performed so far in this age group (71, 85–87).

Table 9.6 Estimated average daily energy requirements (per kg body weight) for children 2–9 years

Age	Girls	Boys
years	kJ/kg BW per day	kJ/kg BW per day
2	355	355
3	330	355
4	320	330
5	320	325
6	320	325
7	305	325
8	285	310
9	265	295

Age 10–17 years

The energy reference values for this age group have been changed from NNR 1996. The new evaluation of energy requirement is calculated from BEE, based on sex and body weight (10) multiplied by PAL values representing three daily levels of physical activity. These values are also based on the overview of studies by Torun and co-workers of children engaged in different forms of activities. As the variation in physical activity is greater among older children and adolescents, the values are given with regard to light, moderate and heavy physical activity (*Table 9.7*). The new evaluation of energy requirement for moderate activity is higher (5–16%) than earlier reference values from 1996, as studies on habitual physical activity have shown that there are sufficient grounds to increase the reference values (71). This is also supported by studies using DLW suggesting an increase in the values for energy requirement in this age-group (88–90), as well as studies using heart rate monitoring (91).

Table 9.7 Estimated average daily energy requirement (per kg body weight) for adolescents 10–17 years for three levels of physical activity. The PAL values used are for ages 10–13 and 14–17 (92)

Age years	Light activity kJ/kg BW/d	Moderate activity kJ/kg BW/d	Heavy activity kJ/kg BW/d
Girls	PAL 1.50/1.45	PAL 1.70/1.65	PAL 1.90/1.85
10	220	250	280
11	200	230	255
12	190	215	240
13	180	200	225
14	165	190	210
15	160	180	205
16	155	180	200
17	155	175	195
Boys	PAL 1.55/1.60	PAL 1.75/1.80	PAL 1.95/2.05
B10	250	285	315
11	235	265	295
12	220	250	280
13	210	235	265
14	205	230	265
15	195	220	250
16	190	215	240
17	185	210	235

Estimated average reference values in relation to age, sex, puberty and body composition

The estimated daily energy requirements according to age for children (*Table 9.8*) are based on calculations and assumptions described below. The values are based on the energy requirement per kilogram body weight (*Tables 9.5–9.7*).

When comparing body weight data from the Nordic countries, the differences between body weights in the same age intervals are small; differences are mainly smaller than one kilogram. Values for body weight related to age in the group 0–5 years are based on the mean of reference values from Denmark (93), Norway (94), Sweden (Swedish growth chart, 2000) and Finland (Finnish growth chart, 1993). No values were available for 2–5 year-olds in Finland and data for 3–5 year-olds in Norway and the other Nordic values were used. Recent values for growth at school age show increasing weight to height and prevalence of overweight (95), therefore values for the age group 6–17 years are

based on mean values from 1973–1977 (93). The increasing incidence of overweight and obesity among young children and adolescents makes the use of means or medians from more recent data to estimate daily energy requirement less satisfactory. Figures from Sweden which are one decade younger than those used for the reference weight show an increase in median weight of up to 4 kg among 10–17 year-old children. Figures from Iceland which are 15 years younger show even larger differences (95). Over a similar time period (1978–1998) the average height has only increased by 1 cm among 9-year-old children in Iceland (96) and less than 1 cm among 18-year-old conscripts in Sweden, Denmark and Finland from 1975–1990 (97). In the US the estimated energy requirements for children are based on the median weight and are slightly higher than the reference weights in the current NNR (98). Children of the same age vary widely in body weight, particularly in the age groups where only a few children have reached puberty. The body weight of children of the same age and sex may differ by a factor of two. The estimated energy requirement in a certain age group, as illustrated in *Table 9.8*, must therefore be used with caution.

The calculated energy requirement for overweight children (> 2 SD weight-for-height) is too high when based on body weight, since the energy requirement is primarily determined by the FFM. Therefore, it is recommended that the energy requirement in overweight children should be based on the weight 1 SD above normal weight for height, or on the weight corresponding to the cut-off value to overweight according to International Obesity Task Force (19).

Reference values for energy requirements in adults

The reference values for energy requirements in adults are based on estimations of basal energy expenditure (BEE) and daily physical activity level (PAL). Energy requirement is equivalent to the product of BEE×PAL. BEE can be calculated from prediction equations (*e.g.* equations presented in *Table 9.1*). The PAL values used in these recommendations (*Table 9.9*) are generalisations based on studies using DLW. By using more detailed information on daily physical activity (time spent in different activities) and the respective metabolic equivalents (MET values = activity EE/resting EE) (99), PAL can be calculated as the daily weighted average MET value (*Table 9.10*). It should be noted, however, that the published MET values may underestimate energy expenditure in very obese individuals.

Table 9.8 Estimated energy requirements (per day) for children 0–17 years based on age-related average weight and moderate physical activity

Age	Average weight, kg		Estimated energy requirements, MJ/d	
	Girls	**Boys**	**Girls**	**Boys**
0–1 mo	3.4	3.6	1.3	1.4
3 mo	5.7	6.1	2.1	2.2
6 mo	7.7	8.2	2.6	2.7
12 mo	9.9	10.6	3.4	3.7
2 y	12.5	13.2	4.4	4.7
3 y	14.9	15.4	4.9	5.5
4 y	16.8	17.3	5.3	5.7
5 y	19.2	19.4	6.1	6.3
6 y	21.1	21.4	6.8	7.4
7 y	23.7	24.8	7.2	8.1
8 y	26.1	26.5	7.4	8.2
9 y	28.7	29.1	7.6	8.6
10 y	31.8	32.2	8.0	9.2
11 y	35.5	35.3	8.2	9.4
12 y	40.4	39.1	8.7	9.8
13 y	45.6	43.5	9.1	10.2
14 y	49.9	49.2	9.5	10.8
15 y	53.2	55.1	9.6	11.3
16 y	54.8	60.0	9.9	12.0
17 y	56.0	63.6	9.9	13.4

Table 9.9 Physical activity level expressed as multiplies of resting energy expenditure according to different levels of occupational and leisure activity (modified from Black *et al.* (100))

	PAL
Bed-bound or chair-bound (not wheelchair)	1.1–1.2
Seated work with no option of moving around and little or no leisure activity	1.3–1.5
Seated work with some requirement to move around but little leisure activity	1.6–1.7
Work including both standing and moving around (*e.g.* housework, shop assistant)	1.8–1.9
Very strenuous work or daily, competitive athletic training	2.0–2.4

Note 1 Moderate, leisure physical activity (*e.g.* brisk walking): 0.025 PAL unit increase for each weekly hour.

Note 2 Strenuous, leisure physical activity (*e.g.* running, competitive soccer): 0.05 PAL unit increase for each weekly hour.

Table 9.10 Two examples of how to estimate daily physical activity level from data on physical activity

Intensity of Activity (MET)		Very inactive day		Active day	
		Time, h	MET×h	Time, h	MET×h
Rest	(1.0)	10	10	8	8
Very light	(1.5)	12	18	8	12
Light	(2.0)	2	4	4	8
Moderate	(5.0)	0	0	1	5
Strenuous	(10.0)	0	0	1	10
Total		24	32	24	43
PAL			1.33		1.79

Explanation. The time spent in different activities is multiplied by the respective metabolic equivalent value (MET value). To obtain the daily physical activity level (PAL), the sum of daily MET×h is divided by 24. Hence, PAL is the weighted average of daily MET×h. Daily energy expenditure is calculated by multiplying PAL by basal (or resting) energy expenditure.

An average PAL for adults in the Nordic countries is assumed to be around 1.6, which is compatible with sedentary work and some physical activity. A totally sedentary lifestyle (PAL 1.4–1.5) is associated with health risks that may be equal to the risk associated with marked obesity (BMI 30–35) or regular smoking. These health risks are offset by approximately 3–4 hours per week moderate physical activity or 2 hours per week more strenuous leisure-time physical activity (101), which would mean an increase of only 0.1 PAL units. However, it is likely that a PAL of roughly 1.8 would be more optimal for overall health. This PAL is approximately the same as observed in moderately active prepubertal children (102). Strenuous athletic training may increase energy requirements to PAL 2.0–2.5 and in extreme cases even up to 3.5 (103, 104). However, it is rare for physical exercise to increase energy requirements by more than 20% compared to energy expenditure during normal daily living.

Table 9.11 shows reference weights based on mean population weights in Denmark, Sweden and Finland, with adjustments for individuals outside BMI range 18.5–25.0. *Table 9.12* shows average estimates of daily energy requirements for men and women with respect to age, body weight and activity level based on data in *Table 9.11*. In other words, the values in *Table 9.12* are estimations assuming that all individuals are at normal weight. It should be noted that these estimations have a large standard error due to inaccuracy in both estimation of BEE and of PAL. Therefore, the results should be used only for estimation on group level.

Few studies with DLW have been carried out with elderly subjects. Therefore, the data for the oldest age group in *Tables 9.11* and *9.12* should be used with special caution.

Due to the age-related weight changes among healthy elderly individuals, 0.5–1.0 kg should be subtracted from the average weights in *Table 9.11* for every 5 years above the age of 75. The reference weights used in *Table 9.12* for aged individuals (> 75 y) are 73.4 kg for men and 61.7 kg for women (both calculated as 1 kg less than the reference weight for the age-group 61–74 y).

Table 9.11 Reference weights (kg) in Denmark, Sweden and Finland. Data from Danskernes kostvaner 1995 (Denmark), Riksmaten 1997–98 (Sweden) and FINRISK 2002 (Finland)

Age years	Danskernes kostvaner		Riksmaten		FINRISK		
Women	weight *	corr. wt **	weight *	corr. wt **	weight ***	corr. wt **	mean corr. weight
18–30	62.7	61.6	65.6	63.3	65.5	62.0	62.3
31–60	65.5	63.1	66.6	63.7	70.1	63.2	63.3
61–74	66.7	62.6	68.2	63.5	72.2	61.9	62.7
Men							
18–30	78.8	76.3	77.4	75.2	81.0	75.7	75.7
31–60	81.7	77.0	82.0	77.3	85.1	76.1	76.8
61–74	80.2	74.6	81.7	75.4	83.2	73.1	74.4

* = self reported weight

** = all individuals with BMI < 18.5 adjusted to 18.5; all with BMI > 25 adjusted to 25

*** = weighed in a laboratory; youngest age range 25–30 y

Table 9.12 Reference energy requirements (MJ/d) in adults based on Nordic reference weights (Table 9.11) and different activity levels

Age years	REE	Sedentary PAL 1.4	Normal PAL 1.6	Active PAL 1.8
Women				
18–30 y	5.9	8.3	9.4	10.7
31–60 y	5.8	8.1	9.2	10.4
61–74 y	5.3	7.4	8.5	9.5
> 75 y	5.1	7.1	8.2	9.3
Men				
18–30 y	7.7	10.7	12.3	13.8
31–60 y	7.4	10.4	11.8	13.3
61–74 y	6.6	9.3	10.6	12.0
≥ 75 y	6.0	8.4	9.6	10.8

REE = resting energy expenditure

PAL = physical activity level

Reference values for energy requirements are based on assumptions of normal weight and energy balance. However, this may not always be the case. For instance, negative energy balance is needed in treatment of obesity. If energy intake is 2.1 MJ/d lower than the energy requirement for energy balance, the estimated weight reduction is 0.5 kg/week. This rate of weight loss is often recommended, although a larger negative energy balance (up to 4.2 MJ/d) leading to a weight loss of 1 kg/week still seems to be compatible with a healthy weight reduction (19, 50). Conversely, some other situations (*e.g.* treatment of malnutrition) may require more energy than estimated for energy balance.

If an estimation of energy requirement is needed for an individual with weight and physical activity different from those presented in *Tables 9.11* and *9.12,* the calculation is carried out as follows: First, the resting energy expenditure (basal metabolic rate) is estimated from the equation given in *Table 9.1.* Then, PAL is estimated either from *Table 9.9*, or by calculation as shown in *Table 9.10*. Finally, the energy requirement is calculated as REE × PAL. It should be noted, however, that the estimation of both REE and PAL may be imprecise, and it is indeed possible to misjudge daily energy requirement by at least 2 MJ.

Energy requirements during pregnancy

Studies in humans and animals show that an insufficient energy and nutrient supply during the foetal lifetime and immediately after birth is related to health later in life. To ensure optimal nutrient supply for the foetus and the baby, it is important that the pregnant and lactating woman eats a well-balanced diet in appropriate amounts. The woman's nutrient intake before conception also seems to be of importance for healthy development of the baby.

When a woman becomes pregnant, her need for energy increases. The higher energy requirement during the pregnancy can be satisfied by an increased food intake but also, at least theoretically, by decreased physical activity resulting in a smaller energy requirement.

During pregnancy several physiological adaptations take place in the woman's body. Besides the growth of the foetus, the placenta and the uterus, the blood volume, the breasts and body fat mass also increase. All those factors together lead to an increased body weight.

There is great variation in how much the woman's body weight increases during pregnancy. A positive association between the woman's weight gain and the health of both baby and mother has been observed. However, a very large weight gain is a health risk for both the mother and the child, especially among pre-pregnancy overweight or obese women (*e.g.* increased risk for breast cancer of the mother, higher risk

for spontaneous abortion, gestational diabetes and gestational hypertension) (105, 106). If the body weight increase during pregnancy is too small, the risk of the baby having low birth weight is increased, as weight gain in pregnancy is positively correlated to size at birth (106). Low birth weight increases the risk for health complications early in life, and small size at birth has been found to be related to higher risk of adult diseases, such as coronary artery disease, hypertension and type 2 diabetes (106–109). Increase in body weight during pregnancy among Nordic women has been on average 14–15 kg (110, 111) but has increased over recent years, for example in Sweden (111). In Iceland, women of normal weight before pregnancy have been found to gain on average 16.7 kg in pregnancy (106). Birth size in the Nordic countries is high >3,500 g, highest in Iceland and the Faeroe Islands and has been increasing for full-term babies in all the Nordic countries over recent years (112). A weight gain up to 18 kg in Icelandic women of normal weight before pregnancy has been found to be safe, both as regards complications during pregnancy and delivery as well as postpartum overweight (106, 113). Similar research has not been performed in the other Nordic countries.

The recommended weight gain during pregnancy should depend on the woman's pre-pregnancy body weight in relation to her height. The weight gain should be higher for leaner women and lower for overweight and obese women. The Institute of Medicine in the USA has developed recommendations for weight gain in pregnancy based on pre-pregnancy weight, established on studies considering the health of both the mother and baby. Women who are underweight before pregnancy (BMI< 19.9 kg/m^2) are recommended to gain 12.5–18 kg in pregnancy, women of normal weight (BMI 19.8–26.0 kg/m^2) are recommended to gain 11.5–16 kg, overweight women (BMI > 26.0–29.0 kg/m^2) 7–11.5 kg and obese women (BMI > 29) > 6–10 kg (111). In support of this, recent studies by Butte and co-workers showed that the increase in maintenance energy metabolism, one of the main components of the energy cost of pregnancy, varies in response to pre-pregnancy body fat content of the woman (114, 115).

The energy cost in pregnancy is due to the foetus, placenta and amniotic fluid, as well as the weight gain of uterus, breasts and blood volume, extracellular water and adipose tissue (116).

Energy requirement during pregnancy based on total energy expenditure and total energy deposition has been estimated for women of normal weight to increase negligibly in the first trimester, by 1,500 kJ (350 kcal) per day in the second trimester and by 2,100 kJ (500 kcal) per day in the third trimester (114). Physical activity influences the energy

requirements during pregnancy. It has been suggested that if a woman decreases her physical activity there should be no need for an extra energy intake. However, Butte *et al.* concluded that extra energy intake is required by healthy pregnant women to support adequate gestational weight gain and increases in BMR, which are not totally offset by reductions in activity energy expenditure in pregnancy (114). Thus in that cohort of healthy women, pregnancy in a real-life situation was associated with increased requirements for dietary energy. A recent Swedish study supports this by showing that activity level among pregnant women is generally unaffected in pregnancy until week 32, when the PAL value decreases slightly (117).

Energy requirements during lactation

The extra need for energy during lactation is estimated in the same way as during pregnancy. The breast milk contains approximately 2.8 kJ per gram (118). A woman who feeds her baby completely with breast milk produces about 700–800 g breast milk per day (118). During a six month period, this corresponds to approximately 386 MJ. The energetic effectiveness of producing milk is suggested to be about 80% (116). In well-nourished women, the BEE appears to be influenced only to a very small degree during the lactation period. The total energy cost for six months' complete breastfeeding of one baby is thus 482 MJ, or 2.6 MJ per day. Since there are large variations between women regarding the amount of milk produced and also some variation in the composition of the milk, the energy cost for lactation differs between women. This energy cost can be satisfied by an increased food intake, by mobilisation of fat from the woman's fat store and/or by a decreased level of physical activity. However, because of a risk for weight gain after pregnancy (119), it is recommended that lactating women increase rather than decrease their amount of physical activity.

In well-nourished women the mobilisation of fat stores during lactation seems to be relatively moderate. Swedish studies (120, 121) show that healthy lactating women mobilise approximately 300 g fat per month. For a woman who is exclusively breastfeeding and with a loss of the body fat gained during pregnancy of approximately 500 g per month, the increment of energy need is 1.8–2.2 MJ (122). In general, women have larger fat stores after the lactation period than prior to the pregnancy, which probably increases the risk for continuing overweight.

There is large variation in the extent to which different women use fat stores to satisfy the energy cost for lactation. The figures mentioned above are therefore not relevant for an individual woman. If the lacta-

tion period is longer than six months, the energy cost for the lactation is calculated in the same way as described above. In general the production of breast milk is smaller, which will result in a smaller energy cost for the lactation. The suggested reference value for an extra energy intake during lactation is 2.0 MJ per day.

Energy requirements in the elderly

Daily energy expenditure declines with age (123, 124), mainly due to decreased fat free mass (FFM) (125, 126) and decreased physical activity (127, 128). Basal energy expenditure (BEE) is strongly related to FFM, mainly organ mass (129). The decrease in BEE is not fully explained by the age-related decrease in FFM (130). Pannemans *et al.* (123) found that 80% of the variation in BEE in elderly subjects was explained by FFM.

Longitudinal (131, 132) and cross-sectional (101, 133, 134) studies have found an age-related decrease in BEE. Knowledge about daily energy expenditure in the elderly (> 75 years) is limited (101). A Swedish study found that BEE among 91–96 year-old subjects was not different from BEE among 70–80 year-old subjects (135). However, a longitudinal follow-up of the 73-year-olds at age 78 showed a decrease in BEE as well as TEE but not in active energy expenditure (AEE) (136). Their PAL values averaged 1.74 at both ages indicating a physically active lifestyle for the age group (136). Diet-induced thermogenesis (DIT) does not seem to be affected by age (134).

Low energy intake

Lowenstein (137) has suggested a reference value of 1,500 kcal/d, corresponding to approximately 6.5 MJ/d, as the minimum daily energy intake necessary for providing an adequate intake of micronutrients from an ordinary diet. In NNR, *very low energy intake* is defined as an energy intake below *6.5 MJ/d,* while an energy intake of 6.5–8 MJ is considered a *low energy intake* with increased risk of an insufficient intake of micronutrients.

A very low energy intake is related to either a very low physical activity level and/or to a low body weight. Low body weight is related to small muscle mass and thereby to low energy expenditure. The age-related decrease in energy expenditure may result in very low energy intake, and very low energy intake is also found among people on slimming diets and among subjects with *e.g.* eating disturbances or food intolerances.

Among healthy subjects an actual very low habitual energy intake is probably rare – even among sedentary elderly subjects the estimated energy requirement is 7–8 MJ, see *Table 9.12*. However, with lower

body weight among the sedentary elderly the energy expenditure may become critically low.

Intake of most micronutrients is positively associated with the energy intake and accordingly, habitual low energy intake is associated with low nutrient intake. In dietary surveys low energy *reporting* is biased by a widespread underreporting independent of age and especially among women and overweight/obese subjects. Thus, it is difficult to explore the consequences of low energy intake on nutritional status based on low energy *reporters.*

Among elderly subjects, low reported energy intakes were not paralleled by biochemical deficiencies (138, 139) indicating underreporting and/or recommendations on the large side and/or insufficient cut-off levels of the biochemical parameters. Among elderly Europeans (138) it was not possible to establish a level of reported energy intake that ensured an adequate reported supply for all of four micronutrients (iron, thiamine, riboflavin and pyridoxine). At a reported intake of 8 MJ 13% of the men and 16% of the women still had an inadequate intake (defined as 2/3 of RI) of at least one of the four micronutrients.

Energy content of foods

Calculation of energy content

The energy available for metabolism – physiologically available energy – is primarily determined by the chemical energy of the food, which is determined by laboratory measurements of the heat produced when its organic molecules are fully oxidized. Not all chemical energy in foods is available to the human and the chemical energy value must therefore be corrected for losses due to insufficient absorption and, as regards protein, even for incomplete oxidation and losses in urea. A precise calculation of the physiologically available energy content in foods requires knowledge of the chemical composition and the digestibility of all the food components. Since those components vary, it is more practical to use standardised factors based on the average composition and digestibility. Due mainly to historical background and tradition, different standard factors still exist, which differ from each other to some extent. In NNR the energy content for a mixed diet is calculated by the following factors: 17 kJ/g protein or available (= glycaemic) carbohydrate, and 37 kJ/g fat. Alcohol (ethanol) is calculated to yield 29 kJ/g. In kcal, these standard factors are: 4 kcal/g protein or carbohydrates, 9 kcal/g fat and 7 kcal/g alcohol. Note that these numbers include some errors caused by rounding off. To transform the figures between the

two different systems of units, the following relationships are used: 1 kcal = 4.2 kJ (or more precisely 4.184) and 1 kJ = 0.24 kcal (or more precisely 0.239).

The standard factors should in principle not be used for calculation of energy content in separate foodstuffs, because both the oxidation heat and the digestibility vary slightly between foodstuffs. In a mixed diet, however, these variations balance each other and the calculated values are very close to the values determined experimentally. Specific factors for calculating energy content in certain foodstuffs have been presented (140, 141).

Carbohydrates and fibre

The values for carbohydrate presented in food composition tables are in many cases determined by the 'difference method', which defines total carbohydrate as the difference between the total dry matter and the sum of protein, fats and ash. The value for carbohydrate then includes digestible mono-, di- and polysaccharides (starch) as well as non-digestible carbohydrate, lignin and organic acids. The glycaemic or 'available' carbohydrate represents total carbohydrates minus total dietary fibre, or the sum of sugars and starch. Available carbohydrate can be calculated by difference (total carbohydrates–dietary fibre). Sugar, alcohols and organic acids are presented separately, but calculated as total carbohydrate. For glycaemic carbohydrate, the oxidation heat is slightly smaller for monosaccharides than for disaccharides and is greatest for polysaccharides (141) but those differences do not have to be considered in practice. When the total carbohydrate is analysed by the difference method, available carbohydrate and dietary fibre contribute the same amount of energy. Therefore the real energy content is overestimated if the diet contains a high amount of fibre. In a diet with around 30 g fibre per day or less, the standardised factors for energy may be used without any significant consequences for the energy calculation (142). Fibre actually contributes a small amount of energy due to the fact that a proportion of the fibre is fermented in the colon to short-chain fatty acids, which can be absorbed and oxidized for energy. This energy contribution depends on the type of fibre but a factor of 8 kJ/g (2 kcal/g) has been suggested as an average value (140, 143). This value is used in NNR when calculating dietary energy. In the regulations for specifying the nutrient content of foods, the energy level for fibre has to be set at zero. However, both the Codex Alimentarius Commission and current suggestions for coming revisions of the European Nutrition Labelling Directive propose that dietary fibre be given an energy factor of 8 kJ/g.

The digestibility of carbohydrate varies between 90% in fruits to approximately 98% in cereals. The digestibility of flour depends on the fractions included, *i.e.* the digestibility decreases with a higher content of fibre.

Protein

Protein does not fully oxidize in the body. The physiologically available energy from protein is therefore reduced due both to incomplete digestibility and urea losses in the urine. The digestibility of protein is lowest in legumes (78%) and highest in animal products (97%) (140, 141).

Fat

The heat of oxidation for fat depends on the fatty acid composition of the triglycerides and the proportion of other lipids in the diet. On average, the available energy from fat is calculated as 95% in most foodstuffs (140, 141).

REFERENCES

1. Westerterp KR, Goran MI. Relationship between physical activity related energy expenditure and body composition: a gender difference. Int J Obes Relat Metab Disord 1997; 2: 184–8.
2. Schoeller DA. Recent advances from application of doubly labelled water to measurement of human energy expenditure. J Nutr 1999; 129: 1765–8.
3. Klausen B, Toubro S, Astrup A. Age and sex effects on energy expenditure. Am J Clin Nutr 1997; 65 (4): 895–907.
4. Muller M, Bosy-Westphal A, Kutzner D, Heller M. Metabolically active components of fat-free mass and resting energy expenditure in humans: recent lessons from imaging technologies. Obes Rev 2002; 3: 113–122.
5. Gallagher D, Allen A, Wang Z, Heymsfield S, Krasnow N. Smaller organ tissue mass in the elderly fails to explain lower resting metabolic rate. Ann N Y Acad Sci 2000; 904: 449–55.
6. Astrup A, Buemann B, Christensen NJ, Madsen J, Gluud C, Bennett P, Svenstrup B. The contribution of body composition, substrates, and hormones to the variability in energy expenditure and substrate utilization in premenopausal women. J Clin Endocrinol Metab 1992; 74: 279–86.
7. Toubro S, Sorensen TI, Ronn B, Christensen NJ, Astrup A. Twenty-four-hour energy expenditure: the role of body composition, thyroid status, sympathetic activity, and family membership. J Clin Endocrinol Metab 1996; 81: 2670–4.
8. Svendsen OL, Hassager C, Christiansen C. Impact of regional and total body composition and hormones on resting energy expenditure in overweight postmenopausal women. Metabolism 1993; 42: 1588–91.
9. Gilliat-Wimberly M, Manore MM, Woolf K, Swan PD, Carroll SS. Effects of habitual physical activity on the resting metabolic rates and body compositions of women aged 35 to 50 years. J Am Diet Assoc 2001; 101 (10): 1181–8.
10. Schofield WN, Schofield C, James WPT. Basal metabolic rate: Review

and prediction. Hum Nutr Clin Nutr 1985; 39 (Suppl 1): 1–96.
11. WHO. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. WHO Technical Report Series; 724. Geneva: WHO, 1985.
12. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community. (Thirty-first series of Food – Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1992.
13. Lof M, Hannestad U, Forsum E. Comparison of commonly used procedures, including the doubly-labelled water technique, in the estimation of total energy expenditure of women with special reference to the significance of body fatness. Br J Nutr. 2003; 90: 961–8.
14. Institute of Medicine. Nutrition during pregnancy. Washington (DC): National Academy Press, 1990.
15. Tataranni PA, Larson DE, Snitker S, Ravussin E. Thermic effect of food in humans: methods and results from use of a respiratory chamber. Am J Clin Nutr 1995; 61: 1013–9.
16. Hellerstein MK. No common energy currency: de novo lipogenesis as the road less travelled. Am J Clin Nutr 2001; 74: 707–8.
17. Caspersen CJ, Powell K, Christenson G. Physical activity, exercise and physical fitness: Definitions and distinctions for Health Related Research. Pub Health Rep 1985; 100: 126–31.
18. Levine JA. Non-exercise activity thermogenesis (NEAT). Best Pract Res Clin Endocrinol Metab 2002; 16: 679–702.
19. WHO, Obesity: Preventing and managing the global epidemic. Report of a WHO consultation on obesity. WHO Technical Report Series 2000; 894: 1–253
20. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000; 320: 1240–3.
21. Kjoller M, Rasmussen NK. Sundhed & sygelighed I Danmark 2000 & udviklingen siden 1987 (Danish Health and Morbidity Survey 2000 and trends since 1987). Statens Institut for Folkesundhed. 2002.
22. Helakorpi S, Patja K, Prättälä R, Aro AR, Uutela A. Health behaviour and health among Finnish adult population 2002. Publications of the National Public Health Institute B_{12}/2002.
23. Steingrimsdottir L, Thorgeirsdottir H, Olafsdottir AS. The diet of Icelanders, dietary survey of the Icelandic Nutrition Council 2002. Main findings. Manneldisráð Íslands 2003.
24. Statistics Norway 2002. www.ssb.no.
25. Statistics Sweden, 2004. www.scb.se.
26. Deurenberg-Yap M, Schmidt G, van Staveren WA, Deurenberg P. The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. Int J Obes 2000;24: 1011–7.
27. Wagner DR, Heyward VH. Measures of body composition in blacks and whites. Am J Clin Nutr 2000; 71: 1392–402.
28. Sorkin JD, Muller DC, Andres R. Longitudinal changes in height of men and women: Implications for interpretation of the body mass index. Am J Epidemiol 1999; 150: 969–77.
29. Dey DK, Rothenberg E, Sundh V, Bosaeus I, Steen B. Height and body weight in the elderly. I. A 25-year longitudinal study of a population aged 70 to 95 years. Eur J Clin Nutr 1999; 53: 905–14.
30. WHO Expert Subcommittee, WHO Expert Subcommittee on the use and interpretation of anthropometry in the elderly. Uses and interpretation of anthropometry in the elderly for the assessment of physical status report to the Nutrition Unit of the World Health Organization. J Nutr Health Aging 1998; 2: 5–17.
31. Beck AM, Ovesen L. At which body mass index and degree of weight loss should hospitalized patients be con-

sidered at nutritional risk? Clin Nutr 1998; 17: 195–8.
32. Rissanen A, Heliövaara M, Knekt P, Aromaa A, Reunanen A, Maatela J. Weight and mortality in Finnish men. J Clin Epidemiol 1989; 42: 781–9.
33. Rissanen A, Knekt P, Heliövaara M, Aromaa A, Reunanen A, Maatela J. Weight and mortality in Finnish women. J Clin Epidemiol 1991; 44: 787–95.
34. Allison DB, Gallagher D, Heo M, Pi–Sunyer FX, Heymsfield SB. Body mass index and all-cause mortality among people age 70 and over: the Longitudinal Study of Aging. Int J Obes 1997; 21: 424–31.
35. Stevens J, Cai J, Pamuk ER, Wiliamson DF, Thun MJ, Wood JL. The effect of age on the association between body-mass index and mortality. N Engl J Med 1998; 338: 1–7.
36. Pedersen AN, Ovesen L, Schroll M, Avlund K, Era P. Body composition of 80-years old men and women and its relation to muscle strength, physical activity and functional ability. J Nutr Health Aging 2002; 6: 413–20.
37. Galanos AN, Pieper CF, Cornoni-Huntley JC, Bales CW, Fillenbaum GC. Nutrition and function: Is there a relationship between body mass index and the functional capabilities of community-dwelling elderly? J Am Geriatr Soc 1994; 42: 368–73.
38. Ensrud KE, Nevitt MC, Yunis C, Cauley JA, Seeley DG, Fox KM, *et al.* Correlates of impaired function in older women. J Am Geriatr Soc 1994; 42: 481–9.
39. LaCroix AZ, Guralnik JM, Berkman LF, Wallace RB, Satterfield S. Maintaining mobility in late life. II. Smoking, alcohol consumption, physical activity, and body mass index. Am J Epidemiol 1993; 137: 858–69.
40. Launer LJ, Harris T, Rumpel C, Madans J. Body mass index, weight change, and risk of mobility disability in middle-aged and older women. J Am Med Assoc 1994; 271: 1093–8.
41. Mikkelsen KL, Heitmann BL, Keiding N, Sorensen TIA. Independent effects of stable and changing body weight on total mortality. Epidemiology 1999; 10: 671–8.
42. Lee IM, Paffenbarger. RS Jr. Is weight loss hazardous? Nutr Rev 1996; 54 (4 Pt 2): S116–24.
43. Byers T. The observational epidemiology of changing weight: an appeal for reasons. Epidemiology 1999; 10 (6): 662–4.
44. Wannamethee SG, Shaper AG, Walker M. Weight change, weight fluctuation, and mortality. Arch Intern Med 2002; 162: 2575–80.
45. Yang D, Fontaine KR, Wang C, Allison DB. Weight loss causes increase mortality: cons. Obes Rev 2003; 4: 9–16.
46. Jeffery RW. Does weight cycling present a health risk? Am J Clin Nutr 1996; 63 (Suppl 3): 452S–5S.
47. Olson MB, Kelsey SF, Bittner V, Reis SE, Reichek N, Handberg EM, *et al.* Weight cycling and high-density lipoprotein cholesterol in women: evidence of an adverse effect: a report from the NHLBI-sponsored WISE study. Women's Ischemia Syndrome Evaluation Study Group. J Am Coll Cardiol 2000; 36: 1565–71.
48. National Task Force on the Prevention and Treatment of Obesity. Weight cycling. JAMA 1994; 272: 1196–202.
49. Han TS, van Leer EM, Seidell JC, Lean ME. Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. BMJ 1995; 31: 1401–5.
50. National Institutes of Health and National Heart, Lung, and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Evidence Report. Obes Res 1998; 6 (Suppl 2): 51S–209S.
51. Chen H, Bermudez OI, Tucker KL. Waist circumference and weight change are associated with disability among elderly Hispanics. J Gerontol A Biol Sci Med Sci 2002; 57: M19–25.
52. Dey DK, Rothenberg E, Sundh V, Bosaeus I, Steen B. Waist circumference, body mass index, and risk for

stroke in older people: a 15 year longitudinal population study of 70-year-olds. J Am Geriatr Soc 2002; 50: 1510–8.
53. Astrup A. Dietary fat and obesity: still an important issue. Scand J Nutr 2003; 47: 50–7.
54. Heitmann BL, Lissner L, Sorensen TI, Bengtsson C. Dietary fat intake and weight gain in women genetically predisposed for obesity. Am J Clin Nutr 1995; 61: 1213–7.
55. McCrory MA, Fuss PJ, Saltzman E, Roberts SB. Dietary determinants of energy intake and weight regulation in healthy adults. J Nutr 2000; 130 (Suppl 2): 276S–9S.
56. Heitmann BL, Lissner L, Osler M. Do we eat less fat, or just report so? Int J Obes Relat Metab Disord 2000; 24: 435–42.
57. Astrup A, Buemann B, Flint A, Raben A. Low-fat diets and energy balance: how does the evidence stand in 2002? Proc Nutr Soc 2002; 61: 299–309.
58. Raben A. Should obese patients be counselled to follow a low-glycemic index diet? No. Obes Rev 2002; 3: 245–56.
59. Raben A, Vasilaras TH, Moller AC, Astrup A. Sucrose compared with artificial sweeteners: different effects on *ad libitum* food intake and body weight after 10 wk of supplementation in overweight subjects. Am J Clin Nutr. 2002; 76: 721–9.
60. Willett W. Dietary fat and obesity: lack of an important role. Scand J Nutr 2003; 47: 58–67.
61. Fogelholm M, Kukkonen-Harjula K. Does physical activity prevent weight gain – a systematic review. Obes Rev 2000; 1: 95–111.
62. Ball K, Mishra G, Crawford D. Which aspects of socioeconomic status are related to obesity among men and women? Int J Obes Relat Metab Disord 2002; 26 (4): 559–65.
63. Helakorpi S, Patja K, Prättälä R, Aro AR, Uutela A. Health behaviour and health among Finnish population. National Public Health Institute, publication series B/7, Helsinki, 2003.
64. Tverdal A. Forekomst av fedme blant 40-42-åringer i to perioder. Tidskr Nor Lægeforen 2001; 121: 667–72).
65. Utviklingen i norsk kosthold 2003. IS-1143. Sosial- og helsedirektoratet. Oslo 2003.
66. Fogelholm M, Männistö S, Vartiainen E, Pietinen P. Determinants of energy balance and overweight in Finland 1982 and 1992. Int J Obes 1996; 20: 1097–104.
67. Prentice AM, Jebb SA. Obesity in Britain: gluttony or sloth? BMJ 1995; 311: 437–9.
68. Fomon SJ, Bell EF. Energy. In: Fomon SJ, editor. Nutrition of normal infants. St. Louis; Mosby, 1993.
69. Butte NF, Wong WW, Hopkinson JM, Heinz CJ, Mehta NR, Smith EOB. Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. Am J Clin Nutr 2000; 72: 1558–69.
70. Davies PS, Wells JCK, Hinds A, Day JME, Laidlaw A. Total energy expenditure in 9 month and 12 month infants. Eur J Clin Nutr 1997; 51: 249–52.
71. Torun B, Davies PSW, Livingstone MBE, Paolisso M, Sackett R, Spur GB. Energy requirements and dietary energy recommendations for children and adolescents 1 to 18 years old. Eur J Clin Nutr 1996; 50 (Suppl 1): S37–81.
72. Butte NF. Energy requirements of infants. Eur J Clin Nutr 1996; 50 (Suppl 1): S24–S36.
73. Davies PS. Energy requirements for growth and development in infancy. Am J Clin Nutr 1998; 68 (Suppl 4): S939–43.
74. Atladottir H, Thorsdottir I. Energy intake and growth of infants in Iceland – a population with high frequency of breastfeeding and high birth weight. Eur J Clin Nutr 2000; 54: 695–701.
75. Butte NF, Henry CJ, Torun B. Report on the working group on energy requirements of infants, children and adolescents. Eur J Clin Nutr 1996; 50 (Suppl 1): S188–9.

76. Tennefors C, Coward WA, Hernell O, Wright A, Forsum E. Total energy expenditure and physical activity level in healthy young Swedish children 9 or 14 months of age. Eur J Clin Nutr 2003; 57: 647–53.
77. Kylberg E, Hofvander Y and Sjölin S. Diets of healthy Swedish Children 4–24 months old. Energy intake. Acta Pædiatr Scand 1986; 75: 932–6.
78. Axelsson I, Borulf S, Righard L, Räihä N. Protein and energy intake during weaning: I. Effects on growth. Acta Pædiatr Scand 1987; 76: 321–7.
79. Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B, Dewey KG. Intake and growth of breast fed and formula-fed infants in relation to the timing of introduction of complementary foods: the Darling study. Acta Pædiatr 1993; 82: 999–1006.
80. Nielsen GA, Thomsen BL, Michaelsen KF. Influence of breastfeeding and complementary food on growth between 5 and 10 months. Acta Pædiatr 1998; 87: 911–7.
81. Butte NF, Wong WW, Ferlic L, Smith EO, Klein PD, Garza C. Energy expenditure and deposition of breast fed and formula-fed infants during early infancy. Ped Res 1990; 28: 631–40.
82. Nordiska näringsrekommendationer 1996. Utarbetad av Sandström B, Aro A, Becker W *et al.* Nord 1996:28. Köpenhamn: Nordiska ministerrådet 1996.
83. Davies PS. Total energy expenditure in young children. Am J Hum Biol 1996; 8: 183–8.
84. Firouzbakhsh S, Mathis RK, Dorchester WL, Oseas RS, Groncy PK, Grant KE, Finklestein JZ. Measured resting energy expenditure in children. J Pediatr Gastroenterol Nutr 1993; 16: 136–42.
85. Goran MI, Nagy TR, Gower BA, Mazariegos M, Solomons N, Hood V, *et al.* Influence of sex, seasonality, ethnicity, and geographic location on the components of total energy expenditure in young children: implications for energy requirements. Am J Clin Nutr 1998; 68: 675–82.
86. O'Connor J, Ball EJ, Steinbeck KS, Davies PSW, Wishart C, Gaskin KJ, *et al.* Comparison of total energy expenditure and energy intake in children aged 6–9 y. Am J Clin Nutr 2001; 74: 643–9.
87. Ekelund U, Sjöström M, Yngve A, Poortvliet E, Nilsson A, Froberg K, *et al.* Physical activity assessed by activity monitor and doubly labeled water in children. Med Sci Sports Exerc 2001; 33: 275–81.
88. Bratteby LE, Sandhagen B, Fan H, Samuelson G. A 7-day activity diary for assessment of daily energy expenditure validated by the doubly labelled water method in adolescents. Eur J Clin Nutr 1997; 51: 585–91.
89. Bratteby LE, Sandhagen B, Fan H, Enghardt H, Samuelson G. Total energy expenditure and physical activity as assessed by the doubly labelled water method in Swedish adolescents in whom energy intake was underestimated by 7-d diet records. Am J Clin Nutr 1998; 67: 905–11.
90. Ekelund U, Åman J, Yngve A, Renman C, Westerterp K, Sjöström M. Physical activity but not energy expenditure is reduced in obese adolescents: a case control study. Am J Clin Nutr 2002; 76: 935–41.
91. Ekelund U, Sjöström M, Yngve A, Nilsson A. Total daily energy expenditure and pattern of physical activity measured by minute-by-minute heart rate monitoring in 14–15 year old Swedish adolescents. Eur J Clin Nutr 2000; 54: 195–202.
92. Torun B. Energy costs of various physical activities in healthy children. In: Schürch B, Scrimshaw NS, editors. Activity, energy expenditure and energy requirements of infants and children. UN ACC-Subcommittee on Nutrition: International Dietary Energy Consultancy Group (I/D/E/C/G). Proceedings of an I/D/E/C/G Workshop, Cambridge, MS, USA, November 1989. Lausanne, 1990.

93. Andersen E, Hutchings B, Jansen J, Nyholm M. Højde og vægt hos danske børn. Ugeskr Læger 1982; 144: 1760–5.
94. Knudtzon J, Waaler PE, Skjaerven R, Solberg LK, Steen J. Nye norske percentilkurver for høyde, vekt, hodeomkrets for alderen 0–17 år. Tidsskr Nor Laegeforen 1988; 108: 2125–35.
95. Dagbjartsson A, Þórsson ÁV, Pálsson GI, Arnórsson VH. Height and weight of Icelandic children 6–20 years. (Hæð og þyngd íslenskra barna og unglinga 6–20 ára.) Icelandic Med J 2000; 86: 509–14.
96. Briem B. Changes in height and weight of 9 year old school-children in Reykjavík 1919–1998. A thesis for a masters degree in health science, University of Iceland, Faculty of Medicine, 1999.
97. Schmidt IM, Jorgensen MH, Michaelsen KF. Height of conscripts in Europe: is postneonatal mortality a predictor? Ann Hum Biol 1995; 22: 57–67.
98. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (Macronutrients). Institute of Medicine of the National Academies, The National Academies Press, Washington DC 2002.
99. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, *et al.* Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000; 32 (Suppl 9): S498–504.
100. Black AE, Coward WA, Cole TJ, Prentice AM. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. Eur J Clin Nutr 1996; 50: 72–92.
101. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, *et al.* Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 1995; 273: 402–7.
102. Fogelholm M. Diet, physical activity and health in Finnish adolescents in the 1990s. Scand J Nutr 1998; 42: 10–12.
103. Westerterp KR, Saris WH, van Es M, ten Hoor F. Use of the doubly labeled water technique in humans during heavy sustained exercise. J Appl Physiol 1986; 61: 2162–7.
104. Sjödin AM, Andersson AB, Hogberg JM, Westerterp KR. Energy balance in cross-country skiers: a study using doubly labeled water. Med Sci Sports Exerc 1994; 26: 720–4.
105. Kieler H. Increased risk of pregnancy complications and fetal death among obese women. Läkartidningen. 2002; 99: 39–40.
106. Thorsdottir I, Torfadottir JE, Birgisdottir BE, Geirsson RT. Weight gain in women of normal weight before pregnancy: complications in pregnancy or delivery and birth outcome. Obstet Gynecol 2002; 99: 799–806.
107. Eriksson JG, Forsen T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJ. Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 2002; 45: 342–8.
108. Gunnarsdottir I, Birgisdottir BE, Benediktsson R, Gudnason V, Thorsdottir I. Relationship between size at birth and hypertension in a genetically homogenous population of high birth weight. J Hyperten 2002; 20: 623–8.
109. Barker DJ. Fetal programming of coronary heart disease. Trends Endocrinol Metab 2002; 13: 364–8.
110. Gunnlaugsson S, Geirsson RT. Weight gain among Icelandic women in pregnancy. Icelandic Med J 1992; 78: 115–7.
111. Forsum E, Boström K, Eriksson B, Olin-Skoglund S. A woman's weight before and during pregnancy is of importance to her infant. USA guidelines would benefit public health in Sweden. Läkartidningen. 2003; 100: 3954–8.
112. Meeuwisse G, Olausson PO. Increased birth weights in the Nordic

countries. A growing proportion of neonates weigh more than four kilos. Läkartidningen 1998; 95: 5488–92.
113. Thorsdottir I, Birgisdottir B. Different weight gain in women of normal weight before pregnancy: Postpartum weight and birth weight. Obstet Gynecol 1998; 92: 377–83.
114. Butte NF, Wong WW, Treuth MS, Ellis KJ, O'Brian Smith E. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr. 2004; 79: 1078–87.
115. Butte NF, Ellis KJ, Wong WW, Hopkinson JM, Smith EO. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. Am J Obstet Gynecol. 2003; 189: 1423–32
116. Hytten FE. Weight gain in pregnancy. I: Clinical physiology in obstetrics. Hytten FE, Chamberlain G, editors. Oxford: Blackwell Scientific Publications, 1991.
117. Lof M, Forsum E. Activity pattern and energy expenditure due to physical activity before and during pregnancy in healthy Swedish women. Submitted for publication.
118. Prentice AM Spaaj CJK, Poppitt SD, Goldberg GR, van Raaij JMA. Energy requirements of pregnant and lactating women. Eur J Clin Nutr 1996; 50 (Suppl 1): S82–111.
119. Rossner S, Ohlin A. Pregnancy as a risk factor for obesity: lessons from the Stockholm Pregnancy and Weight Development Study. Obes Res 1995; 3 (Suppl 2): 267s–75s.
120. Sadurskis A, Kabir N, Wager J, Forsum E. Energy metabolism, body composition and milk production in healthy Swedish women during lactation. Am J Clin Nutr 1988; 48: 44–9.
121. Sohlström A, Forsum E. Changes in adipose tissue volume and distribution during reproduction in Swedish women as assessed by magnetic resonance imaging. Am J Clin Nutr 1995; 61: 287–95.
122. Dewey KG. Energy and protein requirements during lactation. Annu Rev Nutr 1997; 17: 19–36.
123. Pannemans DLE, Westerterp KR. Energy expenditure, physical activity and basal metabolic rate of elderly subjects. Brit J Nutr 1995; 73: 571–81.
124. Henry CJK. Mechanisms of changes in basal metabolism during ageing. Eur J Clin Nutr 2000; 54 (Suppl 3): S77–91.
125. Flynn MA, Nolph GB, Baker AS, Martin WM, Krause G. Total body potassium in aging humans: a longitudinal study. Am J Clin Nutr 1989; 50: 713–7.
126. Young VR. Energy requirements in the elderly. Nutr Rev 1992; 50: 95–101.
127. Vaughan L, Zurlo F, Ravussin E. Aging and energy expenditure. Am J Clin Nutr 1991; 53: 821–5.
128. Poehlman ET. Energy intake and energy expenditure in the elderly. Am J Hum Biol 1996; 8: 199–206.
129. Puggaard L, Bjornsbo KS, Kock K, Luders K, Thobo-Carlsen B, Lammert O. Age-related decrease in energy expenditure at rest parallels reductions in mass of internal organs. Am J Hum Biol 2002; 14: 486–93.
130. Fukagawa NK, Bandini LG, Young JB. Effect of age on body composition and resting metabolic rate. Am J Physiol 1990; 259: E233–8.
131. Keys A, Taylor HL, Grande F. Basal metabolism and age of adult man. Metabolism 1973; 22: 579–87.
132. Tzankoff SP, Norris AH. Longitudinal changes in basal metabolism in man. J Appl Physiol 1978; 45: 536–9.
133. Poehlman ET. Regulation of energy expenditure in aging humans. Annu Rev Nutr 1990; 10: 255–75.
134. Visser M, Deurenberg P, van Staveren WA, Hautvast JGAJ. Resting metabolic rate and diet-induced thermogenesis in young and elderly subjects: relationship with body composition, fat distribution, and physical activity level. Am J Clin Nutr 1995; 61: 772–8.

135. Rothenberg EM, Bosaeus IG, Westerterp KR, Steen BC. Resting energy expenditure, activity expenditure and total energy expenditure at age 91–96 years. Brit J Nutr 2000; 84: 319–24.
136 Rothenberg EM, Bosaeus IG, Steen BC. Energy expenditure at age 73 and 78 – A five year follow-up. Acta Diabetol 2003; 40: Suppl 1: S134–8.
137. Lowenstein FW. Nutritional status of the Elderly in the United States of America, 1971–1974. J Am Coll Nutr 1982; 1: 165–77.
138. deGroot CPGM, van den Broek T, van Staveren W. Energy intake and micronutrient intake in elderly Europeans: seeking the minimum requirement in the SENECA study. Age Ageing 1999; 28: 469–74.
139. Pedersen AN. 80-åriges ernæringsstatus – og relationen til fysisk funktionsevne. 80-årsundersøgelsen 1994/95. Ph.d-afhandling. Fødevaredirektoratet. Københavns Universitet 2001.
140. FAO. Food energy – methods of analysis and conversion factors. FAO Food and Nutrition Paper 77. Food and Agriculture Organization of the United Nations, Rome, 2003. www.fao.org/es/ESN/eims_search/advanced_s_result.asp.
141. Merrill AL, Watt BK. Energy value of foods – basis and derivation. Washington DC: United States Department of Agriculture, Handbook no. 74, 1954, revideret 1973.
142. Livesey G. Energy from food – old values and new perspectives. Nutr Bull 1988; 13: 9–28.
143. Livesey G, Smith T, Eggum BO, Tetens IH, Nyman M, Roberfroid M, *et al.* Determination of digestible energy values and fermentabilities of dietary fibre supplements: a European interlaboratory study *in vivo*. Br J Nutr 1995; 74: 289–302.

CHAPTER 10

Physical activity

Recommended minimum physical activity in addition to normal inactive living	Minutes per day	Intensity
Adults	30*	Moderate
Children and adolescents	60	Moderate/vigorous

* Somewhat less if the activity is vigorous.

There is a scarcity of data that would allow us to compare the level of energy expenditure in the population historically and at present. Furthermore, physical activity is often defined differently in different studies, making comparable analysis difficult. However, the impression that our habitual physical activity level has gradually decreased is supported by studies showing that both average weight and the percentage of women and men in the Nordic countries who are overweight/obese has increased over recent decades (1–4), while results from nutritional surveys imply that the energy intake in the adult population remained relatively stable from the mid-1970s until 1997 (5, 6). This trend is most likely due to structural changes in society, which have resulted in a decrease in physical activity in daily life. As a result, large segments of the population can be characterized as physically inactive.

Physical inactivity is linked to a number of diseases and disorders (for instance certain types of cancer, hypertension, type 2 diabetes, cardiovascular disease and osteoporosis) and the effects of regular physical activity are in many cases similar to those of a well-balanced diet. Changes in physical activity and diet can have an additive effect on these diseases and conditions, while public health strategies also target the same group for the same reason. Furthermore, if diet and physical activity are separated, people may view them as alternatives and hence the additive value of both activity and a wholesome diet as components of a healthy lifestyle is not so easily recognised. Therefore, NNR 2004 integrate nutrition and physical activity.

Definitions

Physical activity is a comprehensive concept that encompasses many terms related to movement of the body. It is defined as any bodily move-

ment achieved by contraction of skeletal muscles that increases energy expenditure (EE) above resting levels (7). *Physical inactivity* equals low levels of physical activity and may be defined as a state where EE approaches resting energy expenditure (REE). Accordingly, people who are sedentary both at work and in their leisure time, who sit or lie a lot and who largely use motorised transportation would be characterised as physically inactive. *Exercise* is planned, structured, and repetitive bodily movement carried out to improve or maintain one or more components of physical fitness (8). *Physical fitness* is a set of attributes related to the ability to perform physical activity that people have or achieve (8). The term includes cardiorespiratory fitness, strength, coordination, flexibility etc. *Cardiorespiratory* fitness relates to the ability of the circulatory and respiratory systems to supply oxygen during sustained physical activity (8). MET (metabolic equivalent) is a unit used to estimate the metabolic cost (oxygen consumption) of physical activity. One MET equals the resting metabolic rate of approximately 3.5 ml O_2/kg and minute. *Light activity* is defined as activity corresponding to an EE below 3 METs, such as fidgeting while sitting or walking slowly (< 3.5 km/h). *Moderate physical activity* is defined as activity that requires three to six times as much energy as the energy needed in a resting state. *Vigorous physical activity* is activity requiring more than six METs. *Resistance training* is exercise designed to increase strength, power and muscle endurance. *Endurance training* is repetitive, aerobic use of large muscles (*e.g.* swimming, walking, bicycling).

Physical activity in the prevention of various diseases

Cardiovascular disease, metabolic syndrome and type 2 diabetes

Several studies show an inverse relationship between coronary heart disease (CHD) and physical activity (9–14) or physical fitness (15–18). This applies to both men and women. People who are physically inactive run twice as great a risk of developing CHD as those who are physically active (19). This is probably an underestimation due to dilution of relative risk (20). The documentation establishing a correlation between physical activity or physical fitness and CHD is convincing, and there seems to be a clear dose-response relationship. Paffenbarger *et al.* demonstrated that those who had an extra energy expenditure of 4–8 MJ (500–1,000 kcal) per week had a 22% lower mortality compared to a group who were physically inactive (21). Leon *et al.* showed that people who were regularly physically active for 30 minutes a day during their leisure time, corresponding to an energy expenditure of 150 kcal (630

kJ), had a 36% lower risk of dying from CHD adjusted for other important CHD risk factors (12). Estimates from Finland (22) indicate that approximately 22–39% of CHD death can be attributed to physical inactivity. The same source shows that physical inactivity is just as important a risk factor as smoking, high serum total cholesterol level or hypertension.

Regular physical activity/physical fitness is favourably associated with plasma lipids (triglycerides, HDL- and LDL-cholesterol) (23, 24) blood pressure (25), insulin sensitivity (26) haemostasis/fibrinolysis (23, 27), and endothelial function (28). Increased physical activity has the potential to influence all these factors in a favourable direction at the same time. The effect 'size' and the amount of physical activity needed to improve these factors are not fully outlined. However, data with respect to plasma lipids, blood pressure and insulin sensitivity are available.

The average expected changes in lipids and lipoproteins following increased physical activity are: An increase in HDL cholesterol of 4.6%, a reduction in LDL-cholesterol of 3.7% and in triglycerides of 5% (29). There is also evidence of a beneficial effect on LDL sub-classes (24). The baseline levels strongly influence the effect of physical activity in that greater beneficial effects are seen in those with poor lipoprotein profile. The improvements are probably more related to the amount of activity and not to the intensity or improvement in cardiorespiratory fitness (24).

A meta-analysis of randomised controlled trials has indicated that the effect of physical activity on systolic/diastolic blood pressure reduction is on average 3/2 mm Hg in normo-tensive and 8/6 mm Hg in hypertensive groups (25). Moderate physical activity on three to five occasions per week with a duration of 30–60 minutes seems to be effective in blood pressure reduction. Physical activity with higher intensity does not seem to be more effective.

There is strong evidence that regular physical activity has a beneficial effect on insulin sensitivity (26). Prospective studies have shown that regular physical activity brings about a linear decrease in the age-adjusted risk of developing type 2 diabetes (30–32). The decrease is in the magnitude of 6% for each 4.1 MJ (500 kcal) expended by physical activity in weekly leisure time (32). The documentation is convincing. It appears that those who are at greatest risk of developing type 2 diabetes benefit the most from regular physical activity (31). The effect of physical activity in patients with type 2 diabetes is conflicting (33), although a meta-analysis concludes that exercise training reduces HbA1c by an amount that should decrease the risk of diabetic complications (34).

Overweight and obesity

Physical activity has profound effects on body composition and metabolism. It increases EE and helps to maintain and increase muscle mass, which may result in an increased basal metabolism and an increased capacity for mobilising and burning fat both while using the muscles and in a resting state (35, 36). Thus, regular physical activity is likely to be of importance in long-term regulation of body weight. Regular physical activity is important for obese people, as health benefits can be achieved through improved physical fitness, regardless of weight loss (37). The mortality and morbidity of being overweight are substantially reduced in people who, despite being overweight, are physically fit (38). Only in short-term studies (16 weeks or shorter duration) is it possible to find evidence of a linear dose-response relationship between the volume of physical activity and the amount of weight loss when diet is controlled. The amount of weight loss is consistent with the excess energy expended (39). In practice, only modest weight loss of around 3 kg might be expected following increased physical activity in obese persons (40). Even though there is a lack of conclusive data, it seems that the amount of activity needed to avoid weight gain is about 60 minutes of moderate intensity or somewhat less of vigorous intensity activity (41).

Cancer

The five types of cancer (colon (42), breast (43–46), prostate, lung and endometrial (47)) for which a convincing or at least a possible relationship between physical activity and cancer has been established comprise a total of 45% of all incidences of cancer. The risk reduction for the most active individuals in relation to inactive groups is about 50% for colon cancer (48). There is evidence of a dose-response relationship between physical activity and colon cancer and pre- and postmenopausal breast cancer (49). It is not possible to determine the shape of the dose-response curve, but the data confirm that moderate activity is effective. The significance of physical activity in the treatment or rehabilitation of cancer patients is unclear.

Musculo-skeletal disorders

Among the many reversible risk factors for falls are muscle weakness in the limbs, poor balance and a poor level of overall physical fitness. These are factors that can be improved through regular physical activity, for instance strengthening exercises (50–52).

Muscle strength and muscle endurance diminish with increasing age and decreasing activity level (53). Physical activity can counter and reverse this trend to a substantial degree (51, 54). Another benefit is fewer muscle complaints in all age groups.

Loss of calcium may lead to osteoporosis. This risk increases with age, particularly in post-menopausal women. Physical activity contributes to increased bone density and can thus counteract osteoporosis. Physical activity during puberty seems to be particularly useful for bone mass and structure (55, 56). Physical activity immediately before and during puberty yields greater maximum bone density in adult life (56–59). For adults and the elderly, physical activity retards bone loss (60). To be beneficial for bone mass and structure, exercise should preferably be weight-bearing (61). Repeated weight-bearing loading, such as walking and running, is more beneficial than *e.g.* swimming and cycling. Even better for bone health are activities with high and odd impacts (*e.g.* tennis, squash, aerobics) or high volume loading (weight training). However, there is a lack of information about the dose-response relationship for osteoporosis (61).

Strengthening exercises – targeting the muscles that stabilize the back – reduce the incidence of back problems, particularly in people with a history of back problems, but also to a certain degree among those who have not previously experienced such problems (62). Regular physical activity may have a preventive effect on low back pain, although the type of the activity has yet to be determined (61).

Mental health and quality of life

A positive association is found between physical activity habits and self-esteem and psychological well-being in children and young and middle-aged adults (8). Furthermore, observational studies have shown that those who are physically inactive are at greater risk of developing depression than those who are physically active (63, 64). However there is no dose-response relationship between physical activity and depression and anxiety (65). Further research is needed to study the volume and mode of physical activity that is most psychologically beneficial and to explore the mechanisms by which physical activity improves mental health.

Recommendations on physical activity

There is strong evidence that vigorous physical activity sufficient to improve cardio-respiratory fitness has a major impact on different health outcomes (8). As a matter of fact, until recently the recommenda-

tions on physical activity were equal to the quantity and quality of exercise sufficient to develop and maintain cardiorespiratory fitness. However, as previously described in this chapter, experimental studies have established that activity of a moderate nature, which does not yield an increase in cardio-respiratory fitness, also produces a favourable effect on several risk factors for CHD and type 2 diabetes (8, 66). Therefore it is important to point out that substantial health gains can be achieved through moderate physical activity. The recommendations should therefore include both modalities of physical activity. Examples of energy requirements corresponding to 3–6 METs (moderate activity) and > 6 METs (vigorous activity) are given in *Table 10.1.* Maximal oxygen uptake decreases as people age and also as a consequence of physical inactivity. Activity of a certain MET value therefore requires a greater percentage of a person's maximal oxygen uptake (*Table 10.1*) as she or he ages. Note that activity of a certain energy cost may be perceived differently by different groups. For instance climbing stairs may be perceived as light activity for a 30-year-old but hard for a 70-year-old.

Table 10.1 Energy requirements for performing various activities in different age groups shown as METs and as percentages of cardio-respiratory fitness (≈ maximal oxygen uptake) *

		Energy requirements as percentages of cardio-respiratory fitness (≈ maximal oxygen uptake)			
Activities	Energy cost	Young	Middle-aged	Old	Very old
	in METs	20–39 y	40–59 y	60–79 y	80+ y
Watching TV/reading	1.3	10	13	15	18
Light household work	2.5	20	25	29	35
Driving car	1.5	12	15	18	21
Moderate physical activity					
Playing with small children	3.5	27	35	41	49
Climbing stairs	5.5	42	55	64	77
Walking (4.8 km/h)	3.5	27	35	41	49
Walking (6.4 km/h)	4.0	31	40	46	56
Snow clearing (snow blower)	3.0	23	30	35	42
Snow clearing (manual)	6.0	47	60	70	84
Lawn mowing (manual)	4.5	35	45	53	63
Vigorous physical activity					
Lifting or carrying 11–20 kg	8.0	62	80	93	>100
Jogging 8.0 km/h	7.0	55	80	93	>100

* Activity of a certain energy cost may be perceived differently by people both as a function of age and physical inactivity. For instance climbing stairs may be perceived as light activity for a 30-year-old and hard for a 70-year-old.

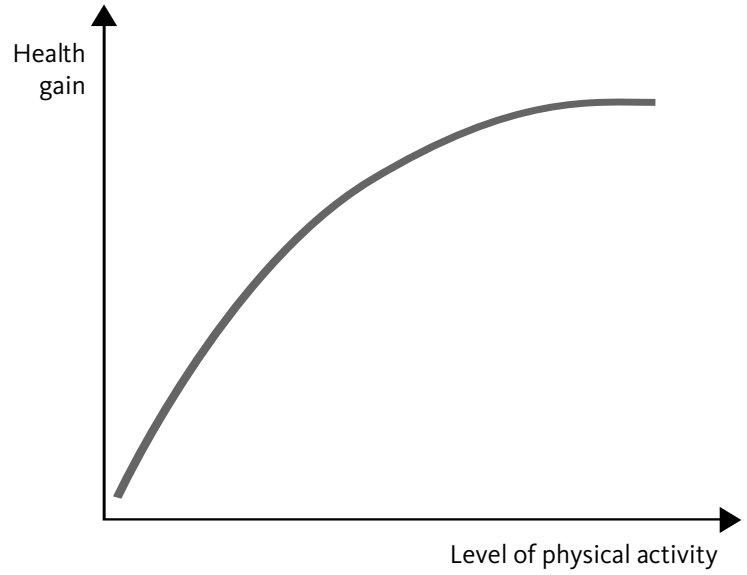

Figure 10.1 Dose-response curve for physical activity and health (69). Different health outcomes probably have different dose-response relationships.

The total amount of physical activity (a combination of intensity, duration and frequency) is related to a number of health variables in a dose-response relationship. The preventive effect (the health gain) increases with increasing activity level, but the relationship is not linear (*Figure 10.1*). Those who are physically inactive may achieve the greatest health gains. This applies even in old age (8, 11, 67). The health gain seems primarily to be dependent on the total energy expenditure, and probably less on the intensity. Another aspect is whether several short bouts of activity are as effective in influencing health outcome as one longer session of the same total duration (68).

The question of how much physical activity is needed to yield health gains is not easily answered, as the answer depends on the group of people in question: the young, older people, overweight individuals, etc. The dose-response relationship between physical activity level and health gains is a continuum that does not seem to have a lower limit. It is important, however, to keep in mind that the different health outcomes probably have different dose-response relationships and also depend on the type of activity.

Children and adolescents

Regular physical activity is necessary for normal growth and the development of cardio-respiratory endurance, muscle strength, flexibility, motor skills and agility (70–74). In addition, physical activity during the formative years strengthens the bones and connective tissues and yields greater maximum bone density in adult life (70, 75, 76). Exercise that gives a high impact loading on bones is important for bone development, particularly during early puberty (55). There is also evidence of an association between cardiorespiratory fitness and cardiovascular disease risk factors in children and adolescents. Furthermore, risk factors such as fatness, insulin : glucose ratio and lipids cluster in children and adolescents with low cardiorespiratory fitness (77).

Regular physical activity is associated with well-being and seems to promote self-esteem in children and adolescents. Furthermore, children and adolescents who are involved in physical activity seem to experience fewer mental health problems (78–81). There is no indication that increased physical activity in school represents any risk of impairing children's cognitive skills as a result of less time for theoretical school subjects (82).

There is little doubt about the health effects of physical activity in children and adolescents. However, the amount of physical activity (including intensity and duration) required for enhancing and maintaining normal development and health in children and young people is rather unknown. Nevertheless the following is recommended for children and adolescents:

- There should be a minimum of 60 minutes of physical activity every day. The activity should include both moderate and vigorous intensity.
- The activity can probably be divided into shorter intervals of physical activity during the course of the day.
- Activities should be as diverse as possible in order to provide optimal opportunities for developing all aspects of physical fitness including cardiorespiratory fitness, muscle strength, flexibility, speed, mobility, reaction time and coordination.

Varied physical activity provides opportunities to develop both fine-motor and gross-motor skills. Active children get the exercise they need while playing in the neighbourhood, at day-care, or on the school playground and by participating in children's sports.

Adults

Following the previous literature review and comments, we can conclude that a 'target dose' that will yield health gains for adults who have been physically inactive for a long time is activity of moderate intensity which corresponds to an additional energy expenditure of approximately 630 kJ (150 kcal) per day (or slightly more than 4.2 MJ (1,000 kcal) per week). An equal health impact can be expected with vigorous activity (such as jogging, cross-country skiing, swimming, circuit training, resistance training, etc.) of shorter duration but equal energy expenditure. Energy expenditure exceeding this 'target dose' yields further health gains. Perhaps the optimal health effects can be expected from the combination of the two modalities; that is 2–3 hours of vigorous exercise per week and daily moderate physical activity corresponding to a total of 8.4 MJ or 2,000 kcal per week (see *Figure 10.2*). The recommendations on physical activity for adults are:

- For inactive adults, daily physical activity of moderate and/or vigorous intensity corresponding to an energy expenditure of about 630 kJ (150 kcal) yields substantial health benefits. This should be in addition to the energy expenditure through normal inactive living. This is equivalent to walking briskly for about 30 minutes.
- The activity can probably be divided into shorter intervals of physical activity during the course of the day, for instance intervals lasting 10 minutes.
- An increase in activity beyond this duration and intensity will yield additional benefits, see *Figures 10.1* and *10.2.* (*Tables 10.2* and *10.3* provide examples of the duration required when engaging in various activities to achieve energy expenditure of about 630 kJ.)
- More physical activity (about 60 min daily) with a moderate and/or vigorous intensity corresponding to an energy expenditure of 1,300 kJ (300 kcal) may be needed for prevention of weight gain.

Elderly

Regular physical activity in elderly people is associated with improved strength and functional ability (83) and inversely related to mortality (84), and was strongly associated with maintaining mobility during a 4-year follow up (85).

Endurance training in the elderly has been found to improve oxygen consumption (VO_2 max) by approximately 23% in a meta-analysis (86). Hard endurance training results in improved VO_2 max, increased muscle mass, unchanged body weight and unchanged daily energy expendi-

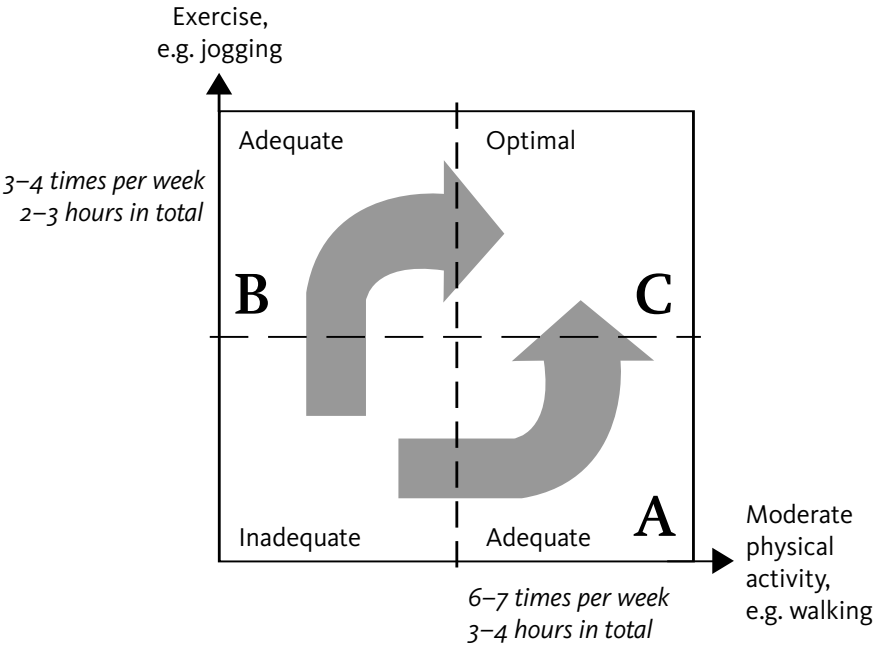

Figure 10.2 Two modalities of physical activity adequate to give health benefits:

A) Physical activity of moderate intensity, for instance walking, household work, playing, with a frequency of 6–7 times per week and a total of 3–4 hours a week;

B) Exercise of moderate to vigorous intensity, for instance jogging, swimming, tennis, resistance training, circuit training, cross-country skiing, with a frequency of 3–4 times a week and a total of 2–3 hours a week.

C) The optimal activity dose may be the combination of a) and b) (both moderate physical activity and moderate to vigorous exercise).

ture because of a compensatory decline in physical activity during the remainder of the day (87, 88) while moderate endurance training increases basal energy expenditure, daily energy expenditure and total energy intake (89).

Resistance training increases basal energy expenditure, muscle mass (67, 90), and daily energy expenditure in the elderly (91) and may counteract the age-related accumulation of fat (92). Frequency of high-resistance training may be less than 3 times a week (93). Low-intensity exercise may be beneficial in the institutionalised elderly (94) and effects of resistance training have been seen even in 85–97 year-old subjects (95).

Table 10.2 Duration of various activities to expend 630 kJ (150 kcal) for an average 77 kg man. The larger the bodyweight, the higher energy expenditure per unit time at equal absolute intensity and vice versa

Intensity	Activity	METs	Approximate duration (minutes)
Moderate	Walking 4.8 km/h	3.5	33
Moderate	Walking 6.4 km/h	4.0	29
Moderate	Bicycling 12 km/h	4.0	29
Moderate	Table tennis	4.0	29
Moderate	Raking leaves	4.5	26
Moderate	Lawn mowing (manual)	4.5	26
Vigorous	Jogging 8.0 km/h	7.0	17
Vigorous	Bicycling 22 km/h	8.0	15
Vigorous	Running 9.7 km/h	10.0	12

Table 10.3 Duration of various activities to expend 630 kJ (150 kcal) for an average 63 kg woman. The larger the bodyweight, the higher energy expenditure per unit time at equal absolute intensity and vice versa

Intensity	Activity	METs	Approximate duration (minutes)
Moderate	Walking 4.8 km/h	3.5	40
Moderate	Walking 6.4 km/h	4.0	36
Moderate	Bicycling 12 km/h	4.0	36
Moderate	Table tennis	4.0	36
Moderate	Raking leaves	4.5	32
Moderate	Lawn mowing (manual)	4.5	32
Vigorous	Jogging 8.0 km/h	7.0	20
Vigorous	Bicycling 22 km/h	8.0	18
Vigorous	Running 9.7 km/h	10.0	14

Healthy elderly people can largely use the recommendations for the adult population at large. This particularly applies to the advice to become more physically active in daily life.

The following recommendations apply:

- Those who have been physically inactive for a long period should start with a programme consisting of walking. The intensity should be increased gradually.
- The intensity can be increased by climbing stairs or hills of increasing steepness, preferably on uneven terrain (which is an advantage for improving balance). Hiking, skiing and orienteering are well suited.

Other forms of aerobic exercise which can be engaged in as an alternative to walking include swimming and other water activities, various types of dance, cycling, rowing, exercise bicycle or rowing ergometers, etc.

Since resistance training is particularly valuable in restoring lost muscle strength, a varied, progressive programme of weight training is recommended for older people, particularly if the muscle condition and/or mass have decreased. Strengthening exercises should be tailored to the needs of the individual with regard to types of exercises, number of sets, repetitions and frequency of training sessions. Strengthening exercises should optimally be combined with aerobic, balance and mobility training.

Pregnancy and lactation

Pregnancy is associated with extensive physiological and anatomical changes. Despite this fact, regular physical activity or exercise has minimal risk and confirmed benefits for most women (96). Women who are moderately physically active during pregnancy experience easier pregnancies and deliveries, have better self-esteem, gain less weight, have more normal deliveries and fewer perinatal complications than women who have not engaged in physical activity during their pregnancy (97–99). There is no evidence of increased risk of miscarriage, premature delivery or foetal growth retardation due to exercise during pregnancy (100–102). Except for complicated pregnancies and a few circumstances in which exercise is contraindicated (see Artal & O'Toole (96) for details), the following recommendations apply:

- Women who have previously not been physically active should engage in moderate physical activity during pregnancy with a gradual progression of up to 30 minutes a day.
- Women who are regular exercisers before pregnancy should continue to engage in physical activity at an appropriate level. They should be able to engage in vigorous intensity exercise, such as jogging, swimming and aerobics.
- Pregnant women must avoid getting overheated. This means that they should be cautious when engaging in prolonged moderate or vigorous physical activity (in excess of 45 minutes of continuous activity) when it is too hot. The concerns related to overheating may be eliminated by performing the activity in shorter bouts, such as 15-minute periods.
- Training the muscles of the pelvic floor is particularly important during pregnancy and after giving birth.

- Activities with a high risk of falling (such as horseback riding, downhill skiing) and activities that include contact sports (such as handball, basketball, ice hockey) may increase the risk of trauma and should be considered undesirable. Scuba diving should be avoided throughout the pregnancy.
- Women who have had an uncomplicated pregnancy can begin moderate physical activity such as walking immediately after delivery. Most women can resume normal physical activity 6–8 weeks after delivery. The recommendations for adults generally apply for breastfeeding women. However, they are advised to nurse before vigorous exercise (96).

REFERENCES

1. Tverdal A. [Height, weight and body mass index of men and women aged 40–42 years]. Tidsskr Nor Laegeforen 1996; 116(18): 2152–2156.
2. Heitmann BL. Ten-year trends in overweight and obesity among Danish men and women aged 30–60 years. Int J Obes Relat Metab Disord 2000; 24(10): 1347–1352.
3. Lahti-Koski M, Vartiainen E, Mannisto S, Pietinen P. Age, education and occupation as determinants of trends in body mass index in Finland from 1982 to 1997. Int J Obes Relat Metab Disord 2000; 24 (12): 1669–1676.
4. Lissner L, Bjorkelund C, Heitmann BL, Lapidus L, Bjorntorp P, Bengtsson C. Secular increases in waist-hip ratio among Swedish women. Int J Obes Relat Metab Disord 1998; 22 (11): 1116–1120.
5 Utvikling av norsk kosthold. 1999. Statens ernæringsråd.
6. Fogelholm M, Mannisto S, Vartiainen E, Pietinen P. Determinants of energy balance and overweight in Finland 1982 and 1992. Int J Obes Relat Metab Disord 1996; 20 (12): 1097–1104.
7. Bouchard C, Shephard RJ. Physical activity, fitness, and health: The model and key concepts. In: Bouchard C, Shephard RJ, Stephens T, editors. Physical activity, fitness, and health. Consensus statement. Champaign: Human Kinetics, 1993: 11–23.
8. US Department of Health and Human services. Physical activity and health: A Report of the Surgeon General. 1–278. 1996. Atlanta GA, Centers for Disease Control and Prevention.
9. Andersen LB, Schnohr P, Schroll M, Hein HO. All-cause mortality associated with physical activity during leisure time, work, sports, and cycling to work. Arch Intern Med 2000; 160 (11): 1621–1628.
10. Paffenbarger RS Jr, Hyde RT, Wing AL, Hsieh C-C. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986; 314: 605–613.
11. Morris JN, Pollard R, Everitt MG, Chave SPW. Vigorous exercise in leisure-time: protection against coronary heart disease. Lancet 1980; 1207–1210.
12. Leon AS, Connett J, Jacobs DR Jr, Rauramaa R. Leisure-time physical activity levels and risk of coronary heart disease and death. The Multiple Risk Factor Intervention Trial. JAMA 1987; 256: 2388–2395.
13. Lee I-M, Hsieh C-C, Paffenbarger RS Jr. Exercise intensity and longevity in men. The Harvard Alumni Health Study. JAMA 1995; 273: 1179–1184.

14. Manson JE, Hu FB, Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC *et al.* A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. N Engl J Med 1999; 341: 650–658.
15. Hein HO, Suadicani P, Gyntelberg F. Physical fitness or physical activity as a predictor of ischaemic heart disease? A 17-year follow-up in the Copenhagen Male Study. J Intern Med 1992; 232: 471–479.
16. Sandvik L, Erikssen J, Thaulow E, Erikssen G, Mundal R, Rodahl K. Physical fitness as a predictor of mortality among healthy, middle-aged Norwegian men. N Engl J Med 1993; 328: 533–537.
17. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality A prospective study of healthy men and women. JAMA 1989; 262: 2395–2401.
18. Blair SN, Kohl HW, Barlow CE. Physical activity, physical fitness, and all-cause mortaility in women: Do women need to be active? Journal of the American College of Nutrition 1993; 12: 368–371.
19. Powell KE, Thompson PD, Caspersen CJ, Kendrick JS. Physical activity and the incidence of coronary heart disease. Ann Rev Public Health 1987; 8: 253–287.
20. Andersen LB. Relative risk of mortality in the physically inactive is underestimated because of real changes in exposure level during follow-up. Am J Epidemiol 2004; 160 (2): 189–195.
21 Paffenbarger RS Jr, Hyde RT, Wing AL, Hsieh CC. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986; 314 (10): 605–613.
22. Haapanen-Niemi N, Vuori I, Pasanen M. Public health burden of coronary heart disease risk factors among middle-aged and elderly men. Prev Med 1999; 28 (4): 343–348.
23. Anderssen SA, Haaland A, Hjermann I, Urdal P, Gjesdal K, Holme I. Oslo Diet and Exercise Study: A one year randomized intervention trial; effect on hemostatic variables and other coronary risk factors. Nutrition, Metabolism and Cardiovascular Diseases 1995; 5: 189–200.
24. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS *et al.* Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 2002; 347 (19): 1483–1492.
25. Fagard RH. Exercise characteristics and the blood pressure response to dynamic physical training. Med Sci Sports Exerc 2001; 33 (6 Suppl): S484–S492.
26. Borghouts LB, Keizer HA. Exercise and insulin sensitivity: a review. Int J Sports Med 2000; 21 (1): 1–12.
27. Rauramaa R, Salonen JT, Seppänen K, Salonen R, Venäläinen JM, Ihanainen M *et al.* Inhibition of platelet aggregability by moderate-intensity physical exercise: a randomized clinical trial in overweight men. Circulation 1986; 74: 939–944.
28. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S *et al.* Effect of exercise on coronary endothelial function in patients with coronary artery disease. N Engl J Med 2000; 342: 454–460.
29. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc 2001; 33 (6 Suppl): S502–S515.
30. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS *et al.* Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991; 338: 774–778.
31. Pan X-R, Li G-W, Hu Y-H, Wang J-X, Yang W-Y, An Z-X *et al.* Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolererance. The Da Qing IGT and Diabetes Study. Diabetes Care 1997; 20: 537–544.
32. Helmrich SP, Ragland DR, Leung RW, Paffenbarger RS. Physical activity and reduced occurrence of non-insulin-

dependent diabetes mellitus. N Engl J Med 1991; 325: 147–152.
33. Ivy JL. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. Sports Med 1997; 24: 321–336.
34. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA 2001; 286 (10): 1218–1227.
35. Martin WH, III, Dalsky GP, Hurley BF, Matthews DE, Bier DM, Hagberg JM *et al.* Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. Am J Physiol 1993; 265 (5 Pt 1): E708–E714.
36. Kiens B. Effect of endurance training on fatty acid metabolism: local adaptations. Med Sci Sports Exerc 1997; 29 (5): 640–645.
37. Blair SN, Brodney S. Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues. Med Sci Sports Exerc 1999; 31 (11 Suppl): S646–S662.
38- Lee CD, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. Am J Clin Nutr 1999; 69: 373–380.
39. Ross R, Janssen I. Physical activity, total and regional obesity: dose-response considerations. Med Sci Sports Exerc 2001; 33 (6 Suppl): S521–S527.
40. Grilo CM. The role of physical activity in weight loss and weight loss management. Med Exerc Nutr Health 1995; 4: 60–67.
41. Saris WH, Blair SN, van Baak MA, Eaton SB, Davies PS, Di Pietro L *et al.* How much physical activity is enough to prevent unhealthy weight gain? Outcome of the IASO 1st Stock Conference and consensus statement. Obes Rev 2003; 4 (2): 101–114.
42. Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma KN *et al.* Energy balance and colon cancer – beyond physical activity. Cancer Res 1997; 57 (1): 75–80.
43. Friedenreich CM, Thune I, Brinton LA, Albanes D. Epidemiologic issues related to the association between physical activity and breast cancer. Cancer 1998; 83 (3 Suppl): 600–610.
44. Bernstein L, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK. Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 1994; 86 (18): 1403–1408.
45. Carpenter CL, Ross RK, Paganini-Hill A, Bernstein L. Lifetime exercise activity and breast cancer risk among post-menopausal women. Br J Cancer 1999; 80 (11): 1852–1858.
46. Hoffman-Goetz L, Apter D, Demark-Wahnefried W, Goran MI, McTiernan A, Reichman ME. Possible mechanisms mediating an association between physical activity and breast cancer. Cancer 1998; 83 (3 Suppl): 621–628.
47. Thune I. Physical activity and risk of cancer. A population based cohort study including prostate, testicular, colorectal, lung, and breast cancer. Institute of Community Medicine, University of Tromsø, 1997. Thesis.
48. Shephard RJ, Futcher R. Physical activity and cancer: how may protection be maximized? Crit Rev Oncog 1997; 8 (2–3): 219–272.
49. Thune I, Furberg AS. Physical activity and cancer risk: dose-response and cancer, all sites and site-specific. Med Sci Sports Exerc 2001; 33 (6 Suppl): S530–S550.
50. Evans WJ. Effects of exercise on body composition and functional capacity of the elderly. J Gerontol A Biol Sci Med Sci 1995; 50 Spec No: 147–150.
51. Meuleman JR, Brechue WF, Kubilis PS, Lowenthal DT. Exercise training in the debilitated aged: strength and functional outcomes. Arch Phys Med Rehabil 2000; 81 (3): 312–318.
52. Sinaki M, Wahner HW, Bergstralh EJ, Hodgson SF, Offord KP, Squires RW *et al.* Three-year controlled, randomized trial of the effect of dose-specified loading and strengthening exercises on bone mineral density of spine and femur in nonathletic,

physically active women. Bone 1996; 19 (3): 233–244.
53. Aniansson A, Grimby G, Rundgren A. Isometric and isokinetic quadriceps muscle strength in 70-year-old men and women. Scand J Rehabil Med 1980; 12 (4): 161–168.
54. Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C *et al.* Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. Acta Physiol Scand 1990; 140 (1): 41–54.
55. Kannus P, Haapasalo H, Sankelo M, Sievanen H, Pasanen M, Heinonen A *et al.* Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med 1995; 123 (1): 27–31.
56. Haapasalo H, Kannus P, Sievanen H, Pasanen M, Uusi-Rasi K, Heinonen A *et al.* Development of mass, density, and estimated mechanical characteristics of bones in Caucasian females. J Bone Miner Res 1996; 11 (11): 1751–1760.
57. Kirchner EM, Lewis RD, O'Connor PJ. Effect of past gymnastics participation on adult bone mass. J Appl Physiol 1996; 80 (1): 226–232.
58. Nichols DL, Sanborn CF, Bonnick SL, Ben Ezra V, Gench B, DiMarco NM. The effects of gymnastics training on bone mineral density. Med Sci Sports Exerc 1994; 26 (10): 1220–1225.
59. Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G. Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 1993; 123 (6): 863–870.
60. Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ, Jr. Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. Ann Intern Med 1988; 108 (6): 824–828.
61. Vuori IM. Dose-response of physical activity and low back pain, osteoarthritis, and osteoporosis. Med Sci Sports Exerc 2001; 33 (6 Suppl): S551–S586.
62. Lahad A, Malter AD, Berg AO, Deyo RA. The effectiveness of four interventions for the prevention of low back pain. JAMA 1994; 272 (16): 1286–1291.
63. Farmer ME, Locke BZ, Moscicki EK, Dannenberg AL, Larson DB, Radloff LS. Physical activity and depressive symptoms: the NHANES I Epidemiologic Follow-up Study. Am J Epidemiol 1988; 128 (6): 1340–1351.
64. Camacho TC, Roberts RE, Lazarus NB, Kaplan GA, Cohen RD. Physical activity and depression: evidence from the Alameda County Study. Am J Epidemiol 1991; 134 (2): 220–231.
65. Dunn AL, Trivedi MH, O'Neal HA. Physical activity dose-response effects on outcomes of depression and anxiety. Med Sci Sports Exerc 2001; 33 (6 Suppl): S587–S597.
66. Després J-P, Lamarche B. Low-intensity endurance exercise training, plasma lipoproteins and the risk of coronary heart disease. Journal of Internal Medicine 1994; 236: 7–22.
67. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME *et al.* Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994; 330 (25): 1769–1775.
68. Hardman AE. Issues of fractionization of exercise (short *vs* long bouts). Med Sci Sports Exerc 2001; 33: 421–427.
69. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C *et al.* Physical activity and public health. A recommendation from centers for disease control and prevention and the American College of Sports Medicine. JAMA 1995; 273: 402–407.
70. Malina RM, Bouchard C. Growth, maturation and physical activity. Champaign III, Human Kinetics, 1991.
71. Rowland TW. Developmental physical activity. Champaign III, Human Kinetics, 1996.

72. Armstrong N. Young people and physical activity. Oxford: Oxford University Press, 1997.
73. Thorstensson A. Muskelstyrka och träningsbarhet hos barn och ungdom. Barn, ungdom och idrott. Malmö: Idrottens Forskningsråd och Sveriges Riksidrottsförbund, 1990: 167–180.
74. Blimkie CRJ, Bar-Or O. Trainability of muscle strength, power and endurance during childhood. In: Bar-Or O, editor. The child and adolescent athlete. (The encyclopedia of sports medicine, bd. 6.) 1996: 122–123.
75. Baily AB. The role of physical activity in the regulation of bone mass during growth. In: Bar-Or O, editor. The child and adolescent athlete. (The encyclopedia of sports medicine, bd. 6.) 1996: 138–152.
76. Inbar O. Development of anaerobic power and muscular endurance. In: Bar-Or O, editor. The child and adolescent athlete. Champaign III, International Olympic Committee, 1996: 42–53.
77. Wedderkopp N, Froberg K, Hansen HS, Riddoch C, Andersen LB. Cardiovascular risk factors cluster in children and adolescents with low physical fitness: The European Youth Heart Study (EYHS). Pediatric Exercise Science 2003; 15 (4): 419–427.
78. Fox KR. The influence of physical activity on mental well-being. Public Health Nutr 1999; 2 (3A): 411–418.
79. Ommundsen Y, Vaglum P. Sport specific influences. Impact on persistence in soccer among adolescent antisocial players. J of Adolescent Research 1992; 7: 507–521.
80. Calfas KJ, Taylor WC. Effects of Physical-Activity on Psychological Variables in Adolescents. Pediatric Exercise Science 1994; 6 (4): 406–423.
81. Steptoe A, Butler N. Sports participation and emotional wellbeing in adolescents. Lancet 1996; 347: 1789–1792.
82. Shepard R. Curricular physical activity and academic performance. Pediatric Exercise Science 1997; 9: 113–126.
83. Ettinger WH, Jr. Physical activity and older people: a walk a day keeps the doctor away. J Am Geriatr Soc 1996; 44 (2): 207–208.
84. Schroll M, Avlund K, Davidsen M. Predictors of five-year functional ability in a longitudinal survey of men and women aged 75 to 80. The 1914-population in Glostrup, Denmark. Aging (Milano) 1997; 9 (1–2): 143–152.
85. LaCroix AZ, Guralnik JM, Berkman LF, Wallace RB, Satterfield S. Maintaining mobility in late life. II. Smoking, alcohol consumption, physical activity, and body mass index. Am J Epidemiol 1993; 137 (8): 858–869.
86. Green JS, Crouse SF. The effects of endurance training on functional capacity in the elderly: a meta-analysis. Med Sci Sports Exerc 1995; 27 (6): 920–926.
87. Goran MI, Poehlman ET. Endurance training does not enhance total energy expenditure in healthy elderly persons. Am J Physiol 1992; 263 (5 Pt 1): E950–E957.
88. Morio B, Montaurier C, Pickering G, Ritz P, Fellmann N, Coudert J *et al.* Effects of 14 weeks of progressive endurance training on energy expenditure in elderly people. Br J Nutr 1998; 80 (6): 511–519.
89. Poehlman ET, Gardner AW, Goran MI. Influence of endurance training on energy intake, norepinephrine kinetics, and metabolic rate in older individuals. Metabolism 1992; 41 (9): 941–948.
90. Campbell WW, Crim MC, Young VR, Evans WJ. Increased energy requirements and changes in body composition with resistance training in older adults. Am J Clin Nutr 1994; 60 (2): 167–175.
91. Hunter GR, Wetzstein CJ, Fields DA, Brown A, Bamman MM. Resistance training increases total energy expenditure and free-living physical activity in older adults. J Appl Physiol 2000; 89 (3): 977–984.
92. Puggaard L, Larsen JB, Ebbesen E, Jeune B. Body composition in 85 year-old women: effects of increased

physical activity. Aging (Milano) 1999; 11 (5): 307–315.
93. Hunter GR, Wetzstein CJ, McLafferty CL, Jr., Zuckerman PA, Landers KA, Bamman MM. High-resistance *versus* variable-resistance training in older adults. Med Sci Sports Exerc 2001; 33 (10): 1759–1764.
94. McMurdo ME, Rennie L. A controlled trial of exercise by residents of old people's homes. Age Ageing 1993; 22 (1): 11–15.
95. Kryger AI. Effects of resistance training on skeletal muscle and function in the oldest old. University of Copenhagen, 2004.
96. Artal R, O'Toole M. Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period. Br J Sports Med 2003; 37 (1): 6–12.
97. Artal R. Exercise and pregnancy. Clin Sports Med 1992; 11 (2): 363–377.
98. Wolfe LA, Davis GAL. Canadian guidelines for exercise in pregnancy. Clin Obstet Gynecol 46: 488–95, 2003.
99. Clapp JF, III. Exercise and fetal health. J Dev Physiol 1991; 15 (1): 9–14.
100. Hartmann S, Bung P. Physical exercise during pregnancy – physiological considerations and recommendations. J Perinat Med 1999; 27 (3): 204–215.
101. Lokey EA, Tran ZV, Wells CL, Myers BC, Tran AC. Effects of physical exercise on pregnancy outcomes: a meta-analytic review. Med Sci Sports Exerc 1991; 23 (11): 1234–1239.
102. Kardel KR, Kase T. Training in pregnant women: effects on fetal development and birth. Am J Obstet Gynecol 1998; 178 (2): 280–286.

CHAPTER 11

Fat

Fat provides the organism with energy in a concentrated form. Lipids, mainly phospholipids and cholesterol, are included in cell membranes and triglycerides are stored in adipose tissue as energy reserves. In addition to energy, dietary fats provide essential fatty acids and fat-soluble vitamins.

Physiology and metabolism

Most of the naturally existing fats are mixtures of triglycerides composed of one molecule of glycerol esterified with three fatty acid molecules, mainly long-chain fatty acids with 16–18 carbon atoms. Non-esterified fatty acids are uncommon in the diet. Fatty acids account for more than 90% by weight of triglycerides. The effects of fatty acids depend on the length of the carbon chain, the degree of saturation, the number, position and structure of the double bonds, and to some extent also on their position in the triglyceride molecule. The unsaturated fatty acids are characterised by the number of double bonds: monounsaturated fatty acids (MUFA) have only one double bond whereas polyunsaturated fatty acids (PUFA) have 2 to 6 double bonds. The positions of the double bonds are calculated either from the carboxy-terminal end of the carbon chain (Δ) or the methyl end (/omega/ or n-). The human organism is capable of synthesising saturated fatty acids (SFA) and MUFA from acetate, whereas PUFA, in both n-6 linoleic acid and n-3 alpha-linolenic acid series, are required from the diet. The n-6 and n-3 fatty acids are metabolised (desaturated and elongated) further in the organism by the same enzyme systems (*Figure 11.1*).

Naturally occurring unsaturated fatty acids in plants and free-living fish are mainly cis-fatty acids. However, they can be transformed into trans-isomers chemically in the food industry by partial hydrogenation of vegetable and fish oils or through the action of bacteria in the forestomach of ruminants. Small amounts of trans fatty acids, mainly Δ11-trans vaccenic acid (18:1 n-7), are found in the milk and meat of cow, sheep and goat. Industrially hydrogenated oils contain varying

amounts of various trans isomers of fatty acids, mainly the Δ8-, Δ9- and 10-isomers but also some vaccenic acid (1). The metabolic effects of individual trans fatty acid isomers have not been studied separately in humans. Studies on the associations between trans fatty acid intake and risk of coronary heart disease do not show clear differences between trans fatty acids of ruminant and industrial origin (2). The main sources of dietary trans fatty acids are dairy products and deep-fried and industrially produced fatty foods containing partially hydrogenated fats.

A particular group of trans fatty acids are conjugated linoleic acids (CLA), which are formed by bacteria in the rumen and by desaturation of trans C 18:1 in the organism. The cis-9, trans-11 CLA that is the predominant isomer in milk fat has exhibited anticarcinogenic properties in experimental animal studies. A chemically produced mixture of CLA isomers reduces fat mass and increases lean body mass in experimental animals. In humans the effect has been less prominent (3). The trans10, cis12 CLA-isomer seems to be responsible for the adipose tissue effects. The same isomer has been found to increase insulin resistance (4) and C-reactive protein levels in humans (5).

In addition to triglycerides, dietary fats comprise phospholipids and cholesterol. The most common dietary phospholipid is phosphatidylcholine (lecithin). Cholesterol is found in foods of animal origin and is synthesised in the human organism, which is true for phospholipids as well. Plants contain small amounts of plant sterols, mainly sitosterol and campesterol, which are poorly absorbed (5–15%) from the intestine, but interfere with the absorption of cholesterol. The absorption of the corresponding saturated sterols sitostanol and campestanol is only 1–3%.

In the gut triglycerides are hydrolysed by lipases to monoglycerides and fatty acids, which together with bile salts, lysophospholipids and unesterified cholesterol form mixed micelles from which the digested lipids are absorbed in the small intestine. Fats are not soluble in water and are therefore transported in the blood as lipoprotein particles. The core of lipoproteins is formed by triglycerides and esterified cholesterol. The surface of the particles is composed of free cholesterol, phospholipids and proteins. The lipoproteins are commonly divided into four classes according to density: chylomicrons, VLDL (very low-density lipoprotein), LDL (low-density lipoprotein) and HDL (high-density lipoprotein). LDL contains about two-thirds of circulating cholesterol and is an important risk factor of atherosclerosis. A high HDL-cholesterol concentration and a low LDL/HDL cholesterol ratio are associated with a reduced risk of atherosclerosis.

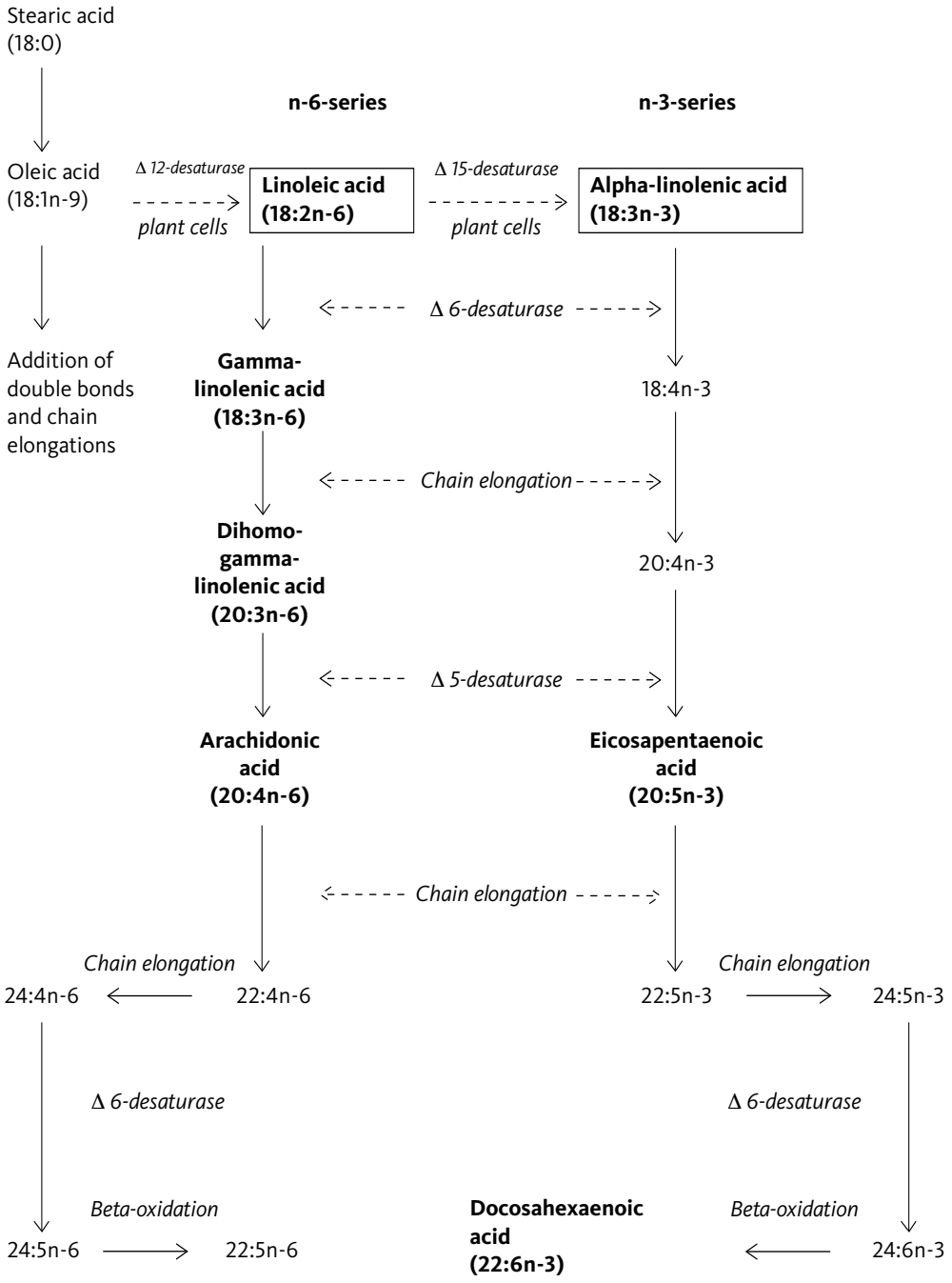

Figure 11.1 Metabolism of polyunsaturated fatty acids

Essential fatty acids

n-6 and n-3 polyunsaturated fatty acids are essential fatty acids (EFA) and must be provided by the food, normally as linoleic acid (C18:2 n-6) and alpha-linolenic acid (C18:3 n-3). They serve important physiological functions in the organism, and the human organism lacks the enzymes Δ11- and Δ15-desaturase that are capable of introducing double-bonds in the n-6 and n-3 positions (*Figure 11.1*). Linoleic acid, when incorporated into skin ceramides, is essential for maintaining the water-permeability barrier of the skin, thereby avoiding excessive trans-epidermal water loss and the accompanying energy loss from water evaporation.

Physiology and metabolism

Both linoleic acid and alpha-linolenic acid can be elongated and desaturated in the organism (*Figure 11.1*). Linoleic acid is metabolised to *e.g.* gamma-linolenic acid (C18:3 n-6), dihomo-gamma-linolenic acid (C20:3 n-6, DHGLA) and arachidonic acid (C20:4 n-6, AA). From alpha-linolenic acid, eicosapentaenoic acid (C20:5 n-3, EPA), docosapentaenoic acid (22:5 n-3, DPA) and, to a limited extent, docosahexaenoic acid (C22:6 n-3, DHA) are formed. DHGLA, AA and EPA can be further transformed to eicosanoids, a group of biologically active substances including prostaglandins, prostacyclins and leukotrienes, which participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions and other functions in tissues.

Furthermore, n-6 and n-3 PUFA, particularly the long-chain metabolites, are important structural components of cell membranes. They are essential for various membrane functions such as fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction.

Linoleic acid and alpha-linolenic acid compete for the same desaturases and elongases. The latter have a higher affinity for the enzymes. Therefore, a very high intake of alpha-linolenic acid reduces the relative proportions of long-chain n-6 fatty acids in tissues. Correspondingly, a very high dietary intake of linoleic acid may disturb the metabolism and distribution of n-3 fatty acids. Because of the competition for metabolic enzymes between the fatty acids of n-6 and n-3 series it is important to maintain a balance between n-6 and n-3 PUFA in the diet. The importance of increasing the proportion of n-3 fatty acids in dietary fats in the USA has been emphasised in review articles (6), but there is no consensus about the optimal n-6/n-3 ratio in the diet.

Deficiency

Clinical symptoms of EFA deficiency (skin changes and growth retardation) have been found in healthy newborn babies fed for 2–3 months with a diet low (< 1 E%) in linoleic acid. EFA deficiency in adults is rare. Reported cases have been associated with chronic diseases or prolonged parenteral or enteral nutrition, either without fat or very low in fat. The minimum requirement for linoleic acid remains unknown. Combined deficiency of linoleic acid and alpha-linolenic acid leads to increased formation of the PUFA C20:3 n-9 and an increased C20:3 n-9/C20:4 n-6 ratio. It has not been confirmed that this ratio is a useful indicator of essential fatty acid deficiency in man.

Clinical signs (skin changes) of insufficient supply of alpha-linolenic acid have been reported at intakes of < 0.05 E% during enteral nutrition and < 0.1 E% during parenteral nutrition, but the specificity of these findings has been challenged. However, there is evidence that alpha-linolenic acid or its metabolite DHA is essential. DHA is found at high concentrations in the synapses of the central nervous system and in the rod outer segment of the photoreceptor cells of the retina, where it is essential for the development of normal visual function (7).

Humans are able to desaturate and elongate alpha-linolenic acid to EPA and DPA but further desaturation to DHA is limited. Conversion is higher in women than in men (8, 9, 10). Studies in preterm infants strongly suggest that fatty acids of n-3 series are essential for normal development of visual function and perhaps for optimal psychomotor development. These findings support the concept that it is necessary to consume n-3 fatty acids at least in amounts sufficient to replace physiological losses.

Several studies indicate that the enzymes that are responsible for the metabolism of EFA cannot synthesize enough long-chain PUFAs from their parent fatty acids to meet the needs at birth, at least not in infants born before term. However the capacity for PUFA synthesis in preterm infants may be higher than for term infants (11). Therefore AA and DHA, which are present in human milk, should be considered conditionally essential for a limited time after birth. It is therefore recommended that a small proportion of AA and DHA, resembling the amounts in human milk, should be included in infant formula intended for preterm infants. There is as yet no consensus whether these fatty acids are conditionally essential also for infants born at term, although it has been recommended that formula intended for term infants should also be supplemented with AA and DHA (12). Supplementation of infant formula with long-chain polyunsaturated fatty acids has been associated with lower blood pressure during later childhood (13). Such

supplementation is in accordance with the European directive on infant formula and follow-on formula intended for term infants, although the directive does not give a specific recommendation for supplementation with AA and DHA (14).

Intake of long chain n-3 fatty acids in pregnancy improves the n-3 status of the foetus and newborn child (15) and may be favourable for the mental development of the child as assessed by the IQ (16). Plasma n-3 long chain fatty acid concentrations that are optimal for both mothers and infants have to be defined before general recommendations for intake are made (17). n-3 content of breast milk is affected by the mother's intake (18), and this in turn may affect the development of visual acuity in the breast fed infant (19).

Upper intake levels and toxicity

Infants have been fed formula containing high (60% of total fatty acids) amounts of linoleic acid without apparent harmful effects. Nonetheless, high intakes of PUFA may have untoward consequences through increased peroxidation, altered immune responses or an increased tendency for bleeding. High concentrations of linoleic acid increase *in vitro* oxidation of LDL, but it is not known whether this has harmful effects on health. Prolonged high intake of EPA and DHA (1.5 E% for several months) has been associated with an increased tendency to nasal bleeding (20). Reduced activity of leucocytes *ex vivo* has been observed after high intakes of alpha-linolenic acid (6.3 E%) or fish oils (1.5 E%) (21, 22, 23).

Estimated requirement

Because minimum requirements of PUFA for adults are not known, the estimates are based on threshold intake data from children. For adults and children from 2 years of age, the lower level of intake of PUFA (sum of n-6 and n-3) is kept unchanged compared to NNR 1996, *i.e.* 3 E%, including at least 0.5 E% from n-3 fatty acids. This will provide some margin above the minimum requirement. For pregnant and lactating women, the contribution of PUFA should be at least 5 E%, including about 1 E% from n-3 fatty acids. For children 6–11 months and 1–3 years of age, the lower level of intake of n-6 fatty acids in NNR 1996 was set at 4.5 and 3 E%, respectively, and that of n-3 fatty acids at 0.5 E%. These estimates are now changed and for children 6–11 months of age the total amount of PUFA should comprise at least 5% and n-3 fatty acids at least 1% of the total energy intake.

Cholesterol

Cholesterol is formed in various types of cells in the human organism. It is used for the production of bile acids and steroid hormones and for cell membrane structures. Its synthesis is effectively regulated. Cholesterol uptake by cells reduces endogenous synthesis. About 1 gram of cholesterol is synthesised in the adult human organism daily. This is 3–4 times the amount absorbed from an average Nordic diet of adults.

The most important dietary sources of cholesterol are meat and offal, eggs and dairy products. The fractional absorption of cholesterol is reduced when the intake increases. On average, 40–50% of dietary cholesterol is absorbed, but absorption varies between individuals. According to a meta-analysis of studies published after 1974 (24), 100 mg of dietary cholesterol increased serum total cholesterol by 0.056 mmol/L and HDL-cholesterol by 0.008 mmol/L, and slightly increased the total/HDL-cholesterol ratio by 0.020 units. In a minor part of the population (*e.g.* subjects who have the apoprotein E 4/4 genotype) dietary cholesterol has a more pronounced effect on serum cholesterol whereas in most people dietary cholesterol only negligibly influences the serum cholesterol concentration.

Several expert groups, mainly in the USA, have recommended that cholesterol intake in adults should be kept below 300 mg/day. The average cholesterol intake in the Nordic countries is 250–350 mg/day. It is anticipated that the dietary guidelines promoting increased consumption of vegetable foods and limiting excessive intake of fatty dairy and meat products will lead to a reduction in cholesterol intake. Therefore, the current recommendation does not set an upper intake level for cholesterol. Apparently, the endogenous capacity to synthesise cholesterol is sufficient to meet the needs even in preterm infants. Therefore, there is no recommendation that infant formula should contain cholesterol, although cholesterol is a natural constituent of human milk.

Dietary fat and health

The relatively high fat content of the diet in the Nordic countries may have contributed to the high prevalence of cardiovascular diseases, certain types of cancer, obesity and gallstones. There has been a strong association between the intake of saturated fat, serum cholesterol concentration (LDL-cholesterol) and the incidence of coronary heart disease in cross-cultural studies. In prospective studies within populations, the associations between saturated fat intake and risk of coronary heart disease have been less significant. By reducing total fat intake, or by modifying dietary fatty acid composition in order to reduce the proportion

of SFA and increase that of unsaturated fatty acids, the risk of coronary heart disease (CHD) is reduced. In studies exceeding six months in duration, reduction or modification of dietary fat intake reduced cardiovascular events by 16%. In studies of more than 2 years' duration, the risk reduction was 24% (25). In a review of epidemiological studies and dietary intervention trials, Hu *et al.* (26) concluded that replacing saturated fat with unsaturated fat is more effective in lowering the risk of CHD than simply reducing total fat consumption.

When cis-MUFA and PUFA are substituted for the SFA and trans fatty acids, LDL-cholesterol concentration in serum is reduced while HDL-cholesterol remains unchanged (27, 28). If total fat intake is reduced as well, the HDL-cholesterol concentration also declines. Increased physical activity may counterbalance the effects of reduced fat intake on HDL-cholesterol. Studies with dietary intervention in free-living individuals have shown a mean reduction in serum total cholesterol of 8.5% in 3 months and 5.5% in 12 months, but the dietary goals were seldom achieved (29). In metabolic ward studies the compliance has been better, and the serum cholesterol levels have been reduced by 10–15% (30). A high intake of trans fatty acids has been associated with increased risk of CHD in some prospective studies (31, 32, 33).

Detailed recommendations on intakes of individual fatty acids are so far poorly justified. The effects on lipoprotein lipids of individual saturated fatty acids differ to some extent. Myristic acid (C14:0), palmitic acid (C16:0) and lauric acid (C12:0) increase both LDL- and HDL-cholesterol levels (C14:0>C16:0>C12:0) but stearic acid (C18:0) has a neutral effect comparable to that of oleic acid (C18:1). Trans fatty acids from partially hydrogenated vegetable oils or fish oils increase LDL-cholesterol almost as much as the C12–16 SFAs but may in high amounts reduce HDL-cholesterol (34, 35, 36). In one study trans fatty acids from partially hydrogenated fish oils affected LDL- and HDL cholesterol concentrations more than partially hydrogenated soybean oil (34).

The cholesterol-raising fatty acids are generally found in the same foods that are the main sources of total saturated fatty acids. For practical reasons it is sufficient to divide dietary fats into saturated plus trans fatty acids, which should contribute about 10 E%, and cis-unsaturated fatty acids contributing about 20 E% (MUFA 10–15% and PUFA 5–10%). Population studies indicate that the risk of atherosclerosis can remain quite low even though the total fat intake varies between 10 and 40 E%, as long as the intake of SFA does not exceed 10 E%. However a restricted total fat intake may be beneficial for body weight control in populations with low physical activity and high prevalence of obesity. Population studies indicate a clear association between fat intake and obesity, and

intervention studies have shown that fat-reduced diets consumed *ad libitum* contribute to weight reduction, although the effect is limited, on average 2.5 kg (37). For these reasons and for the balance between different nutrients in the diet, a moderate reduction of total fat intake to about 30 E% intake seems prudent, in order to ensure sufficient intake of other nutrients that are important for health maintenance, and possibly to prevent weight gain (38).

To sustain the rapid growth rate during infancy, fat accounts for about 50% of the total energy in human milk and infant formula. After 6 months of age this high energy density should be reduced by successively lowering the fat content of the complementary food to meet the recommendations for adults. If the proportion of fat and thereby the energy density of the food becomes too low, this must be compensated for by more voluminous servings, which in turn might result in insufficient energy intake. Some studies in children indicate that the recommendation of a fat content around 30% of the total energy is applicable for children already after the age of 1 year, since fat intakes at these levels did not adversely influence children's growth and neurological development (39). Therefore it should be safe to conclude that from the age of 2 years the total fat intake can be reduced to 30 E%, which is a change compared to NNR 1996. This adjustment reflects newer data on energy expenditure in infants using the doubly labelled water method (40).

Dietary fat composition may also influence blood pressure, insulin sensitivity, blood coagulation and risk of cancer. High intakes of EPA and DHA reduce high blood pressure values, increase bleeding time and reduce high serum triglyceride levels, but they may increase serum LDL-cholesterol levels as well. Dietary supplementation with n-3 fatty acids has reduced mortality in patients with CHD (41, 42, 43, 44). The effect may be mediated by reduced risk of cardiac arrhythmias, and fish may be more beneficial than fish oils (45). In a recent Finnish study, alpha-linolenic acid showed similar inverse associations with the risk of CHD death as eicosapentaenoic acid and docosahexaenoic acid in the fatty acid composition of serum lipids (46). It is possible that very low intake of PUFA increases blood pressure, but at intakes exceeding the minimum requirement, blood pressure is not affected by linoleic acid intake.

Recent studies including subjects from the Nordic countries suggest that replacing saturated fatty acids with unsaturated fat improves insulin sensitivity in healthy subjects when total fat intake is kept below 37 E% (47) and that reduction of total and saturated fat intake in conjunction with modest weight reduction and increased physical activity reduces the risk of diabetes in subjects with glucose intolerance (48).

Regular physical activity improves insulin sensitivity and reduces the risk of CHD.

In experimental animal studies, fat intake promotes the development of breast cancer and colon cancer. In epidemiological studies, the associations between dietary fats and the risk of different types of cancer have remained controversial.

Recommendations

Adults and children from 2 years of age

It is recommended that the intake of saturated plus trans fatty acids be reduced to about 10 E% (calculated as fatty acids). Even lower levels may be desirable in persons with hypercholesterolaemia. The intake of trans fatty acids from partially hydrogenated, industrially produced, fats should be reduced as much as possible. To achieve the recommended intake values, the intake of saturated plus trans fatty acids should be reduced by roughly one-third in the Nordic countries. Saturated and trans fatty acids are mainly found in the same foods, and therefore the intakes of both classes of fatty acids are expected to be reduced proportionally. A reduction of SFA intake will generally also reduce cholesterol intake.

Cis-MUFA should contribute 10–15 and PUFA 5–10 E% intake, including about 1 E% from n-3 fatty acids. Because of the potentially harmful effects of very high PUFA intakes, higher intakes of PUFA are not recommended. A ratio of n-6 to n-3 polyunsaturated fatty acids between 3 and 9 is considered to be adequate.

Consequently, the recommended total fat intake is 25–35 E%, calculated as triglycerides. Reducing total fat intake below 30 E% may be desirable for obese people whereas intakes of 30–35 E% are acceptable for lean people, provided that the consumption of saturated plus trans fatty acids does not exceed the recommendation. It is difficult to ensure sufficient intake of fat-soluble vitamins and EFA if total fat intake is below 15 E%. Generally, a reduction of fat intake below 25 E% does not provide additional benefits, since very low-fat diets tend to reduce HDL-cholesterol and increase triglycerides in serum and impair glucose tolerance in susceptible individuals.

From studies in adults it has been estimated that a reduction in the intake of SFA by 5 E% would reduce the risk of CHD by 17–42%, depending on whether carbohydrates or unsaturated fatty acids are substituted for the SFA (32), and a reduction in the intake of trans fatty acids by 2 E% would decrease the risk of CHD by 25% (33). Correspondingly, in the Nordic countries, the risk of CHD would be reduced by about 30% by

a one-third reduction (4–5 E%) in SFA intake and by about 3% through a one-third reduction (0.2–0.3 E%) in trans fatty acid intake.

Children 6–23 months

Due to the rapid growth the energy requirement per kg body weight is higher during infancy than later in life. To meet this, close to 50% of the energy in human milk is provided by fat. Infants who are not breast fed or are partially breast fed should be fed an infant formula as a substitute during the first 4–6 months, whereafter the diet successively becomes more diversified. According to the proposed revision of the EU directive (14) for infant formula and follow-on formula, fat should comprise 40–55 E% in infant formula and 35–55% in follow-on formula, intended as the major liquid part of an increasingly diversified diet from the fifth month of age. As exclusive breastfeeding is recommended during the first 6 months of life, and because the fat composition of infant formula and follow-on formula is regulated, no further recommendations are given for the first 6 months of life. With increasing amounts of complementary foods the fat intake often declines rapidly to around 30 E% at the end of infancy, depending on the composition of the complementary food. It is also common that after the first year the proportion of fat increases gradually until 3 years of age to levels common among adults.

It is recommended that the proportion of total fat be kept between 30 and 45 E% for infants between 6 and 11 months of age and between 30 and 35 E% from 12 to 23 months of age. A gradual decrease of the fat content with age from 6 months to 2 years of age is recommended. From 6–23 months of age the total amount of PUFA should constitute 5–10% of the total energy intake, including at least 1 E% from n-3 fatty acids. The optimum ratio of n-6/n-3 fatty acids is not known for various age groups. From 6–11 months of age it is recommended that the ratio of 5–15:1 for infant formula and follow-on formula (14), gradually approaches the ratio for adults of 3–9:1.

Trans fatty acids interfere with the metabolism of PUFA (49). The intake of trans fatty acids from partially hydrogenated, industrially produced fats should be kept as low as possible as long as the child's capacity to desaturate and elongate fatty acids may be limited. From the age of 12 months, the intake of saturated plus trans fatty acids should be limited to about 10% of total energy.

Dietary sources and intake

Habitual fat intake in the Nordic countries today is higher than it used to be 100 years ago. It was highest during the 1960s and 1970s but has

declined during the past 20–30 years. *Table 11.1* shows the mean fatty acid intake in the Nordic countries according to recent surveys. The ratios of linoleic acid (LA) to alpha-linolenic acid (ALA) intakes were among the lowest values of the 14 European countries participating in the European TRANSFAIR study (50).

Table 11.1 Dietary intake (E%) of total fat and fatty acid clusters in the Nordic countries in 1997–2002

	Denmark 2000–02	Finland 2002	Iceland 2002	Norway 1997	Sweden 1997–8
Total fat	33	33.6	35.3	31	34
SFA	14.3	14.0	14.7	12.1	14
TFA	1.2	0.5	1.4	< 1.0*	1
MUFA	11.5	11.2	11.2	10.8	12
PUFA	4.7	5.1	4.7	5.4	4.5
n-6/n-3 ratio	4.2	4.0	3	5.5	5

* Household consumption survey 2000

The intake of fat from butter and milk products declined by 20% in Iceland between 1968 and 1985. In Finland the intake of saturated fatty acids was reduced from 19 to 14 E% between 1982 and 2002, and in Norway the intake of saturated plus trans fatty acids was reduced from 21 to 15% between 1975 and 2001.

Mortality in CHD has declined among people of working age in the Nordic countries during the past 30 years, but there are differences between the countries, particularly for men (*Table 11.2*). In people of working age the CHD mortality has been reduced by more than one-half since 1970 both in Finland and in Norway. Of the reduction, 30–50% has been estimated to result from the decline in serum cholesterol levels (51, 52, 53, 54).

Table 11.2 Ischaemic heart disease mortality rates per 100,000 in women and men aged 25–64 years in the Nordic countries (www.who.dk)

	Women		Men	
	1971	1999	1971	1999
Denmark	44.25	15.18	174.36	58.67
Finland	63.20	16.48	373.73	105.99
Iceland	52.74	13.71*	221.76	76.24*
Norway	36.08	15.76	196.74	67.24
Sweden	38.20	16.43	158.22	64.16

* 1997

The most important sources of fat are 1) margarines, butter, oils and other fats, 2) milk and milk products, and 3) meat and meat products. Dairy products, hard margarines and hydrogenated fats in processed products are the main sources of saturated and trans fatty acids. Soft margarines and vegetable oils are the main sources of polyunsaturated fatty acids, while monounsaturated fatty acids are derived from all three product groups.

Since January 2004, Danish legislation prohibits sale of products containing > 2% trans fatty acids (based on the total amount of fatty acids) produced by industrial hydrogenation (39). Trans fatty acids from ruminant animal products are not considered in the Danish regulations.

REFERENCES

1. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids (adopted on 8 July 2004). The EFSA Journal 2004; 81, 1–49. www.efsa.eu.int/science/nda/nda_opinions/588/opinion_nda09_ej81_tfa_en1.pdf.
2. Weggemans RM, Rudrum M, Trautwein EA. Intake of ruminant *versus* industrial trans-fatty acids and risk of coronary heart disease: what is the evidence? Eur J Lipid Sci Technol 2004; 106: 390–397.
3. Terpstra AH. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. Am J Clin Nutr. 2004; 79: 352–61.
4. Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary trans10-cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care. 2002; 25: 1516–21.
5. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation. 2002; 106: 1925–9.
6. Harper CR, Jacobson TA. The fats of life. The role of omega-3 fatty acids in the prevention of coronary heart disease. Arch Intern Med 2001; 161: 2185–2192.
7. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001; 40: 1–94.
8. Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. Br J Nutr. 2002; 88: 355–363.
9. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr. 2002; 88: 411–420.
10. Gerster H. Can adults adequately convert α-linolenic acid (18: 3n-3) to eicosapentaenoic acid (20: 5n-3) and docosahexaenoic acid (22: 6n-3)? Internat J Vit Nutr Res 1998; 68: 159–173.
11. Uauy R, Mena P, Wegher B, Nieto S, Salem N Jr. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age

and intrauterine growth. Pediatr Res. 2000; 47: 127–35.
12. Koletzko B, Agostoni C, Carlson SE, Clandinin T, Hornstra G, Neuringer M, Uauy R, Yamashiro Y, Willatts P. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. Acta Paediatr. 2001; 90: 460–4.
13. Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P, Boehm G. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ 2003; 326: 953–955.
14. Scientific Committee on Food. Report on the Revision of Essential Requirements of Infant formulae and Follow-on Formulae. SCF/CS/NUT/IF/65/Final 18 May, 2003. http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.
15. Velzing-Aarts FV, van der Klis FR, van der Dijs FP, *et al.* Effect of three low-dose fish oil supplements, administered during pregnancy, on neonatal long-chain polyunsaturated fatty acid status at birth. Prostaglandins Leukot Essent Fatty Acids 2001; 65: 51–7.
16. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003; 111: 39–44.
17. Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. Am J Clin Nutr 2000; 71: 307S–11S.
18. Lauritzen L, Jorgensen MH, Hansen HS, Michaelsen KF. Fluctuations in human milk long-chain PUFA levels in relation to dietary intake. Lipids 2002; 37: 237–244.
19. Jorgensen MH, Hernell O, Hughes E, Michaelsen KF. Is there a relation between docosahexaenoic acid concentration in mothers' milk and visual development in term infants? J Pediatr Gastroenterol Nutr 2001; 32: 293–6.
20. Clarke JTR, Cullen-Dean G, Regelink E, Chan L, Rose V. Increased incidence of epistaxis in adolescents with familial hypercholesterolemia treated with fish oil. J Pediatr 1990; 116: 139–41.
21. Kelley DS, Branch LB, Love JE, Taylor PC, Rivera YM, Iancono JM. Dietary α-linolenic acid and immunocompetence in humans. Am J Clin Nutr 1991; 53: 40–6.
22. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, Spur BW *et al.* Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1989; 312: 1217–24.
23. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonneman G, van der Meer JWM, *et al.* The effect of dietary supplementation with n-3 polyunsaturated fatty acid on the synthesis of interleukin-1 and tumor necrosis factory by mononuclear cells. N Engl J Med 1989; 320: 265–71.).
24. Weggemans RM, Zock PL, Katan MB. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density cholesterol in humans: a meta-analysis. Am J Clin Nutr 2001; 73: 885–891.
25. Hooper L, Summerbell CD, Higgins JPT, *et al.* Dietary fat intake and prevention of cardiovascular disease: systematic review. Br Med J 2001; 322: 757–763.
26. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001; 20: 5–19.
27. Müller H, Kirkhus B, Pedersen JI. Serum cholesterol predictive equations with special emphasis on trans and saturated fatty acids. An analysis from designed controlled studies. Lipids. 2001; 36: 783–791.
28. Sacks FM, Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am J Med 2002; 113: 13S–24S.

29. Tang LJ, Armitage JM, Lancaster T, Silagy CA, Fowler GH, Neil HAW. Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects. Br Med J 1998; 316: 1213–1220.
30. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. Br Med J 1997; 314: 112–117
31. Aro A. Trans fatty acids: health effects. In: Encyclopedia on food sciences and nutrition. Caballero B, Trugo L, Finglas P, eds. Academic Press, London 2003.
32. Hu FB, Stampfer MJ, Manson JE, *et al.* Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med 1997; 337: 1491–1499.
33. Oomen CM, Ocké MC, Feskens EJM, van Erp-Baart M-A, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet 2001; 357: 746–751.
34. Almendingen K, Jordal O, Kierulf P, Sandstad B, Pedersen JI. Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. J Lipid Res 1995; 36: 1370–1384.
35. Mauger J-F, Lichtenstein AH, Ausman LM, Jalbert SM, Jauhiainen M, Ehnholm C, Lamarche B. Effect of different forms of dietary hydrogenated fats on LDL particle size. Am J Clin Nutr 2003; 78; 370–5.
36. Zock PL, Katan MB. Hydrogenation alternatives: effects of trans fatty acids and stearic *versus* linoleic acid on serum lipids and lipoproteins in humans. J Lipid res 1992; 33: 399–410.
37. Astrup A. The role of dietary fat in the prevention and treatment of obesity: efficacy and safety of low-fat diets. Int J Obes 2001; 25: S46–S50.
38. Foreyt JP, Carlos Poston WS. Consensus view on the role of dietary fat and obesity. Am J Med 2002; 113: 60S–62S.
39. Rask-Nissilä L, Jokinen E, Terho P, Tammi A, Hakanen M, Rönnemaa T, Viikari J, Seppänen R, Välimäki I, Helenius H, Simell O. Effects of diet on the neurological development of children at 5 years of age: the STRIP project. J Pediatr. 2002; 140: 328–333.
40. Tennefors C, Coward WA, Hernell O, Wright A, Forsum E. Total energy expenditure and physical activity level in healthy young Swedish children 9 or 14 months of age. Eur J Clin Nutr 2003; 57: 647–53.
41. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med 2002; 112: 298–304.
42. Burr ML, Gilbert JF, Holliday RM, *et al.* Effects of changes in fat, fish, and fibre intakes on death and myocardial infarction: Diet and Reinfarction Trial (DART). Lancet 1989; 2: 757–761.
43. GISSI Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione Trial. Lancet 1999; 354: 447–455.
44. de Lorgeril M, Renaud S, Mamelle N, *et al.* Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994; 343: 1454–1459.
45. Marckmann P. Fishing for heart protection. Am J Clin Nutr 2003; 78: 1–2.
46. Erkkilä AT, Lehto S, Pyörälä K, Uusitupa MIJ. n-3 fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary heart disease. Am J Clin Nutr 2003; 78: 65–71.
47. Vessby B, Uusitupa M, Hermansen K, *et al.* Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. Diabetologia 2001; 44: 312–319.
48. Tuomilehto J, Lindström J, Eriksson JG, *et al.* Prevention of type 2 diabetes mellitus by changes in lifestyle

among subjects with impaired glucose tolerance. N Engl J Med 2001; 344: 1343–1350.
49. Aggett PJ, Haschke F, Heine W, Hernell O, Koletzko B, Lafeber H, Ormisson A, Rey J, Tormo R. Committee report: Childhood diet and the prevention of coronary heart disease. ESPGAN Committee on Nutrition. European Society of Pediatric Gastroenterology and Nutrition. J Pediatr Gastroenterol Nutr 1994; 19: 261–9.
50. Hulshof KFAM, van Erp-Baart MA, Anttolainen M, Becker W, *et al.* Intake of fatty acids in Western Europe with emphasis on trans fatty acids: The TRANSFAIR study. Eur J Clin Nutr 1999; 53: 143–157.
51. Johansson L, Drevon CA, Bjorneboe GEA. The Norwegian diet during the last hundred years. Eur J Clin Nutr 1996; 50: 277–283.
52. Pedersen JI, Tverdal A, Kirkhus B. Kostendringer og dødelighetsutvikling av hjerte- og karsygdommer i Norge. Tidskr Nor Laegeforen 2004; 124: 1532–1536.
53. Pietinen P, Lahti-Koski M, Vartiainen E, Puska P. Nutrition and cardiovascular disease in Finland since the early 1970s: a success story. J Nutr Health Aging. 2001; 5: 150–154.
54. Vartiainen E, Puska P, Pekkanen J, Tuomilehto J, Jousilahti P. Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. Br Med J 1994; 309: 23–27.
55. Stender S, Dyerberg J. Influence of trans fatty acids on health. Ann Nutr Metab 2004; 48: 61–66.

CHAPTER 12

Carbohydrates

Carbohydrates provide 40–80% of the energy in the human diet and are the single most important source of energy worldwide. Chemically, carbohydrates are heterogeneous and include polyhydroxy aldehydes, ketones, alcohols and acids, as well as their derivatives and polymers. Analytically, for instance for labelling of foods, carbohydrates are still often expressed 'by difference', which means that moisture, protein, fat and ash are determined and the rest named 'carbohydrates'. This obviously non-specific procedure includes all kinds of carbohydrates regardless of their physiological and nutritional properties, as well as variable amounts of non-carbohydrate material, *e.g.* organic acids, lignin and polyphenols (1–3).

Chemical classification

The chemical classification of carbohydrates is usually based on molecular size and monomeric composition, three principal groups being sugars (1–2 monomers), oligosaccharides (3–9 monomers) and polysaccharides (10 or more monomers) (1). As shown in *Table 12.1*, the most important food carbohydrates are glucose, fructose and galactose (monosaccharides), sucrose, lactose and trehalose (disaccharides), oligosaccharides and polysaccharides. There are two main classes of polysaccharides, starch and non-starch polysaccharides (NSP). Starch is a homopolymer of glucose with two main forms, amylose (basically unbranched) and amylopectin (highly branched). NSP include a host of different polymers, highly variable in terms of molecular size and structure, as well as in monomeric composition. Main classes of NSP are cellulose, hemicelluloses, pectins, and hydrocolloids. Due to the structural variability, different NSP may have very different physical-chemical properties, which are of key importance for their physiological effects. Whereas cellulose is insoluble in water, pectins and hydrocolloids, *e.g.* guar gum and mucilages, may form highly viscous water solutions.

Table 12.1 Classification of main food carbohydrates. Adapted from (4)

Class (DP*)	Sub-Group	Components	Monomers	Digestibility**
Sugars (1–2)	Monosaccharides	Glucose		+
		Galactose		+
		Fructose		+
	Disaccharides	Sucrose	Glu, Fru	+
		Lactose	Glu, Gal	+ (–) ***
		Trehalose	Glu	+
		Maltose	Glu	+
Oligosaccharides (3–9)	Malto-oligosaccharides	Maltodextrins	Glu	+
	Other oligosaccharides	α-Galactosides	Gal, Glu	–
		Fructo-oligosaccharides	Fru, Glu	–
Polysaccharides (> 9)	Starch	Amylose	Glu	+ (–)
		Amylopectin	Glu	+ (–)
		Modified starch	Glu	+ –
	Non-starch poly-saccharides	Cellulose	Glu	–
		Hemicelluloses	Variable	–
		Pectins	Uronic acids	–
		Hydrocolloids, *e.g.* gums, mucilages, β-glucans	Variable	–

* DP = Degree of polymerisation
** Denotes digestibility in the small intestine (+ digestible, – non-digestible, + (–) mainly digestible, + – partly digestible)
*** Lactose is more or less non-digestible by individuals with low intestinal lactase activity
Fru Fructose
Glu Glucose
Gal Galactose

Nutritional classification

Nutritionally, it is useful to differentiate in the first place between two broad categories of carbohydrates – those digested and absorbed in the human small intestine, providing carbohydrates to body cells, and those passing to the large intestine, forming substrate for the colonic microflora. The importance of this differentiation was recognised already in the 1920s by McCance and Widdowson who classified carbohydrates as 'available' or 'unavailable'. The term 'dietary fibre' was then coined and increasingly used more or less synonymously with 'unavailable carbohydrates' (4). A recent FAO/WHO Expert Consultation on Carbohydrates in

Human Nutrition (1) recommended introduction of the concept 'glycaemic carbohydrate', meaning 'providing carbohydrate for metabolism'.

Glycaemic carbohydrates

The main glycaemic carbohydrates are:

- Glucose and fructose (monosaccharides)
- Sucrose and lactose (disaccharides)
- Malto-oligosaccharides
- Starch (polysaccharide)

Main sources of glucose and fructose are fruits, berries, juices and some vegetables. Free galactose is rare in foods, except in fermented and lactose hydrolysed milk products. Fruits, berries and juices are also natural sources of sucrose, although sugar added to foods, carbonated drinks and sweets or in the household usually provides most of the dietary sucrose. More or less completely hydrolysed starch or high fructose syrup, in which about half the glucose is isomerised to fructose, have been increasingly used to replace sucrose in confectionary and carbonated drinks. Lactose occurs exclusively in milk and milk products. Human milk has the highest lactose content, 7 g/100 g, which is a main source of energy in the milk. The lactose content in cow's milk is around 5 g/100 g. Malto-oligosaccharides originate mainly from partly hydrolysed starch. Main sources of starch are bread and other cereal products, potatoes and tubers (1).

Dietary fibre

The main types of dietary fibre are:

- Non-starch polysaccharides – cellulose, hemicelluloses, pectins, hydrocolloids, etc.
- Resistant oligosaccharides – fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides
- Resistant starch
  1) Physically enclosed starch,
  2) Some types of raw starch granules,
  3) Retrograded amylose
  4) Chemically modified starches
- Lignin (and other usually minor components associated with the dietary fibre polysaccharides)

The term dietary fibre was originally defined as 'that portion of food which is derived from cellular walls of plants which are digested very poorly by human beings' (5). The recognition that polysaccharides

added to foods, notably hydrocolloids, could have effects similar to those originating from plant cell walls led to a redefinition of dietary fibre to include 'polysaccharides and lignin that are not digested in the human small intestine' (6).

The definition and delimitation of 'dietary fibre' has been much debated and related both to physiological considerations and to methods that can be used for dietary fibre analysis in foods. There have been two slightly different standpoints in this debate:

A) In view of the key importance of small-intestinal digestibility for the nutritional effects of carbohydrates, dietary fibre should be defined to include all non-digestible carbohydrates (NDC). Lignin and other non-digestible but quantitatively minor components that are associated with the dietary fibre polysaccharides and may influence their physiological properties should be included as well (4, 7, 8).
B) In keeping with the original definition focusing on the importance of plant cell walls, the dietary fibre concept should be limited to non-starch polysaccharides of plant cell wall origin (9).

In 2002, the Food and Nutrition Board's report Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients) (10), differentiated two kinds of fibre: 1) *Dietary Fibre* (consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants); and 2) *Functional Fibre* (consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans). *Total Fibre* is defined as the sum of these two kinds of fibre. The rationale behind this differentiation is that there is epidemiological evidence for beneficial effects of foods naturally high in dietary fibre, such as wholegrain cereals, fruits and vegetables, and that dietary fibre can be regarded as a marker of such foods. Consequently, some form of documentation on the beneficial effects of added, functional fibre should be required. However, the report (10) stated that it is not anticipated that the new definitions will significantly impact on recommended levels of intake, but that information on both dietary fibre and functional fibre will more clearly delineate the source of fibre and the potential health benefits.

With any definition of dietary fibre, NSP from plant cell walls, such as cellulose, hemicelluloses and pectins, are the dominant components. Hydrocolloids can be either naturally occurring cell wall or storage components, or added to foods as ingredients to obtain specific technological and/or nutritional benefits. Recently, there has been a steadily in-

creasing interest in the importance of two other classes of NDC, resistant oligosaccharides and resistant starch. In view of the partly similar physiological and nutritional effects of these food components and non-starch polysaccharides, both scientists and regulators increasingly tend to regard dietary fibre as synonymous with non-digestible carbohydrates.

Average dietary fibre intake in Western countries is typically around 20 g/day. Cereals are the predominant source of dietary fibre in Nordic diets (11) and wholegrain cereals contain 3–4 times more fibre than products made from white flour. Fruits and vegetables provide a larger proportion of the fibre in southern Europe (12). Cellulose is insoluble in water and occurs together with hemicelluloses in cereals. The lignified outer layers are the predominant source in wholegrain products, and this type of fibre is most resistant to fermentation by the colonic microflora. Oats and barley contain high levels of a soluble viscous polysaccharide, β-glucan. Pectins – a main type of dietary fibre in fruits and vegetables – have similar properties.

Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, etc. are often included in 'sugars'. The small-intestinal absorption of polyols depends on their structure and on the amount. Thus, polyols may add variably to NDC.

Considerable amounts of lactose may reach the colon in young children, due to the high content in breast milk, and in children and adults with low intestinal lactase activity. A limited capacity to absorb fructose seems to be rather common, especially if this sugar is consumed alone without glucose, which may be another cause of diarrhoea due to carbohydrate malabsorption (13). Foods naturally containing fructose such as fruits and berries, however, generally also contain glucose and other sugars.

Organic acids such as lactic acid, citric acid, malic acid, which occur in fermented foods, fruits and berries, respectively, may contribute to carbohydrates measured 'by difference'.

The nutritional classification of food carbohydrates cannot be based on the chemical properties alone, although a chemical classification is necessary as a basis for the specific carbohydrate analysis. The FAO/WHO Consultation (1) recommended the term 'glycaemic carbohydrates' and stressed that 'use of the term dietary fibre should always be qualified by a statement itemising those carbohydrates and other substances intended for inclusion. Dietary fibre is a nutritional concept, not an exact description of a component of the diet'. Another important aspect in practice, not least when used for food tables and labelling, is that the definitions of various classes of carbohydrates have to be complemen-

tary to avoid accounting for any fraction twice or not at all. Thus, the sum of glycaemic carbohydrates and dietary fibre should ideally equal 'total carbohydrate'. With the methods currently used some overlap may occur and some components may be missed, as shown in *Table 12.2.*

Dietary fibre is usually analysed using enzymatic gravimetric or enzymatic chemical methods that include non-starch polysaccharides (NSP), analytically resistant starch and lignin. Methods measuring NSP alone give lower estimates in foods containing resistant starch and/or lignin, *e.g.* wholegrain flour and cereals processed in a way that generates resistant starch. Resistant oligosaccharides are not included in any of the current dietary fibre methods, and therefore have to be measured separately and added to the total fibre estimate. Dietary fibre methods including resistant starch measure the fraction resistant to the enzymes used in the assay. Such 'analytically resistant starch' includes mainly retrograded amylose, and the analytical methods need fine-tuning to correspond better to the physiologically resistant starch (4, 14).

In both epidemiological studies and mechanistic intervention studies, the term dietary fibre is usually used for non-digestible plant material as measured by official analytical methods approved by the Association of Official Analytical Chemists. This means inclusion of non-starch polysaccharides (NSP) as the main component, lignin and analytically resistant starch. Other NDC such as resistant oligosaccharides and inulin are not included and usually make up a small part of the NDC in Nordic diets. Therefore, the dietary fibre recommendation in NNR, based on the available studies, refers to dietary fibre naturally occurring in plant foods as measured by AOAC methods for total dietary fibre.

Physiology and metabolism

Glycaemic carbohydrates

Digestion and absorption

The glycaemic carbohydrates provide carbohydrate to body cells, mainly in the form of glucose. In practice, only monosaccharides can be absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and is continued in the small intestine by pancreatic amylase. The degradation products – mainly maltose and oligosaccharides – are hydrolysed further to glucose by a set of enzymes, 'disaccharidases', bound to the brush border membrane of the enterocytes. The same enzymes hydrolyse the dietary disaccharides. Glucose and galactose are absorbed efficiently by a secondary active carrier coupled with sodium (glucose transporter, GLUT 2), whereas fructose is absorbed by facilitated diffusion that does not

Table 12.2 Classification and measurement of main carbohydrates and related substances, all included in carbohydrate 'by difference'

Class (DP*)	Components	Analysis methods			
		'Sugars'	'Starch'	'Dietary fibre' AOAC **	Digestibility in the small intestine
Sugars (1–2)	Glucose	+			+
	Galactose	+			+
	Fructose	+			+
	Sucrose	+			+
	Lactose	+			+ (–) [1]
	Trehalose	+			+
Oligosaccharides (3–9)	Maltodextrins	+	+		+
	α-Galactosides	+/–			–
	Fructo-oligosaccharides	+/–			–
Polyols	Maltitol				+ (–) [2]
	Sorbitol				+ (–) [2]
	Xylitol				+ (–) [2]
Polysaccharides (>9)	Starch (amylose amylopectin)		+		+
	Chemically modified starch (food additives)		+/–	+ (–) [3]	+ (–)
	Resistant starch		+/–	+ (–) [3]	–
	Cellulose			+	–
	Hemicelluloses			+	–
	Pectins			+	–
	Hydrocolloids *e.g.* alginates, gums, mucilages, β-glucans			+	–
	Inulin			+ (–) [3]	–
Related substances	Lignin			+	–
	Tannins/polyphenols			+ (–) [3]	+ (–) [4]
	Phytate			+ (–) [3]	–
	Organic acids			–	+

* DP: Degree of polymerisation
** AOAC: Association of Official Analytical Chemists
+/– Determined in some but not all methods
1 Adults with low intestinal lactase activity have a limited capacity to digest and absorb lactose
2 Polyols are partly and variably absorbed
3 Partly determined
4 Includes soluble, partly absorbable, and insoluble, un-absorbable forms.

involve sodium co-transport (GLUT 5). The monosaccharide absorption is generally regarded as the rate-limiting step. However, down-regulation of lactase occurs in most humans between 1–2 years to teenage (primary low lactase activity, hypolactasia), resulting in a limited lactose absorption capacity. The same is true for sucrose in the rare cases of sucrase deficiency (15, 16).

Absorbed sugars are transported to the liver and then to the systemic circulation. The cellular uptake is mediated by a number of glucose transporters (GLUT 1–4), variously expressed in different tissues. Insulin is a key hormone for the uptake and metabolism of carbohydrates. The plasma insulin concentration increases immediately after ingestion of carbohydrates. One important effect is to increase the translocation of glucose transporters (GLUT 4) to cell membranes, which increases the peripheral uptake, counteracting an excessive rise in blood glucose. Glucose is a preferred fuel for most body cells, and can be stored as glycogen in the liver and in the muscles. The storage capacity is limited, in total to around 500 g, of which 3–400 g can be stored in the muscles. Liver glycogen is used to maintain normal blood glucose levels between meals, whereas muscle glycogen is used primarily as source of energy within the muscles. Unlike glucose, fructose enters mainly liver cells without the need for insulin. The metabolism of fructose favours lipogenesis more than glucose. Galactose, arising from hydrolysis of lactose, is also transformed to glucose mainly in the liver. This transformation is inhibited by alcohol.

There is no absolute need for food carbohydrates to sustain life, provided that adequate amounts of protein, for *de novo* synthesis of glucose, and fat are consumed. Only cells in the central nervous system, red blood cells and some other cells dependent on anaerobic glycolysis have an absolute requirement for glucose. At prolonged deficit of glucose brain cells can adapt partially to utilise fat-derived metabolites, *i.e.* β-hydroxybutyric acid and acetoacetic acid. A very low carbohydrate diet, however, results in a chronically increased production and plasma level of these acids, referred to as ketosis, and absence of glycogen stores, with adverse effects on high-intensity energy production by muscles (17). Other possible adverse effects of diets very low in carbohydrates are bone mineral loss, hypercholesterolaemia and increased risk of urolithiasis, but such effects are not well documented (10, 18).

Rate of absorption and the glycaemic index (GI)

The glycaemic carbohydrates reach the peripheral circulation mainly as glucose. Insulin is secreted in response to the elevated blood glucose concentration after a meal. Vagal signals, gastrointestinal hormones

(incretins) and certain non-carbohydrate food components, notably amino acids, contribute to stimulating the insulin secretion. The blood glucose level is determined by three main factors : The rate of intestinal carbohydrate uptake, the net liver uptake or elimination, and the peripheral glucose uptake, which is in turn dependent upon the insulin level and the peripheral insulin sensitivity/resistance. With a constant dietary carbohydrate load, there is a range of blood glucose responses between individuals, from low responses with a continuum to what is defined as 'impaired glucose tolerance, IGT' and 'type 2 diabetes'.

Avoiding excessive blood glucose elevations and concomitant high post-prandial insulin levels, with rapid decline and risk of subsequent fall of the blood glucose concentration below the fasting level, has long been regarded as important for metabolic control in diabetes. A stable blood glucose level has also been considered advantageous in relation to satiety and mood. The concept glycaemic index (GI) was introduced by Jenkins and co-workers in 1981, in order to rank foods in a standardised way regarding their effects on blood glucose levels after a meal. The FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition (1) defined GI as the incremental area under the blood glucose response curve during 1.5–3 hours after a 50g carbohydrate portion of a test food, expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject. Glucose or white bread are used as standards. GI values obtained with the white bread standard are about 40% higher than those obtained with the glucose standard. GI values for about 750 foods have been published (19).

In practice, the blood glucose response is determined by both the GI and the amount of carbohydrate in a normal portion of the food. Consequently, the glycaemic load (GL) concept was introduced in 1997 by Harvard epidemiologists to quantify the glycaemic effect of a portion of food (20). GL is defined as the amount of glycaemic carbohydrate in a food times the GI of the food/100, and addition of GL values for foods and meals has been used to estimate the glycaemic load of the whole diet. The glycaemic response to a meal can be influenced, in addition, by protein and fat content, as well as the size of the meal and the amount of drink taken with the food. Several groups, however, have demonstrated that the glycaemic response to a meal can be predicted from properly determined GI of the constituent foods (*e.g.* 21–22). Lack of consistency in other studies (*e.g.* 23) using published GI values may be due to these values not being applicable to the foods in question. Therefore, further application of the GI concept is dependent on validated GI determinations for specific food products. In this connection the beneficial effect of physical activity on glucose metabolism should be

kept in mind (See *Chapter 10*, (26) and (33)). Physical activity has the potential to influence insulin sensitivity and thereby the glycaemic response to any meal.

Whereas it was previously assumed that sugars are rapidly absorbed and polysaccharides (starch) slowly absorbed, research in recent years has identified a number of food-related factors determining the GI. These factors are generally unrelated to the molecular size of the carbohydrate. For instance, fructose has a low GI (30 with the white bread reference as 100) and sucrose an intermediate GI (24), *i.e.* lower than white bread. Starchy foods, on the other hand, can have low, intermediate or high GI, depending on their composition (amylose/amylopectin ratio) and physical/chemical state. The swelling and dissolution of starch at wet heat treatment, known as gelatinisation, is particularly important in making starch more readily accessible to digestive enzymes (24).

Physical barriers such as in intact cereal grains, cellular structures in leguminous seeds, parboiled rice and whole fruits, and the protein network in pasta products are food-related factors lowering the glycaemic index (24). Organic acids (acetic, propionic and lactic acid) decrease the glycaemic response to foods or meals, mainly due to inhibition of gastric emptying (25). Viscous soluble types of dietary fibre may also delay gastric emptying, in addition to their inhibitory effect on diffusion and transport in the small intestine (26).

The glycaemic index may influence the metabolic response to a subsequent meal. Short-term studies have demonstrated such 'second meal-effects' on blood glucose and insulin levels at a lunch meal following a low-GI breakfast or at breakfast following intake of slow-released and/or indigestible carbohydrate meals in the late evening. Improved glucose tolerance in the morning has thus been demonstrated after low GI/high fibre meals late in the evening. Such effects may be partly related to the colonic fermentation of NDC (27–30).

There are epidemiological findings suggesting that excessive postprandial glycaemia may be related to increased all-cause mortality in diabetics as well as in people with normal fasting blood glucose concentration. This could be related to effects on established risk factors such as LDL- and HDL-cholesterol, triglycerides and coagulation/fibrinolysis factors, but also to protein glycation and possibly increased oxidative stress (31). However, the extent to which the differences in glycaemia are related to differences in GI or to insulin resistance determined by fatness, genetics and physical activity cannot be determined.

Medium-term (3–8 week) intervention studies have shown that low GI diets can improve metabolic control in diabetes. Improvements in insulin sensitivity, blood lipid levels and fibrinolysis (PAI1) have been

reported in patients with cardiovascular disease and diabetes (for review see *e.g.* 31). In a recent controlled intervention study (32) a low fat, high carbohydrate diet with low GI also reduced LDL cholesterol in healthy subjects.

Attenuation of postprandial blood glucose levels, with concomitant lower insulin levels, could be expected to influence satiety. Studies on the importance of low GI foods for satiety and body weight regulation, however, have given variable results and intervention studies have so far failed to substantiate clear benefits of low GI, in addition to those obtained by a high carbohydrate diet (see below) (33, 34). The satiating effect of foods is not simply related to GI (35). For instance, boiled potatoes have a high GI, but nevertheless a very high satiating effect (36).

More long-term intervention studies are needed to establish the role of low GI foods and diets for maintenance of health and prevention of chronic disease. In order to perform such studies in a realistic way, a variety of low GI foods are needed.

Effects of carbohydrates on plasma lipids

A number of short- to medium-term studies have shown a transient increase in fasting triglycerides and decreased HDL-cholesterol levels after introduction of high-carbohydrate diets (37). Furthermore, epidemiological studies (38, 39) have shown a significant negative association between GI and HDL-cholesterol. In an eight-month intervention study with a controlled, fat modified diet rich in fruit and vegetables, complex carbohydrates and dietary fibre conforming to *e.g.* NNR recommendations, an initial increase in trigyceride levels was seen, which diminished with time (40). A specific trigyceride elevating effect of fructose, and hence sucrose, has been demonstrated in animal experiments. In some, but not all, human studies a high intake of refined sugars (> 20 E% sucrose or > 5 E% fructose) has resulted in elevated triglyceride levels (37, 41). A recent well-controlled study with 17 E% as fructose showed increased fasting and daylong triglyceride levels in men but not in women (42). On the other hand, a three-month multi-centre study did not show any adverse effects on plasma lipids of high-carbohydrate diets (with 51–56 E% carbohydrates) either with predominantly sugars, including fructose, or predominantly starch (43). Restriction of refined sugar intake may, however, lead to reduced triglyceride levels in hypertriglyceridaemic subjects, even at relatively moderate intake levels (44).

In summary, effects on plasma lipids do not motivate specific restrictions in sucrose (which is half fructose) or fructose for the general population, other than those based on general nutritional considerations, *i.e.* a limitation of added refined carbohydrates to 10 E%.

Carbohydrate malabsorption

Congenital deficiency of sucrase is a rare cause of sucrose malabsorption, which can cause gastrointestinal complaints, including diarrhoea, when larger amounts of sucrose are introduced into the diet (15). Congenital lactase deficiency is a rare cause of severe diarrhoea and malnutrition in infants due to the high content (7 g/100 g) of lactose in breast milk.

The common form of low intestinal lactase activity is due to a down-regulation of the enzyme in most people in the world, usually at the age of 2–15 years. Individuals with low lactase activity experience symptoms such as increased intestinal gas production, diarrhoea and pain, due to fermentation of malabsorbed lactose in the large intestine, *i.e.* lactose intolerance, when their lactose absorption capacity is exceeded. However, most adults can drink 1–2 dl milk (corresponding to 5–10 g lactose) or more without symptoms, especially when taken with meals. Fermented milk products are usually tolerated better, and hard cheese is in practice lactose free. Reports on sensitivity to very small amounts of lactose, a few grams or less, have not been reproduced in controlled studies. Furthermore, some malabsorption of lactose would add to the fermentation substrate available for the colonic flora with possible beneficial effects as long as the lactose does not give intolerance symptoms. Lactose malabsorption should therefore not be a reason for a general exclusion of milk products from the diet. The individual sensitivity is quite variable and should be tested (45, 46).

Resistant oligosaccharides are almost completely malabsorbed. The rapid fermentation in the colon may give intolerance symptoms similar to those characteristic for disaccharide malabsorption.

About 10% of dietary starch is estimated to be resistant to enzymatic degradation in the small intestine, *i.e.* resistant starch, RS. Since various starchy products are staple foods in most diets, the amount of resistant starch can be estimated to 10–20 g/d or more, *i.e.* in the same order of magnitude as NSP (47). The fermentation of RS is slow, and therefore generally does not cause intolerance symptoms (48).

Dietary fibre

The interest in dietary fibre as a health-promoting constituent of foods started from epidemiology. The inverse relationship between intake of dietary fibre and the prevalence of chronic 'Western' diseases led to the hypothesis that dietary fibre would be protective against a number of chronic diseases prevalent in populations with low fibre intake. Mechanistic studies have revealed a range of physiological effects of dietary

fibre components, providing support for this hypothesis (for review, see *e.g.* 49).

Physiological effects of dietary fibre

The main physiological effects of dietary fibre are summarised in *Table 12.3.* Beneficial effects on lipid and carbohydrate metabolism are shared with, and complementary to, those exerted by other food components and properties.

LAXATION. Insoluble, especially lignified types of fibre, such as in wheat bran, have the most prominent effects on faecal bulk. The increase in faecal weight ranges from 1.3 g/g ingested pectin to 5.7 g/g wheat bran fibre (50). Oligosaccharides and resistant starch may also provide some faecal bulk (51).

BLOOD LIPIDS. Viscous types of soluble fibre lower plasma total and LDL-cholesterol levels. Although fasting triglyceride levels are generally not affected, different kinds of fibre, especially soluble, viscous types,

Table 12.3 Main physiological effects of dietary fibre as related to other components or properties of foods

Physiological effect	Type of dietary fibre				Examples of food components or properties with similar effects
	NSP		RS	NDO	
	Sol	Insol			
Faecal bulking effect	+	++	+	+	
Fermentation substrate	++	+	++	++	Lactose, fructose in some individuals and instances
Blood glucose response	↓	+/– 0	? 1)		Food form Cellular structure Starch gelatinisation Amylose/amylopectin ratio Organic acids
LDL-cholesterol level	↓	+/– 0	? 2)	+/– 0	Unsaturated fatty acids Plant sterols
Triacylglycerol level	+/– 0	+/– 0	+/– 0	? 2)	n-3 fatty acids (–) Carbohydrates (+)

1) RS is not absorbed but may or may not influence the glycaemic response of digestible starch
2) Lipid lowering effects seen in animals but not demonstrated in man. Refers to fasting triacyl glycerol levels. Post-prandial levels may be influenced

NSP Non-starch polysaccharides
RS Resistant starch
NDO Non-digestible (resistant) oligosaccharides

can reduce post-prandial hyperlipidaemia (52). These effects are related to diminished cholesterol and/or bile acid absorption (53) and hypothetically also to products of colonic fermentation. Effects on lipid metabolism of resistant starch and resistant oligosaccharides demonstrated in experimental animals have so far not been reproduced in man.

BLOOD GLUCOSE ATTENUATION. Viscous, soluble type of fibre is one factor contributing to a lower post-prandial blood glucose (lower GI) and insulin response. This was originally demonstrated by incorporating isolated viscous fibre into test meals (54). When occurring in foods, the structural properties to which dietary fibre contribute are more important in this respect, unless *e.g.* special cereal varieties or fractions enriched in soluble viscous fibre are used (25, 55).

FERMENTATION. Dietary fibre components are subject to anaerobic fermentation by the colonic microflora. The extent of fermentation is dependent on both substrate and host factors, *e.g.* molecular structure and physical form of the substrate, bacterial flora and transit time. Less fermentable types of fibre, such as in lignified outer layers of cereal grain, generally have the most prominent faecal bulking effects due to their ability to bind water in the distal colon. Fermentable fibre also contributes to the faecal bulk through increased microbial mass. The importance of fermentation and fermentation products for colonic health is increasingly recognised (56).

The main fermentation products are short-chain fatty acids (SCFA) such as acetate, propionate and butyrate, and gases, notably hydrogen and methane. The decrease in pH of the colonic content has been implicated as protective against colon cancer, *e.g.* through reduced formation of bile salt metabolites implicated in carcinogenesis. Furthermore, butyrate is recognised as a main source of energy for colonocytes with effects on cell differentiation and apoptosis that might be protective (56, 57). Acetate and propionate are absorbed with possible systemic effects on carbohydrate and lipid metabolism. Propionate has been shown to inhibit liver cholesterol synthesis in experimental animals, but the importance of such a mechanism in humans remains to be established (52). The proportion of different SCFA differs with the fermentation substrate. Resistant starch and oat fibre are types of dietary fibre that have been shown to produce high proportions of butyrate (for review, see *e.g.* 51).

Probiotics are 'live microbial food ingredients that are beneficial to health' and prebiotics have been defined as 'non-digestible food components that beneficially affect the host by selectively stimulating the

growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health' (58). Fructo-oligosaccharides (FOS, *i.e.* inulin and shorter) were first shown to increase the count of bifidobacteria, but this now seems to be a more general effect of increased amounts of non-digestible carbohydrates, such as other oligosaccharides (galacto-oligosaccharides, GOS; resistant malto-oligosaccharides, MOS) and resistant starch (48, 51).

SOLUBLE AND INSOLUBLE DIETARY FIBRE. The distinction between 'soluble' and 'insoluble' dietary fibre was based on the variable physiological effects described above. However, this differentiation is method dependent, and solubility does not always predict physiological effects. For instance some types of soluble fibre may be resistant to fermentation, and the blood glucose and LDL-cholesterol lowering effect may be lost if soluble viscous fibre is depolymerised, losing its viscosity. Therefore, the distinction between soluble and insoluble fibre should be phased out (1).

Dietary fibre and chronic disease

COLON CANCER. The reduced transit time, increased faecal weight with dilution of the intestinal content and improved laxation were factors behind the early hypothesis that an appropriate intake of dietary fibre would reduce the risk of both colonic cancer and diverticular disease. There is an inverse relationship between faecal weight (influenced by non-starch polysaccharide intake) and risk of colon cancer (59). Recent epidemiological evidence further supports a protective effect (60), but more refined case control studies and intervention studies with alternate end-points have so far been unsuccessful in substantiating such a relationship.

CORONARY HEART DISEASE. A number of epidemiological studies have consistently shown a lower risk of coronary heart disease (CHD) with increased intake of dietary fibre from wholegrain cereals and fruits and vegetables (for review see *e.g.* 61). Correlations with wholegrain cereals are consistently stronger than with dietary fibre or cereal fibre. Viscous types of dietary fibre, such as pectin, guar gum and oat β-glucans, lower serum cholesterol, but it is not known to what extent the protective effects are due to dietary fibre or to other constituents of wholegrain cereals, fruits and vegetables. Furthermore, other lifestyle factors associated with the consumption of wholegrain foods and fruits and vegetables may contribute to the protective effects.

OBESITY. Several physiological effects of foods rich in dietary fibre may be important for body weight regulation, including diminished energy density, slower gastric emptying, short-term increase in satiety and decreased rate of nutrient absorption. Two recent reviews of randomised trials concluded that a high intake of dietary fibre (or NSP) resulted in weight loss, with no difference between fibre types or between fibre in foods or as supplements (61, 62). The recent FAO/WHO recommendations (63) judged the evidence that NSP (dietary fibre) might protect against weight gain and obesity as 'convincing'.

OTHER DISEASES. The possible protective effects of dietary fibre against breast cancer, diabetes, duodenal ulcers and gastric cancer need further substantiation, as well as the possible effects of fermentation on immune functions.

Refined sugars

Nutrient density

Nutrient density is the amount of essential nutrients in foods per unit energy content. An adequate nutrient density is essential for providing recommended intakes of nutrients, especially in individuals with a low energy intake. Fat and refined sugars mainly provide energy and no or few nutrients and thus tend to decrease the nutrient density. Studies among both children and elderly nursing home residents (64–67) have shown that a high intake of refined sugars (> 10–15 E%) may adversely affect the intake of essential nutrients. This is a main reason for maintaining the previous recommendation of an upper limit of 10 E% from refined sugars (68). Refined sugars include sucrose, fructose, glucose, starch hydrolysates, *i.e.* glucose syrup, high-fructose syrup etc., as food ingredients or added during food manufacturing. This limit is of special importance for children and adults with low energy intake. It is also applicable for planning diets for heterogeneous groups to obtain recommended intakes of nutrients and dietary fibre (69).

Dental caries

Historically, introduction of refined sugars into the diet led to a pronounced increase in dental caries, and later experimental and epidemiological studies confirm the association (70). Caries develops as tooth tissues demineralise upon pH decrease due to fermentation of carbohydrates by tooth-colonising bacteria. Thus, dental caries is an infectious disease but sucrose and other easily fermentable mono- and disaccharides play a key role (70). Foods rich in starch may also contribute, especially when the starch molecule is easily available to degradation by

amylase. The presence of sucrose intensifies the cariogenic potential of starch, whereas acid production from lactose is normally low (71).

Rapid and efficient fermentation, mainly into lactic acid, upon sugar exposure causes pH decreases well below 5.5, which is considered critical for caries development in enamel (the tooth crown). In tooth roots the critical pH for demineralisation is approximately 6.5, a pH reached already when bread without added sugar is consumed. In addition to lactic acid, sucrose induces production of insoluble extracellular glucose polymers, *i.e.* glucans and mutans, leading to voluminous biofilms that favour colonisation of cariogenic streptococci on the teeth surfaces.

Dental caries prevalence has declined in the Nordic counties during recent decades, whereas no corresponding reduction in sugar intake has been noted. Accordingly, newer studies in the Nordic countries do not identify a significant correlation between reported sugar intake and caries prevalence (72, 73), whereas some older studies do (74). The impact of fluoride and other life-style variables seem to override variations in sugar intake in these studies. High sugar intake has been associated with an increased risk of caries only when oral hygiene is simultaneously poor and at low level fluoride prophylaxis (68, 75). Comparisons between countries with highly deviating sugar intake, however, display a strong correlation between average sugar intake and caries prevalence (70).

Though there has been a considerable improvement in caries status among children and adolescents in recent decades, it is noteworthy that most of the disease today is found in a fraction (15–20%) of the population, and that substantial socio-economic and geographical differences persist in caries prevalence. The polarised distribution of dental caries, seen already at the age of one year (76), and an increase in caries development among some groups of children call for a focus on individually designed caries preventive programmes, in addition to the population-directed primary prevention by fluoride-containing toothpaste.

A general level of safe sugar intakes cannot be stated, since net caries development upon sugar challenge is modified by various other life-style factors (exposure to fluoride, meal frequency and diet composition), heredity, illness, medication, malnutrition, and flow and composition of saliva.

Sweeteners

Sweeteners are used as substitutes for sucrose for various reasons: 1) As low-energy alternatives, 2) For people with diabetes, and 3) In tooth-friendly products (substitution diminishes the risk of caries but not of erosions due to a low pH). Different kinds of sweeteners are allowed for

use in foods, marked E950–E967. These substances are either sugars or sugar alcohols, or natural or synthetic compounds chemically unrelated to carbohydrates (77).

Fructose is somewhat sweeter than sucrose, whereas lactose and partially hydrolysed starch are less sweet alternatives. These sugars are regarded as food raw materials and do not carry any E-number. Their energy value is similar to sucrose, and they are fermented by dental plaque bacteria, implying increased risk for caries at frequent consumption.

Sugar alcohols, such as sorbitol, mannitol, xylitol, isomalt, lactitol and maltitol are somewhat less sweet than sucrose. They are not fermented by plaque bacteria and are therefore tooth-friendly. Their absorption is limited and the metabolisable energy contribution therefore less than that of readily absorbable sugars, usually 8–12 kJ/g. Products with more than 10% sugar alcohols have to carry a label warning for a laxative effect at high consumption.

Saccharin, cyclamate, aspartam and acesulfam K are approved synthetic, high-intensity sweeteners that do not provide energy. Neohesperidindihydrochalcon and taumatin are produced from plant material. With due consideration of the legal limitations for their use, these sweeteners can be regarded as safe alternatives to avoid the energy contribution of traditional sweeteners. From the point of view of dental decay, products such as drugs, lozenges, chewing gums and soft drinks with sweeteners can be regarded as safe. However, frequent consumption of *e.g.* soft drinks with high acidity may lead to erosion of the teeth.

Carbohydrates and obesity

The potential of low-fat (< 30 E%)/high carbohydrate (> 50–55 E%) diets for weight reduction under free-living conditions has been demonstrated in a number of medium-term intervention studies (less than one year). Meta analyses of such studies have shown consistently lower body weight on the fat-reduced high carbohydrate diets, compared with normal or high fat diets (78–81). However, the long-term effect of a reduced fat intake on weight-loss is generally less documented and available studies vary in design and size (81). In addition, most of the studies showing lack of good long-term results lack active intervention during the entire trial (82). Furthermore, two recent large diabetes prevention trials (83, 84) demonstrated that fat-reduced, high-fibre diets together with 20–30 min daily physical activity produced sustained weight loss over four years and a 58% reduction in type 2 diabetes onset.

The main potential in a low fat/high carbohydrate diet may be in preventing weight gain, as well as weight regain after a weight loss (85).

Diets with a higher fat content, and restricted high glycaemic index carbohydrates in particular, have been advocated as an alternative. However, the effects on body weight and the safety of such diets have not been documented in intervention studies. As mentioned above, there is strong evidence that dietary fibre helps to decrease/maintain the body weight.

The importance of the type of carbohydrate ('simple' *versus* 'complex' carbohydrates, in practice sucrose *versus* starch) in a high carbohydrate diet (51–56 E%) was investigated in a three-month multi-centre trial (43). Both groups lost weight on the high carbohydrate diets, and there were no significant effects on plasma lipid levels. In another 10-week intervention study, on the other hand, a high sucrose consumption (28 E%) mostly as beverages, increased energy intake, body weight, fat mass, and blood pressure in moderately overweight subjects, compared with the intake of artificially sweetened beverages (86). Together with epidemiological observations showing a relationship between consumption of sugar-sweetened drinks and childhood obesity (87), this study supports the importance of limiting carbohydrate intake from beverages sweetened with sugar.

Fructose has recently been suggested to play a specific role in weight gain and insulin resistance syndrome (88). Unlike glucose, fructose is preferentially metabolised to lipids in the liver. On the other hand, fructose has a low glycaemic index. Fructose induces metabolic alterations typical for insulin resistance (metabolic) syndrome in animal models, but data in humans are less clear. Until more human studies have been performed, there is no basis for specific recommendations regarding fructose, beyond the general limitation of refined sugars.

Low-carbohydrate/high-fat/high-protein diets have been increasingly advocated recently and are very popular in the media, without detailed evidence of their efficacy or safety. A systematic review (89) concluded that there is insufficient evidence to make recommendations for or against the use of low-carbohydrate diets. The safety of long-term adherence to such diets has not been documented. Among the published studies, participants' weight loss while using low-carbohydrate diets was principally associated with decreased energy intake and increased diet duration but not with reduced carbohydrate content. On the other hand, increasing protein content to 20–25 E% while keeping a low fat/high carbohydrate content may assist weight reduction (90).

Carbohydrates and physical performance

Carbohydrate is the most readily available substrate for muscle activity, and dietary carbohydrates are essential for muscle and liver glyco-

gen formation (see *Chapter 10*). The general recommendation that carbohydrates should provide at least 50–55 E% is generally also sufficient for optimum glycogen storage. Low GI carbohydrates may be advantageous before strenuous long-duration exercise, although evidence in human studies is conflicting. High GI foods may provide the most rapid restoration of glycogen levels after exercise. Adequate supply of carbohydrates during exercise (0.5–1.0 g/kg body weight/hour) prolongs maximal performance during extended exertion (> 2 hours) and may even improve shorter (1–2 hours) aerobic and intermittent exercise (91, 92).

Requirement and recommended intake

Although there is no absolute need for food carbohydrates to sustain life, dietary supply of carbohydrate is essential to avoid ketosis. The US Food and Nutrition Board estimated the average requirement of (glycaemic) carbohydrate to 100 g/d for children and boys and girls up to 18 years, as well as adults (10), based mainly on data regarding carbohydrate utilisation by the brain. This corresponds to about 15–20 E% in adult females and males, respectively. A Dutch expert group (93) used similar calculations, arriving at carbohydrate requirements corresponding to 40–45 E%. As reviewed above, available evidence indicates that diets with a total carbohydrate content of > 50–55 E% and with a fat content of 25–30 E% are advantageous with respect to maintenance of body weight in prevention of weight gain.

A restriction in the intake of added, refined sugars is important to ensure an adequate nutrient density of the diet, especially for children and persons with a low energy intake. Frequent consumption of sugar-containing foods should be avoided to reduce the risk of dental caries. Consumption of sugar-sweetened drinks has been associated with an increased risk of excess weight-gain (see above) and should therefore be limited.

In NNR 1996, the recommendations on dietary fibre intake were based on an evaluation of some international expert reports, which mainly considered effects on the intestine. A quantitative recommendation of dietary fibre intake still has to be based primarily on amounts required for bowel regularity and for keeping a faecal bulk associated with a diminished risk of colon cancer (50, 59). Since then a number of studies have been published supporting the beneficial effects of dietary fibre and/or dietary fibre-rich foods such as wholegrain cereals, fruit and vegetables, on a number of diseases. A protective effect of dietary fibre against colon cancer was supported by the EPIC study (60), and

there is epidemiological evidence of protective effects on the risk of coronary heart disease, as well as obesity.

The previous recommendation of 25–35 g dietary fibre/d, corresponding to about 3 g/MJ, is kept unchanged. The lower figure can be regarded as a minimum intake for women and the higher figure for men. Similar intake levels were set as reference intakes by the US FNB, 38 g/d for men and 25 g/d for women, respectively. These are based mainly on perceived protective effects against coronary heart disease, supported by data indicating protective effects also against type 2 diabetes (10).

Total carbohydrate content

50–60 percent of the energy should be provided by carbohydrates. For planning purposes 55 E% is to be used.

Refined sugars

Refined sugars (sucrose, glucose, fructose, starch hydrolysates and other carbohydrates that do not carry essential nutrients) should not exceed 10 E% to ensure adequate nutrient density.

Frequent consumption of sugar-rich foods should be avoided in order to reduce the caries risk.

Dietary fibre

The dietary fibre intake for adults should be at least 25–35 g/d or 3 g/MJ (AOAC methods for total dietary fibre). This intake should be derived primarily from foods naturally rich in dietary fibre, such as wholegrain cereals, pulses, vegetables, potatoes and tubers, fruits and berries. Such foods provide a range of nutrients in addition to the dietary fibre. Added, isolated fibre may provide some specific benefits in relation to gastro-intestinal and/or metabolic functions. Low GI foods may confer benefits in addition to those related to their fibre content.

Intake of appropriate amounts of dietary fibre from a variety of foods is important for children as well. By school age an otherwise balanced diet is likely to provide at least 10 g dietary fibre/d (94). The intake should then gradually increase to reach the recommended level during adolescence.

Dietary sources and intake

Cereals and potatoes are the major sources of carbohydrates in the Nordic diet. Fruit, berries and milk provide, in addition to refined sugars, mono- and disaccharides. Cereals (wholegrain), fruits, berries, veg-

etables and potatoes provide the main proportion of the dietary fibre intake. Total carbohydrates contribute 45–52 E% and the fibre intake is 19–27 g/10 MJ.

REFERENCES

1. Joint FAO/WHO Expert Consultation. Carbohydrates in human nutrition. Food and Agriculture Organization. World Health Organization. FAO Food and Nutrition Paper 66. Rome, 1998.
2. Southgate DAT. Digestion and metabolism of sugars. Am J Clin Nutr 1995; 62: 203S–210S.
3. Asp N-G L. Classification and methodology of food carbohydrates as related to nutritional effects. Am J Clin Nutr 1995; 61 (Suppl): 930S–7S.
4. Asp N-G. Dietary carbohydrates: classification by chemistry and physiology. Food Chemistry 1996; 57: 9–14.
5. Trowell HC. Ischemic heart disease and dietary fiber. Am J Clin Nutr 1972; 25: 926–32.
6. Trowell HC, Southgate DAT, Wolever TMS, Leeds AR, Gassull MA, Jenkins DJA. Dietary fibre redefined. Lancet 1976; 1: 967.
7. Cho S, DeVries J, Prosky L. Dietary fiber analysis and applications. AOAC International, S. Gaithersburg, Maryland, USA, 1997
8. The Definition of Dietary Fiber. Report from the Dietary Fiber Definition Committee, American Association of Cereal Chemists, 2001.
9. Englyst HN, Hudson GJ. The classification and measurement of dietary carbohydrates. Food Chemistry 1996; 57: 15–21
10. Dietary reference intakes for energy, carbohydrates, fiber, fat, protein and amino acids (Macronutrients). 7. Dietary, functional, total fiber. The National Academy of Sciences, USA, 2002, pp 7-1–7-69.
11. Dietary habits and nutrient intake in Sweden 1997–98. Riksmaten. National Food Administration, Sweden, 2002.
12. Cummings JH, Frolich W (Eds). Dietary fibre intakes in Europe. Commission of the European Communities, Directorate-General Science, Research and Development, Brussels, Luxembourg, 1993, pp 11–19.
13. Rumessen JJ, Gudamnd-Hoyer E. Fructans of chicory: intestinal transport and fermentation of different chain lengths in relation to fructose and sorbitol malabsorption. Am J Clin Nutr 1998; 68: 357–64.
14. Champ M, Kozlowski F, Lecannu G. *In-vivo* and *in-vitro* methods for resistant starch measurement. In BV McCleary and L Prosky (Eds) Advanced dietary fibre technology. Blackwell Science, Oxford, UK, 2001, pp 106–19.
15. Gudmand-Höyer E, Skovbjerg H. Disaccharide digestion and maldigestion. Scand J Gastroent Suppl 1996; 216: 111–21.
16. Lactose intolerance revisited. Scand J Nutr 2001; 45: 154–177.
17. Hultman E, Harris RC, Spriet LL. Diet in work and exercise performance. In: Shils ME, Olson JA, Shike M, Ross AC (Eds) Modern nutrition in health and disease, 9th edition, Baltimore, MD: Williams and Wilkins. 1999, pp 761–782.
18. Vining EPG. Clinical efficacy of the ketogenic diet. Epilepsy Res 1999; 37: 181–90.
19. Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr 2002; 76: 5–56.
20. Salmeron J, Ascherio A, Rimm E *et al.* Dietary fiber, glycemic load and risk of NIDDM in men. Diabetes Care 1997; 20: 545–50.
21. Wolever TMS, Jenkins DJA. The use of the glycemic index in predicting

the blood glucose response to mixed meals. Am J Clin Nutr 1986; 43: 167–72.
22. Järvi AE, Karlström BE, Granfeldt YE, Björck IME, Asp N-G, Vessby B. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. Diabetes Care 1999; 22: 10–18.
23. Møller BK, Flint A, Pedersen D, Raben A, Tetens I, Holst JJ, Astrup A. Prediction of glycaemic index in mixed meals. Int J Obes Relat Metab Disord 2003; 27 (Suppl 1): S38. A J Clin Nutr in press.
24. Björck I, Liljeberg H, Östman E. Low glycemic-index foods. British J Nutr 2000; 83 Suppl.1: S149–155.
25. Liljeberg H, Björck I. Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. European J Clin Nutr 1998; 52: 368–371.
26. Jenkins DJ, Axelsen M, Kendall CW, Augustin LS, Vuksan V, Smith U. Dietary fibre, lente carbohydrates and the insulin resistance disease. British J Nutr 2000; 83 Suppl 1: S157–S163.
27. Wolever TM, Jenkins DJ, Ocana AM, Rao VA, Collier GR. Second-meal effect: low glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am J Clin Nutr 1988; 48: 1041–1047.
28. Liljeberg HGM, Åkerberg A, Björck IME. Effect of the glycemic index and content of indigestible carbohydrates of cereal-based breakfast meals on glucose tolerance at lunch in healthy subjects. Am J Clin Nutr 1999; 69: 647–655.
29. Axelsen M, Arvidsson Lenner R, Lönnroth P, Smith U. Breakfast glycaemic response in patients with type 2 diabetes: effects of bedtime dietary carbohydrates. European J Clin Nutr 1999: 53: 706–710.
30. Björck I, Elmståhl HL. The glycaemic index: importance of dietary fibre and other food properties. Proc Nutr Soc 2003; 62: 201–206.
31. Augustin LS, Franceschi S, Jenkins DJA, Kendall CWC, La Vecchia C. Glycemic index in chronic disease: a review. Eur J Clin Nutr 2002; 56: 1049–1071.
32. Sloth B., Krogh-Mikkelsen I, Flint A, Tetens I, Björck I, Vinoy S, Elmståhl H, Astrup A, Lang V, Raben A. No difference in body weight decrease between a low-glycemic-index and a high-glycemic-index diet but reduced LDL cholesterol after 10-wk *ad libitum* intake of the low-glycemic-index diet. Am. J. Clin. Nutr 2004; 80: 337–47.
33. Pawlak DB, Ebbeling CB, Ludwig DS. Should obese patients be counselled to follow a low-glycaemic index diet? Yes. Obesity Reviews 2002; 3: 235–243.
34. Raben A. Should obese patients be counselled to follow a low-glycaemic index diet? No. Obesity Reviews 2002; 3: 245–256.
35. Holt SH, Miller JC, Petrocz P, Farmakalidis E. Interrelationship among postprandial satiety, glucose and insulin responses and changes in subsequent food intake. Eur J Clin Nutr 1996; 50: 788–797.
36. Holt SH, Miller JC, Petrocz P, Farmakalidis E. A satiety index of common foods. Eur J Clin Nutr 1995; 49: 675–690.
37. Truswell AS. Food carbohydrates and plasma lipids – an update. Am J Clin Nutr 1994; 59 Suppl.3: 710S–718S.
38. Frost G, Leeds AR, Dore CJ, Madeiros S, Bradling S Dornhorst A. Glycaemic index as a determinant of HDL-cholesterol concentration. Lancet 1999; 353: 1045–1048.
39. Leeds AR. Glycemic index and heart disease. Am J Clin Nutr 2002; 76: 286S–289S.
40. Sandström B, Marckmann P, Bindslev N. An eight-month controlled study of a low-fat high-fibre diet: effects on blood-lipids and blood pressure in healthy young subjects. Eur J Clin Nutr 1992; 46: 95–109.
41. Frayn K, Kingman S. Dietary sugars and lipid metabolism in humans.

Am J Clin Nutr 199562 (Suppl. 1): 250S–63S.
42. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. Am J Clin Nutr 2000; 72: 1128–1134.
43. Saris WHM, Astrup A, Prentice AM, Zunft FHJ, Formiguera X, Verboeket-van de Venne, Raben A, Poppitt SD, Seppelt B, Johnston S, Vasilaris TH, Keogh GF. Randomized controlled trial of changes in dietary carbohydrate/fat ratio and simple vs complex carbohydrates on body weight and blood lipids. Int J Obesity 2000; 24: 1128–1134.
44. Smith JB, Niven BE, Mann JI. The effect of reduced extrinsic sucrose intake on plasma triglyceride levels. Eur J Clin Nutr 1996; 50: 498–504.
45. Vesa TH, Seppo LM, Marteau Pr, Sahi T, Korpela R. Role of irritable bowel syndrome in subjective lactose intolerance. Am J Clin Nutr1998; 67: 710–715.
46. Korpela R. Symptoms of 'lactose intolerance'. Scand J Nutr 2001; 45: 171–173.
47. Langkilde A-M. Substrate for fermentation in man. Scand J Nutr 2001; 45: 169–170.
48. Conway PL. Prebiotics and human health: The state-of-the-art and future perspectives. Scand J Nutr 2001; 45: 13–21.
49. Eastwood (ed) Vahouny symposium dietary fiber 2002. Proceedings Nutr Society suppl (kompl).
50. Cummings JH. The effect of dietary fiber on faecal weight and composition. In: Spiller GA, ed. CRC Handbook of Dietary Fiber in Human Nutrition, 2nd ed. Broca Raton, FL: CRC Press., pp 263–349.
51. Nyman M. Fermentation and bulking capacity of indigestible carbohydrates. The case of inulin and oligofructose. Br J Nutr 2002; 87: S163–S168.
52. Lairon D. Dietary fibres and dietary lipids. In BV McCleary, L Prosky, eds. Advanced dietary fibre technology. Blackwell Science, Oxford, 2001, 177–185.
53. Andersson H. Diet and cholesterol metabolism in the gut – implications for coronary heart disease and large bowel cancer. Scand J Nutr/Näringsforskning 1996; 40: 11.15.
54. Jenkins DJA, Wolever TMS, Leeds AR *et al.* Dietary fibres, fibre analogues and glucose tolerance: Importance of viscosity. British Med J 1978: 1: 1392–1394.
55. Liljeberg HG, Granfeldt YE, Björck IM. Products based on high fiber barley genotype, but not on common barley or oats lower postprandial glucose and insulin responses in healthy humans. J Nutr 1996; 126: 458–466.
56. Cummings JH, Rombeau J, Sakata T, eds. Physiological and clinical aspects of short-chain fatty acids. Cambridge University Press, Cambridge, 1995.
57. Sakata T. Effects of short-chain fatty acids on gastro-intestinal epithelial cells. In: Dietary Fibre: Mechanisms of action in human physiology. Cherbut C, Barry JL, eds. John Libbey, Eurotext, 1995, pp 61–68.
58. Salminen S, Bouley C, Boutron-Ruault M-C *et al.* Functional food science and gastrointestinal physiology and function. British J nutr 1998; 80 Suppl 1: S147–S171.
59. Cummings JH, Bingham SA, Heaton KW, Eastwood MA. Fecal weight, colon cancer risk, and dietary intake of nonstarch polysaccharides (dietary fiber). Gastroenterology 1992; 103: 1783–1789.
60. Bingham SA, Day NE, Luben R *et al.* Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition. Lancet 2003; 361: 1496–1501.
61. Pereira MA, Ludwig DJ. Dietary fiber and body weight regulation. Observations and mechanisms. Pediatr Clin North Am 2001; 48: 969–980.
62. Howarth NC, Salzman E, Roberts SB. Dietary fiber and weight regulation. Nutrition Reviews 2001; 59: 129–139.
63. WHO Technical report series 916. Diet, nutrition and the prevention of

chronic diseases. World Health Organization, Geneva 2003.
64. Lyhne N, Ovesen L. Added sugars and nutrient density in the diet of Danish children. Scandinavian Journal of Nutrition/Näringsforskning 1999, 43: 4–7.
65. Beck AM, Ovesen L. Added sugars and nutrient density in the diet of elderly Danish nursing home residents. Scandinavian Journal of Nutrition 2002; 46: 68–72.
66. Alexy U. Kersting M, Schultze-Pawlitschko V. Two approaches to derive a proposal for added sugars intake for German children and adolescents Publ Health Nutr 2003; 6: 697–702.
67. Øverby NC, Lillegaard IT, Johansson L, Anderssen LF. High intake of added sugar among Norwegian children and adolescents. Pub Health Nutr 2004; 7: 285–93.
68. Sukkers sundhedsmæssige betydning (Health effects of sugar). Danish Nutrition Council 2003. Publication no 33.
69. De svenska näringsrekommendationerna översatta till livsmedel, SNÖ-rapporten (Swedish Nutrition Recommendations Expressed as Food). National Food Administration Report no 1, 2003.
70. Navia JM. Carbohydrates and dental health. Am J Clin Nutr. 1994; 59: 719S–27S.
71. Lingstrom P, Johansson I, Birkhed D. Carbohydrates and dental caries – the influence of individual factors. Scandinavian Journal of Nutrition/ Näringsforskning. 1997; 41: 4: 170–74.
72. Sundin B. Dental caries and sugar-containing products. Analytical studies in teenagers. Dissertation. Malmö 1994.
73. Larsson B, Johansson I, Ericson Th. Prevalence of caries in adolescents in relation to diet. Community Dent. Oral Epidemiology 1992; 20: 133–7.
74. Martinsson T. Socio-economic investigation of school children with high and low caries frequency. III. A dietary study based on information given by the children. Odontologisk Revy 1972; 23: 93–114.
75. Kleemoja-Kujala E, Rasanen L. Relationship of oral hygiene and sugar consumption to risk of caries in children. Community Dent Oral Epidemiol. 1982; 10 (5): 224–33.
76. Olika villkor – olika hälsa. En studie bland invandrare från Chile, Iran, Polen och Turkiet. (Different conditions – different health) SoS-rapport 2000:3, Swedish National Board of Health and Welfare, Stockholm.
77. Swedish National Food Administration. www.slv.se
78. Bray GA, Popkin BM. Dietary fat does affect obesity. Am J Clin Nutr 1998; 68: 1157–1173.
79. Yo-Poth S, Zhao G, Etherton T *et al.* Effects of the National Cholesterol Education Programs Step I And Step II Dietary Intervention Programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr 1999; 69: 632–646.
80. Astrup A, Grunwald GK, Melanson EL *et al.* The role of low-fat diets in body weight control: a meta-analysis of *ad libitum* intervention studies. Int J Obes 2000; 24: 1545.
81. Pirozzo S, Summerbell C, Cameron C, Galsziou P. Should we recommend low-fat diets for obesity? Obes Rev 2003; 4.83–90.
82. Hill JO, Astrup A. What diets should we be recommending for obesity? Obes Rev. 2003 May; 4 (2): 77–8.
83. Tuomilehto J *et al.* Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001; 344: 1343–50.
84. Knowler WC *et al.* Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346: 393–403.
85. Toubro S, Astrup A. Randomised comparison of diets for maintaining obese subjects' weight after major weight loss: ad lib, low fat, high carbohydrate diet v fixed energy intake. BMJ 1997 Jan 4; 314 (7073): 29–34.
86. Raben A, Vasilaras TH, Møller AC, Astrup A. Sucrose compared with

artificial sweetners: different effects on *ad libitum* food intake and body weight after 10 wk of supplementation in overweight subjects. Am J Clin Nutr 2002; 76: 721–29.
87. Ludwig DS, Peterson KE, Gortmarker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective observational analysis. Lancet 2001; 357: 505–508.
88. Elliot SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain. And the insulin resistance syndrome. Am J Clin Nutr 2002; 76: 911–922.
89. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD *et al.* Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA 2003; 289: 1837–1850.
90. Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein *versus* carbohydrate in *ad libitum* fat reduced diet for the treatment of obesity. Int J Obes Related Disord 1999; 23: 528–536.
91. el-Sayed MS, MacLaren D, Rattu AJ. Exogenous carbohydrate utilisation.: effects on metabolism and exercise performance. Comp Biochem Physiol A Physiol 1997; 118: 789–803.
92. Shi X, Gisolfi CV. Fluid and carbohydrate replacement during intermittent exercise. Sports Med 1998; 25: 157–172.
93. Dietary reference intakes, energy, proteins, fats and digestible carbohydrates. Gesondheidsraad, The Nederlands, 2002, pp 137–50.
94. Aggett P, Agostoni C, Axelsson I, Edwards CA, Goulet O, Hernell O *et al.* Nondigestible carbohydrates in the diets of infants and young children: a commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 2003; 36: 329–337.

CHAPTER 13

Protein

Proteins are a constituent of all organic material in cells of animals and plants, and the protein molecules are built of 20 amino acids. Proteins perform specific functions within the body, such as building the major cellular structural elements, repairing processes, enzyme action, antibody action and transport of various substances. Dietary protein has two roles in nutrition, a specific role as source of nitrogen and amino acids and a non-specific role as an energy source. In individuals in energy balance and with a moderate physical activity level, the protein requirement is defined as the lowest intake of protein to maintain nitrogen balance.

Physiology and metabolism

During digestion and absorption dietary proteins are split into amino acids. Within the body, amino acids absorbed into the blood build tissue protein and other nitrogen-containing compounds. Thus, the requirement of protein is actually a requirement of amino acids and nitrogen.

Body proteins are continually broken down and synthesised. The protein turnover is many times higher than the amount of proteins added from dietary protein, which indicates an extensive reutilisation of amino acids in protein metabolism. Nitrogen from the amino acids leaves the body via the urine in the form of urea, uric acid, creatinine, etc. Small quantities of nitrogen are also lost from faeces, sweat and other secretions, and from the skin, hair and nails. The body needs amino acids to compensate for these losses, and amino acids are also needed for protein synthesis during anabolism, *e.g.* growth, pregnancy and lactation.

It is usually assumed that almost all of the dietary nitrogen takes the form of protein. Dietary nitrogen x 6.25 is accepted as a reasonable approximation of the amount of protein in the diet, since the average protein contains 16% nitrogen and should be referred to as 'crude protein' (1). However, as the nitrogen content of amino acids varies from 7.7% to 32.2%, the nitrogen content of individual foods depends on the

amino acid composition. The conversion factor can vary from 5.30 (sunflower) and 5.83 (wheat) in foods that contain protein with high nitrogen content to 6.38 (milk) in foods containing proteins with lower nitrogen content (1). Nitrogen balance is the difference between nitrogen intake and nitrogen output. A negative nitrogen balance (*i.e.* losses greater than intake) is seen during fasting and starvation. A positive nitrogen balance is seen during active growth. Over a long-term basis, healthy adult subjects should have nitrogen equilibrium, *i.e.* intake and losses should be equal.

Amino acids from dietary protein are classified as either essential (indispensable) amino acids that cannot be synthesised in the human body and thus must be provided in the diet, or nonessential (dispensable) amino acids that are synthesised within the body from other amino acids (transamination) provided an adequate nitrogen supply. In man the essential amino acids are: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine. Histidine is considered essential although it does not fulfil the criterion of reducing protein deposition and inducing negative nitrogen balance when removed from the diet (2, 3).

In addition, the nonessential amino acids are: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine and tyrosine (4). Conditionally essential amino acids are amino acids the synthesis of which requires the provision of another amino acid, either as the carbon donor or as the donor of an accessory group, *e.g.* the sulphur group of cysteine (5). Under normal conditions they are synthesised in sufficient amounts but during certain conditions, such as prematurity (6) and during illness (7), synthesis cannot support the metabolic needs. These amino acids include arginine, cysteine, glutamine, glycine, proline and tyrosine.

Amino acids essential for adults and the elderly are also essential for infants and children.

Dietary proteins differ in their nutritional quality. This reflects the differences in amino acid composition of proteins and the amount of each amino acid. High-quality protein indicates an optimal amino acid composition of dietary protein according to the human needs for each amino acid. Dietary proteins of animal origin (meat, fish, milk, and egg) are considered high quality and are almost fully absorbed, while dietary proteins of plant origin (cereals and vegetables) are considered lower quality due to their less optimal amino acid composition and lower digestibility (legumes and oilseeds). Dietary proteins of plant origin complement each other and the quality of the protein is usually not a nutritional problem in complex meals. A high intake of dietary fibre may inhibit the digestion of dietary proteins. The typical Nordic mixed

diet includes meat, milk and fish, and thus the quality of the dietary proteins is usually high. In practice the differences in quality between proteins may be unimportant in diets containing a mixture of proteins (8).

Protein constitutes 15–20% of the human body, which corresponds to approximately 12 kg in a person with a body weight of 70 kg.

Requirement and recommended intake

FAO/WHO/UNU defines the protein requirement of an individual as 'the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in persons maintaining energy balance at modest levels of physical activity' (9). FAO/WHO/UNU sets the average daily protein requirement for adults to 0.6 g good-quality protein per kg body weight based on published data from short-term (1–2 weeks) and long-term (1–3 month) nitrogen balance studies. The requirements of protein and especially amino acids are under heavy discussion. The controversy is focusing on quality rather than quantity, since diets throughout the industrialised world provide much higher intakes of both protein and essential amino acids than considered adequate. Measurement of nitrogen balance is considered relatively imprecise but despite its limitations the nitrogen balance method is considered the main technique to evaluate the minimum protein needs in studies over relatively short periods (10). A new approach that estimates the requirements of amino acids determined by tracer balance has proposed considerably higher requirements than FAO/WHO/UNU (11). Millward and Rivers (12) have proposed a diurnal cycling model that combines nitrogen balance and amino acid tracer balance studies. However, methods deducing requirements from amino acid oxidation (tracer balance method) also have limitations (13).

Protein deficiency results in oedema, muscle weakness and changes in hair and skin. Protein deficiency is often linked to energy deficiency, protein-energy malnutrition (PEM), as well as deficiency of other nutrients based on a general nutrition deficiency At present, there are no specific biochemical markers of protein deficiency. Based on anthropometry (BMI, skinfolds), weight loss and biochemistry, the prevalence of PEM has been estimated to be approximately 28% among 5,000 Swedish patients (14).

Adults

FAO/WHO/UNU recommends 0.75 g of good-quality protein per kg body weight per day based on an estimated average requirement (EAR) of 0.6 g per kg per day increased by two standard deviations (2×12.5%) to

allow for individual variability. The 2002 US recommendations (15) for protein are based on a meta-analysis of nitrogen balance studies (16) and cite an EAR of 0.65 g and RDA of 0.83 g good-quality protein for healthy adults with no significant differences according to adult age or sex. In addition FNB 2002 recommends an Acceptable Macronutrient Distribution Range (AMDR) of 10–35 percent of energy intake (E%) from protein.

Higher satiety after protein intake than after carbohydrate and fat intake has been shown, but not consistently (17). A recent meta-analysis of the effect on weight loss of high protein-low carbohydrate diets in the treatment of obesity showed that weight loss was associated with decreased energy intake and not with the macronutrient composition of the diet (18).

At reference energy intakes a protein intake of 0.8 g per kg body weight corresponds to 8–10 E% from protein. Most foods have a protein content higher than 10 E%, which makes it difficult to compose a diet with a protein content less than 10 E%. The average protein intake in the Nordic countries ranges from 14 E% to 18 E%.

According to the Nordic dietary habits, a protein intake corresponding to 10–20 E% is recommended. For food planning purposes the recommendation is 15 E%. This intake of protein should more than adequately meet the requirements for essential amino acids.

At very low energy intakes (< 6.5 MJ) a protein content above 15 E% may be necessary for food planning purposes.

Protein and physical exercise

Whether there is an increased protein requirement as a result of heavy physical exercise is still somewhat an ongoing matter of controversy. Physical exercise leads to an increased protein oxidation in the muscle in absolute terms. However, the contribution of protein to energy turnover is remarkably reduced in relation to that of fat and carbohydrate. As the body gives priority to covering its energy needs – even when protein turnover is increased – one must be certain that energy needs are met before discussing increased protein needs during physical exercise. Critical analysis of the background data in many studies that give support for increased protein needs indicates that energy needs are not met.

An increased demand for protein during physical exercise may be due essentially to three reasons: 1) increased muscle mass as result of training; 2) increased breakdown of muscle tissue and protein turnover during hard physical activity, especially endurance training; 3) increased gluconeogenesis from muscle protein if energy needs are not met, lead-

ing to muscle protein catabolism and negative nitrogen balance (19, 20). Studies using both the nitrogen balance technique and stable isotope technique have suggested that the daily protein requirement may be as high as 1.4–1.8 g per kg body weight in athletes with heavy training (21, 22, 23, 24). Recent long-term studies using stable isotope techniques (25, 26, 27) indicate, however, that there seems to exist a compensatory reduction in leucine oxidation during the recovery phase post-exercise, indicating a homeostatic response in order to conserve body protein, an effect that has not been observed in short-term studies (3–4 hours) on protein turnover during physical exercise (23, 24). Consequently, the fact that protein turnover is increased during exercise does not necessarily mean that protein need over a 24-hour period is increased in athletes, as protein utilisation may be improved and more efficient as a result of training (28, 29).

However even though physical training would double the daily protein requirements (as suggested by earlier studies), the daily energy needs would also be very high. Therefore, there are no data supporting the use of protein supplements in athletes consuming a variable, mixed diet (19, 20).

A protein intake corresponding to the intake for adults, *i.e.* 10–20 E%, is recommended since no special consideration is given to physical activity levels in the present dietary recommendations.

Elderly

Chronic disease is more frequent in the elderly, and may lead to periodic temporary losses of body protein through fever or simply loss of appetite. Such losses have to be replaced from the diet and thus represent an added need for dietary protein (30). In addition, older individuals exhibit a gradual loss of lean body mass with age (sarcopenia) estimated to be 0.5 mg nitrogen per kg body weight per day (10), not only as a result of decreased physical activity (31).

In 1985 FAO/WHO/UNU concluded that healthy elderly people have a dietary protein requirement that is not less than the need established for younger adults, and that this amount is higher than for younger adults in relation to lean body mass 'because it is an accepted fact that protein utilisation is less efficient in the elderly'. However the basis of this 'accepted fact' is unclear, since little is known about the extent and regulation of protein utilization in humans (32). Previous balance studies in the elderly are conflicting, with some supporting (33, 34) and others questioning (35, 36, 37) the adequacy of 0.8 g protein per kg body weight per day. A retrospective re-analysis (38) of shorter-term nitrogen balance studies (33, 34, 35) supports the conclusion that this recommen-

dation may not be adequate for many elderly persons, while others have questioned this conclusion (10, 32). Recent long-term nitrogen balance studies have found protein needs at or above 0.8 g (39, 40, 41). Only two studies specifically addressed the question of the extent of age-related changes in protein requirements by studying both younger and older subjects (33, 42, 43). No differences were identified.

A recent balance study indicated that in healthy elderly individuals there is a lower metabolic demand for dietary protein and no significant impairment in protein utilization and thus on average a lower apparent protein requirement than in younger subjects (44). A meta-analysis by Rand *et al.* (16) did not find age-related differences in requirements and the recommendation for adults +51 years of age. Thus, the data to establish an average protein requirement and a suggested safe and adequate protein allowance for older persons are conflicting.

A protein intake corresponding to the intake for adults, *i.e.* 10–20 E%, is recommended since this amount also seems abundant for the elderly.

For food planning purposes for elderly people with a very low energy intake (< 6.5 MJ), the protein intake should be at least 1.0 g protein per kg body weight per day.

Infants and children

Recommended protein intakes in infants and children are based on the factorial method. The calculation is based on estimates of the need for maintenance and growth, the efficiency of conversion from dietary protein to body protein and intra-individual variation in growth. There is, however, considerable discussion about the appropriate values to use for these calculations during the first year of life, leading to large differences in the recommendations for protein intake during the first year of life, especially the first 6 months. The 2002 US recommendations (15) give an adequate intake (AI) of 1.52 g protein per kg body weight for the whole 0–6 month period, and a RDA of 1.5 g protein per kg body weight for the 6–12 month period. For comparison, the revised estimates of the FAO/WHO/UNU 1985 figures (45) decline gradually from 2.7 g/kg during the first month of life to 1.0 g/kg for the 6–12 month period. These figures were used as the 1996 NNR. For the present NNR no adequate intake is given for the first 6 months. During this period infants are either breast fed or receive infant formula. The protein content of breast milk is considered adequate in term infants and the protein content of infant formula is regulated by an EU directive. According to the current directive (91/321/EEC), the protein content of an infant formula should be between 0.45 and 0.7 g/100 kJ. For the 6–11 month and 1–1.9 year age groups, the new adequate intakes are equal to 1996 NNR, which

were based on the revised estimates of the FAO/WHO/UNU 1985 figures (45), and close to new values from other European countries (46, 47).

In relation to body weight for children between 2 and 18 years of age, there is agreement among the values in recent recommendations (15, 46, 47) and these values have been used in the current adequate intake (*Table 13.1*).

If the recommended protein intakes are combined with the average energy requirements for the same sex and age groups, the protein energy percentage necessary to cover the adequate protein intake can be calculated. At 6 months this value is 5.3 E%, followed by a decline to 4.3% at two years. Thereafter, there is a gradual increase to 7.3 E% for boys and 8.7 E% for girls at the age of 17 years.

During the first 1–2 years of life, the protein E% increases considerably when the infant gradually changes from breast milk with an E% about 5 to the family diet with an E% typically around 15. The average protein E% at the age of 12 months in selected European countries is quite high, between 15% and 19.5 E% (48).

In NNR 1996 the recommended protein E% in the 6–11 month period was 7–10 E%; this has been expanded in the present recommendation to 7–15 E%.

The reasons are that very few infants during the last months of infancy have an E% below 10, and also to balance the decrease in the recommendation for fat E% during this age in the present NNR.

The recommended protein E% for the 12–23 month period is the same as for the 1996 NNR, *i.e.* 10–15 E%.

Table 13.1 Adequate protein intake in infants and children per kg body weight *

Age	Protein g/kg BW
6–11 mo	1.1
12–23 mo	1.0
2–17 y	0.9

* The origins of the values are given in the text

Pregnant and lactating women

In pregnancy, the average protein requirement is increased to provide additional protein for deposition in the maternal (*i.e.* blood, uterus, breasts), foetal and placental tissues. Recent observational studies indicate a relationship between a low protein intake in late pregnancy and suppressed placental and foetal growth (49, 50). FNB (15) recommends an increase in the average intake of 25 g protein per day added to the non-pregnant allowance during the second and third trimester or 1.1 g

protein per kg body weight per day. Adaptive responses in nitrogen metabolism aimed at nitrogen and protein accretion by the mother and the foetus are evident in early gestation (51). In the NNR 1996, 6 g protein per day through pregnancy added to the non-pregnant allowance was recommended after correction for digestibility. The average protein intake of Nordic women is high and many women do not need to add protein to their diet during pregnancy. Since total energy expenditure changes little and weight gain is minor during the first trimester compared to in the non-pregnant woman, the additional energy and protein intake is recommended during the second and third trimester only. In NNR 2004 an energy intake of approximately 1.5 MJ and 2 MJ above the needs for non-pregnant women is recommended during the second and third trimester of pregnancy, respectively.

A protein intake of 10–20 E% throughout pregnancy will thus provide sufficient protein and is recommended to pregnant women.

During lactation the average protein requirement is increased in relation to the milk composition and volume. The average protein content of breast milk (nitrogen × 6.25) has been taken as 1.1 g protein* per 100 ml. During the first month the protein content is higher, approximately 1.3 g per 100 ml (9). The increased requirement of protein and energy is based on the average amount and content of breast milk. FAO/WHO/UNU suggests a safe level of extra protein intake of about 16 g per day during the first 6 months of lactation, 12 g per day during the second 6 months, and 11 g per day thereafter. FNB (15) recommends an increase in the average intake of 25 g protein per day added to the non-lactating allowance or 1.1 g protein per kg body weight per day.

For a woman who is exclusively breastfeeding and with a loss of the body fat accumulated during pregnancy of approximately 500 g per month, the increment of energy need is 1.8–2.2 MJ (52). During an increased energy intake of about 2.2 MJ, a protein content of 20 g corresponds to 15 E% from protein. Thus, a diet containing 10–20 E% during an increased energy intake of approximately 2 MJ should have sufficient protein content for lactating women.

During lactation the recommendation is also 10–20 E% from protein.

Upper intake levels

A high intake corresponding to two or three times more than recommended intake of protein has been associated with increased risk of

* *I.e.* crude protein. The real nitrogen conversion factor is 5.18 as approximately 25% of the nitrogen content in breast milk is derived from non-protein nitrogen that corresponds to a real protein content of 0.8 g protein per ml.

juvenile diabetes and adiposity in children, increased calcium losses and increased homocysteine levels (53). A high intake of protein has also been associated with progression of renal disease, but at present there is no evidence of impaired renal function in healthy individuals (53, 54). A high intake of protein increases the calcium losses via urine but there is no evidence of an increased risk of osteoporosis provided adequate calcium intake (55, 56). Recent analyses found no influence on calcium balance in young adults but improved balance in the elderly with increased protein intake (57), and bone mineral density improved by increased protein intake in elderly subjects supplemented with calcium and vitamin D, but no association between protein intake and change in bone mineral density in un-supplemented subjects (58). In addition, a study found (59) that a high protein intake (24 E%) was associated with a decreased risk of ischaemic heart disease compared to a lower protein intake (15 E%). There is no evidence of adverse effects of an intake of 15–20 E% from protein, and a recent meta-analysis found no adverse effects on health at a protein intake up to 25 E% (60). However, there does not seem to be any advantage at this level and there is little long-term information on the health effects (61). Thus, there seems to be a need for a better understanding of the functional implications of dietary protein intake, which might lead to protein requirements based on optimal health.

Considering the recommendations for fat and carbohydrates, the protein intake of adults should not exceed 20 E% from protein.

A high protein intake results in a high renal solute load. However, it is only during the first months of life that the kidneys cannot handle a high solute load (62). It has been suggested that a high protein intake during the first years of life can increase the risks of obesity later in childhood (63), but the present evidence is not convincing. However, in a recent study from Iceland, 9–12 month olds with the highest protein intake, *i.e.* more than 17 E%, had higher BMI at the age of 6 years than their peers consuming less protein (64).

Although the possible negative consequences of a high protein intake are not clearly demonstrated, the following upper limits for protein intake are suggested, assuming sufficient intake of other nutrients: 0–6 mo: 10 E%, 6–12 mo: 18 E%, and above 1 y: 20 E%.

Dietary sources and intake

Dietary proteins are found in almost all foods of animal and plant origin. Meat, fish, milk and eggs have a high content of protein of high quality. Pulses, nuts and seeds also have a high protein content, which

makes them important sources of proteins in vegetarian diets, especially for vegans who also exclude milk and eggs from their diet.

The average protein intake is high in the Nordic countries, ranging from 14 E% in Denmark to 18 E% in Iceland.

According to the Danish National Dietary Surveys 1995 and 2001 (65), the average dietary intake of protein among 4–14 year-old boys and girls was 14 E% in 1995 and 13 E% in 2000/01. Among healthy elderly people, the average protein intake is estimated at 71–97 g per day in men and 55–80 g in women, corresponding to 13–14 E% from protein (66). In the Danish elderly this corresponds to 0.98 g per kg body weight per day (65).

The Icelandic Dietary Survey 2002 shows that energy percent from protein increases with age, *i.e.* 15 E% for 15–19 year olds and 20 E% for 60–80 year olds (see *Chapter 43*).

REFERENCES

1. Hambraeus L, Lönnerdal B. Nutritional aspects of milk proteins. Chapter 12 in: Advanced dairy chemistry – 1 Proteins. 3rd edition Part B (eds. PF Fox & PLH McSweeney). Kluwer Academic/Plenum Publishers New York 2003. pp 605–645.
2. Kriengsinyos W, Rafii M, Wykes LJ, Ball RO, Pencharz PB. Long-term effects of histidine depletion on whole-body protein metabolism in healthy adults. J Nutr 2002; 132: 3340–3348.
3. Laidlaw SA, Kopple JD. Newer concepts of the indispensable amino acids. Am J Clin Nutr 1987; 46: 593–605.
4. Crim MC, Munro HN. Proteins and amino acids. In: Shils ME, Olson JA (eds). Modern nutrition in health and disease. Eighth edition. 1994. Lea & Fibiger. Malvern, USA.
5. Reeds PJ. Dispensable and indispensable amino acids for humans J Nutr 2000; 130: 1835S–1840S.
6. Uauy R, Greene HL, Heird WC 1993. Conditionally essential nutrients. In: Nutritional needs of the preterm infant (Tsang *et al.* eds.) Willims and Wilkins, Baltimore, p 267–280.
7. Williams JZ, Abumrad N, Barbul A. Effect of specialized amino acid mixture on human collagen deposition. Ann Surgery 2002; 236: 369–375.
8. Waterlow JC. The requirements of adult man for indispensable amino acids. Eur J Clin Nutr 1996; 50, suppl.1: S151–S179.
9. FAO/WHO/UNU. Energy and protein requirements. WHO Techn Rep Series 724. WHO, Geneva, 1985.
10. Millward DJ, Roberts SB. Protein requirements of older individuals. Nutr Res Rev 1996; 9: 67–87.
11. Young VR. Adult amino acid requirements: the case for a major revision in current recommendations. J Nutr 1994; 124: 1517S–1523S.
12. Millward DJ, Rivers JP. The nutritional role of indispensable amino acids and the metabolic basis for their requirements. Eur J Clin Nutr 1988; 42: 367–393.
13. Fuller MF, Garlick PJ. Human amino acid requirements: can the controversy be resolved? Annu Rev Nutr 1994; 14: 217–241.
14. Socialstyrelsen. Näringsproblem i vård och omsorg. Prevention och behandling. SoS-rapport 2000: 11.
15. FNB (Food Nutrition Board). Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients). 2002.

16. Rand WR, Pellett PL, Young VR. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. Am J Clin Nutr 2003; 77: 109–127.
17. Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. Am J Clin Nutr 2003; 77: 91–100.
18. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, Bravata DM. Efficacy and safety of low-carbohydrate diets: a systematic review. J Am Med Assoc 2003; 289: 1837–1850.
19. Lemon PW. Effects of exercise on protein metabolism. In: Maughan RJ (ed.). Nutrition in sport. Oxford: Blackwell science, 2000: 133–152.
20. Hambraeus L, Branth S, Raben A. Nutrition and fluid intake with training. Capter 2.4 in Textbook of Sports Medicine. (eds M Kjaer, M Krogsgaard, P Magnusson, L Engebretsen, H Roos, T Takala, S L-Y Woo) Blackwell Sciences Ltd Oxford 2003.
21. Tarnopolsky MA, MacDougall JD, Atkinson SA. Influence of protein intake and training status on nitrogen balance and lean body mass. J Appl Physiol 1988; 64: 187–193.
22. Friedman JE & Lemon PW. Effect of chronic endurance exercise on retention of dietary protein. Int J Sports Med 1989; 10: 118–123.
23. Lemon PW, Tarnopolsky MA, MacDougall JD, Atkinson SA. Protein requirements and muscle mass/strength changes during intensive training in novise bodybuilders. J Appl Physiol 1992; 73: 767–775.
24. Tarnopolsky MA, Atkinson SA, MacDougall JD, Cheskey A, Phillips S, Schwarcz HP. Evaluation of protein requirements for trained strength athletes. J Appl Physiol 1992; 73: 1986–1995.
25. El-Khoury AE, Forslund A, Olsson R, Branth S, Sjödin A, Andersson A, Atkinson A, Selvaraj A, Hambreus L, Young VR. Moderate exercise at neutral energy balance does not affect total 24h leucine oxidation or nitrogen excretion in healthy men. Am J Physiol 1997; 273: E394–E407
26. Forslund A, Hambraeus L, Olsson R, El-Khoury AE, Yu-M & Young VR: The 24h status of whole body leucine and urea kinetics at 'normal' and 'high' protein intakes, with exercise, in healthy adults. Am J Physiol 1998; 275 (Endocrin Metabol 38) E 310–320.
27. Forslund AH, El-Khoury AE, Olsson RM, Sjödin AM, Hambreus L,Young V. Effect of protein intake and physical activity on 24h pattern and rate of macronutrient utilization. Am J Physiol 1999; 276. E964–E976.
28. Butterfield, GE & Calloway, DH: Physical activity improves protein utilization in young men. Br J Nutr 1984; 51; 171–184.
29. Rennie MJ, Tipton KDF: Protein and amino acid metabolism during and after exercise and the effects of nutrition. Annu Rev Nutr 2000; 20; 457–483
30. Munro H. Protein nutriture and requirement in elderly people Biblthca Nutr Dieta 1983; 33: 61–74.
31. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Fiatarone Singh MA. Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. Am J Clin Nutr 2002; 76: 473–481.
32. Millward DJ, Fereday A, Gibson N, Pacy PJ. Aging, protein requirements, and protein turnover. Am J Clin Nutr 1997; 66: 774–786.
33. Cheng AHR, Gomez A, Bergan JG, Lee T, Monckeberg F, Chichester CO. Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination wheat-soy-milk mixture. Am J Clin Nutr 1978; 31: 12–22.
34. Zanni E, Calloway DH, Zezulka AY. Protein requirements of elderly men. J Nutr1979; 109: 513–524.
35. Uauy R, Scrimshaw NS, Young VR. Human protein requirements: nitro-

gen balance response to graded levels of egg protein in elderly men and women. Am J Clin Nutr 1978; 31: 779–785.
36. Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR. Human protein requirements: assessment of the adequacy of the current Recommended Dietary Allowance for dietary protein in elderly men and women. Am J Clin Nutr 1982; 35: 6–14.
37. Bunker VW, Lawson MS, Stansfield F, Clayton BE. Nitrogen balance studies in apparently healthy elderly people and those who are housebound. Brit J Nutr 1987; 57: 211–221.
38. Campbell WW, Crim MC, Dallal GE, Young VR, Evans WJ. Increased protein requirements in elderly people: new data and retrospective reassessments. Am J Clin Nutr 1994; 60: 501–509.
39. Castaneda C, Charnley JM, Evans WJ, Crim MC. Elderly women accomodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. Am J Clin Nutr 1995; 62: 30–39.
40. Morse MH, Haub MD, Evans WJ, Campbell WW. Protein requirement of elderly women: nitrogen balance responses to three levels of protein intake. J Gerontol 2001; 56A: M724–M730.
41. Campbell WW, Trappe TA, Wolfe RR, Evans WJ. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. J Gerontol 2001; 56A: M373–M380.
42. Pannemans DLE, Halliday D, Westerterp KR. Whole body protein turnover in the elderly men and women: responses to two levels of protein intake. Am J Clin Nutr 1995; 61: 33–38.
43. Pannemans DLE, Halliday D, Westerterp KR, Kester ADM. Effect of variable protein intake on whole-body protein turnover in young men and women. Am J Clin Nutr 1995; 61: 69–74.
44. Fereday A, Gibson NR, Cox M, Pacy PJ, Millward DJ. Protein requirements and aging: metabolic demand and efficiency of utilization. Brit J Nutr 1997; 77: 685–702.
45. Dewey KG, Beaton G, Fjeld C, Lonnerdal B, Reeds P. Protein requirements of infants and children. Eur J Clin Nutr 1996; 50 (Suppl 1): S119–S147.
46. Gezondheidsraad. Dietary reference intakes. Energy, proteins fats and digestible carbohydrates. Publication No. 2001/19E, The Hague, 18 July 2001.
47. DACH. Referenzwerte für die Nährstoffzufuhrt. 1. Auflage. Deutsche Gesellschaft für Ernährung, Österreichsche Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung, Schweizerische Vereinung für Ernährung, Umschau Braus, Frankfurt am Main 2000.
48. Rolland-Cachera MF, Deheeger M, Bellisle F. Increasing prevalence of obesity among 18-year-old males in Sweden: evidence for early determinants. Acta Paediatr 1999; 88: 365–367.
49. Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. Brit Med J 1996; 312: 410–414.
50. Godfrey K, Barker DJ, Robinson S, Osmond C. Maternal birth weight and diet in pregnancy in relation to the infant's thinness at birth. Br J Obstet Gynaecol 1997; 104: 663–667.
51. Kalhan SC. Protein metabolism in pregnancy Am J Clin Nutr 2000; 71 (Suppl): 1249S–1255S.
52. Dewey KG. Energy and protein requirements during lactation. Annu Rev Nutr 1997; 17: 19–36.
53. Becker W, Hambræus L, Samuelson G. Hög proteinhalt i svensk mat. En hälsorisk? Läkartidningen 1996; 93: 37–40.
54. Klahr S. Effects on protein intake on progression of renal disease. Ann Rev Nutr 1989; 9: 87–108.
55. Heaney RP. Protein intake and bone health: the influence of belief systems on the conduct of nutritional science. Am J Clin Nutr. 2001; 73: 5–6.

56. Dawson-Hughes B. Interaction of dietary calcium and protein in bone health in humans. J Nutr. 2003; 133: 852S–854S.
57. Pannemans DLE, Schaafsma G, Westerterp KR. Calcium excretion, apparent calcium absorption and calcium balance in young and elderly subjects: influence of protein intake. Brit J Nutr 1997; 77: 721–729.
58. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr 2002; 75: 773–779.
59. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999; 70: 221–227.
60. Astrup A, Ryan L, Grunwald GK, Storgaard M, Saris W, Melanson E, Hill JO. The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of *ad libitum* low-fat dietary intervention studies. Brit J Nutr 2000; 83, supp 1: S25–S32.
61. Eisenstein J, Roberts SB, Dallal G, Saltzman E. High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data. Nutr Rev 2002; 60 (I): 189–200.
62. Fomon SJ. Nutrition of normal infants. St.Louis: Mosby 1993.
63. Rolland-Cachera MF, Deheeger M, Akrout M, Belliste B. Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 month to 8 years of age. Int J Obes 1995; 19: 573–578.
64. Gunnarsdóttir I, Thorsdottir I. Relationship between growth and feeding in infancy and body mass index at the age of 6 years. Int J Obes Relat Metab Disord 2003; 27: 1523–1527.
65. Fagt S, Matthiessen J, Trolle E, Lyhne N, Christensen T, Hinsch H-J, Hartkopp H, Biltoft-Jensen A, Møller A, Daae A-S. The Danish Dietary Survey 2000–2001. In Danish. Abstract in English [2. danskernes kostvaner 2000–2001. Udviklingen i danskernes kost – forbrug, indkøb og vaner]. Fødevardirektoratet, Afdelingen for Ernæring. Fødevarerapport 2002:10.
66. Pedersen AN. Nutritional status of 80-year-old Danish men and women – and the relation to functional ability. [80-åriges ernæringsstatus – og relationen til fysisk funktionsevne. 80-årsundersøgelsen 1994/95]. PhD dissertation. (In Danish, abstract in English). Fødevaredirektoratet. Københavns Universitet 2001.

CHAPTER 14

Alcohol

Alcohol (ethanol) is generally consumed as beer (2.5–6 vol-% alcohol), wine (about 12%) or spirits (about 40%). The energy liberated upon oxidation of alcohol in the organism corresponds to 29 kJ per g. At high alcohol consumption the energy efficiency appears to be lower, with relatively higher heat dissipation than with the other energy yielding nutrients (1). Alcohol is efficiently absorbed through passive diffusion in the intestine and is distributed in the total water compartment of the body. Most of it is oxidized in the body but a small amount (5–10%) is lost with expired air and in the urine.

Alcohol is a toxic substance that affects all organs of the body. Both acute and chronic alcohol damage contribute significantly to morbidity and mortality in the population. From a public health perspective it is important to bear in mind that overall consumption is a main determinant of the alcohol-related harm rates in the population (2). The negative health effects of alcohol are primarily determined by the total amount of alcohol to which the body is exposed. This means that alcohol damage may develop in individuals who have not been visibly drunk. It is likely that a daily consumption of 70 g alcohol per day will result in alcohol damage (3). Alcohol is associated with blood pressure in a J-shaped manner (more marked in women than in men) (4). An intake as small as 25 g per day may be followed by an increase in blood pressure (5).

In the Nordic countries, alcohol accounts for about 5% of energy intake in persons above 15 years of age, somewhat higher in Denmark than in the other countries. The intake is very unevenly distributed and about half the population is responsible for most of the consumption. Estimates suggest that every third man and tenth woman obtain as much as 10% of energy intake from alcohol. A moderate intake of alcohol may generally (6, 7), but not always (8), provide a surplus of energy in addition to that from food and may thus contribute to overweight.

Replacing part of the food intake with alcohol reduces the quality of the diet, which often leads to a limited choice of food items with an increased proportion of fat and refined carbohydrate. In particular the

consumption of milk products, fruits and vegetables appears to decrease when the intake of alcohol is increased. Some exceptions to this pattern may be noted. A Danish study showed a strong association between fruit and vegetable consumption and wine intake (9). A high alcohol consumption may result in impaired absorption of nutrients and increased loss in the urine. From a nutritional point of view it is therefore reasonable to recommend moderation in alcohol intake. Among high alcohol consumers, nutritional status is always affected (10). In particular, deficiencies in ascorbic acid, thiamine, magnesium, phosphorus, vitamin D and protein are frequent (11, 12).

Alcohol consumption even in moderate amounts is associated with increased mortality among both men and women under the age of 45 years (13–17). For older age groups several epidemiological studies have shown that moderate intake of alcohol is associated with reduced mortality due to reduced incidence of cardiovascular diseases (14, 17) and stroke (18). However, mortality increases at high intake. In women, the decreased mortality has been observed in the age group above 50 and those at high risk of coronary heart disease (19). The preventive effect has been ascribed to the HDL cholesterol increasing effect of alcohol. In an intervention study it was shown that moderate alcohol intake (15 and 30 g per day), in addition to increasing HDL cholesterol also decreased LDL cholesterol and triacylglycerol (20). A reduction in C-reactive protein as a marker for systemic inflammation has also been observed after an intake of 40 g and 30 g alcohol per day for men and women respectively (21). Increased C-reactive protein is also considered a predictor for cardiovascular disease. In a Danish prospective study, the preventive effect was associated only with consumption of wine, while consumption of beer was neutral and that of spirits associated with increased mortality (22). It was suggested that this pattern could explain the U-formed association between alcohol intake and mortality. Antioxidants in red wine (flavonoids, tannins, polyphenols, etc.) might possibly explain the protective effect of wine. Furthermore, a large meta-analysis concludes that moderate wine consumption is inversely associated with vascular risk (23). On the other hand, a systematic review of a large number of different epidemiological studies concluded that the protective effect is connected to alcohol *per se* (24).

Quantity as well as frequency of alcohol consumption are important in the risk assessment of alcohol intake (26). Binge drinking is related to acute impairment and has numerous adverse health consequences compared to steady drinking (25–27).

Even in old age the relationship between alcohol and mortality is U-shaped (28), and the benefit is mainly found among elderly people

with coronary heart disease (17). The level at which the risk of mortality is lowest is age-related and reaches the highest intake of alcohol at age 65+ (17). On the other hand, elderly subjects may become more sensitive to alcohol because of the age-related changes in body composition (*i.e.* decreased body water and increased body fat). Light-to-moderate alcohol consumption has recently been shown to be associated with reduced risk of vascular dementia in elderly individuals in a prospective follow-up study (29).

The effect of alcohol on the risk of cardiovascular and other diseases is dependent on the distribution of other risk factors in the population. It is uncertain whether the beneficial effects of moderate alcohol consumption might apply only to individuals at risk of cardiovascular diseases. No studies have analysed the effect on mortality risk when subjects change their alcohol intake from abstention to a moderate intake. In addition, several other nutrients (*e.g.* fibre, certain unsaturated fatty acids, antioxidant vitamins) and physical activity have been shown to reduce the risk of cardiovascular mortality and morbidity. Therefore there is no good reason to increase alcohol consumption with the motivation of preventing cardiovascular disease.

The increased blood pressure and triacylglycerol may at least partly explain the increased cardiovascular mortality at high alcohol intake.

In the European Comparative Alcohol Study, no support was found for the notion that increases in *per capita* consumption of alcohol have any cardioprotective effect at the population level (2).

High alcohol intake has been associated with cancer in the mouth, pharynx and oesophagus (30) as well as with breast cancer (31, 32).

Foetal alcohol syndrome (FAS) is the most important identified cause of mental retardation in the Western world (33). FAS is characterised by defects in the central nervous system, growth retardation, facial deformities and malformations of different kinds. Based on self-recorded intake it has been shown that an intake above 30 g per day is associated with increased risk of FAS (34). A daily intake of > 25 g late in pregnancy has been shown to result in reduced birth weight (35). Alcohol intake during pregnancy is also associated with increased frequency of abortion. Although the effect of alcohol consumption during lactation has not been established, some studies (36), but not all (37), have suggested impaired development of infants whose mothers consumed alcohol when lactating. Reduced milk production (38), reduced milk intake (39) and sleep disturbances in the child (40) have been described. Because the lowest intake of alcohol not associated with health risk is unknown, pregnant and lactating women should abstain from alcohol.

Recommendation

Considering that alcohol has negative health effects and affects nutritional status, it is recommended to limit alcohol intake. Based on estimates of the maximal mortality risk reduction associated with moderate alcohol consumption (16, 17), the intake should not exceed 10 g (approximately 1 unit*) per day for women and 20 g (approximately 2 units*) per day for men. In relation to energy intake, the consumption of alcohol should not exceed 5 energy percent in adults.

Pregnant women, children and adolescents are recommended to abstain from alcohol.

* 1 unit is defined as 12 g alcohol (41) corresponding to the alcohol content in one bottle of beer (330 ml), one glass of wine (120 ml) or one glass of spirits (40 ml). The definition of a unit may differ in different countries from approximately 8 g to 12 g (17).

REFERENCES

1. Lieber CS. Metabolism and metabolic effects of alcohol. Med Clin North Am 1984; 68: 3–31.
2. Nordstrom T, Skog OJ. Alcohol and mortality: methodological and analytical issues in aggregate analyses. Addiction 2001; 96 (Suppl 1): S5–17.
3. Pequignot G, Tuyns AJ, Berta JL. Ascitis cirrhosis in relation to alcohol consumption. Int J Epidemiol 1978; 7: 113–20.
4. Klatsky AL, Friedman GD, Armastong MA. The relationship between alcoholic average use and other traits to blood pressure: a new Kaiser Permanente study. Circulation 1986; 73: 628–36.
5. Gordon T, Doyle JT. Alcohol consumption and its relationship to smoking, weight, blood pressure, and blood lipids. Arch Intern Med 1986; 146: 262–5.
6. Suter PM, Häsler E, Vetter W. Effects of alcohol on energy metabolism and body weight regulation: is alcohol a risk factor for obesity? Nutr Rev 1997; 55: 157–171
7. Buemann B, Toubro S, Astrup A. The effect of wine or beer *versus* a carbonated soft drink, served at a meal, on *ad libitum* energy intake. Int J Obes 2002; 26: 1367–1372.
8. Männistö S, Uusilato K, Roos E, Fogelholm M, Pietinen P. Alcohol beverage drinking, diet and body mass index in a cross-sectional survey Eur J Clin Nutr 1997; 51: 326–332
9. Tjonneland A, Grønbæk M, Stripp C *et al.* Wine intake and diet in a random sample of 48763 Danish men and women. Am J Clin Nutr 1999; 69: 49–54.
10. Lieber, CS. The influence of alcohol on nutritional status. Nutr Rev 1988; 46: 241–54.
11. Bøhmer T, Utzon P, Tallaksen CME. Skjørbuk med samtidig våt beriberi hos to pasienter. Tidsskr Nor Lægefor 1994; 114: 3181–3.
12. Halvorsen S, Jørgensen J, Skausig OB. Avitaminoser. I: Medicinsk årbog. København: Munksgaard, 1983: 111–9.
13. Andreasson S, Allebech P, Romelsjö A. Alcohol and mortality among young men: longitudinal study of Swedish conscripts. Br Med J 1988; 296: 1021–5.

14. Klatsky AL, Armstrong MA, Friedman GD. Alcohol and mortality. Ann Intern Med 1992; 117: 646–54.
15. Romelsjö A, Leifman A. Association between alcohol consumption and mortality, myocardial infarction, and stroke in 25-year follow-up of 49,618 young Swedish men. Br Med J 1999; 319: 821–822.
16. Rehm J, Gutjahr E, Gmel G. Alcohol and all-cause mortality: a pooled analysis. Contemp Drug Problems 2001; 28: 337–361.
17. White IR, Altmann DR, Nanchahal K. Alcohol consumption and mortality: modelling risks for men and women at different ages. Brit Med J 2002; 325: 191–197.
18. Reynolds K, Lewis JB, Nolen JDL, Kinney GL, Sathya B, He J. Alcohol consumption and risk of stroke. J Am Med Assoc 2003; 289: 579–588.
19. Fuchs CS, Stampfer MJ, Colditz GA, Giovannucci EL, Manson JE, Kawachi I, *et al.* Alcohol consumption and mortality among women. N Engl J Med 1995; 332: 1245–50.
20. Baer DJ, Judd JT, Clevidence BA *et al.* Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr 2002; 75: 593–9.
21. Sierksma A, van der Gaag MS, Kluft C, Hendriks HFJ. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur J Clin Nutr 2002; 56: 1130–1136
22. Grønbæk M, Deis A, Sørensen TI, Becker U, Schnohr P, Jensen G. Mortality associated with moderate intakes of wine, beer, or spirits. Br Med J 1995; 310: 1165–9.
23. Castelnuovo AD, Rotondo S, Iacoviello L, Donati MB, Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation, 2002; 105: 2836–44.
24. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine or spirits? Brit Med J 1996; 312: 731–6.
25. Tolstrup JS, Jensen MK, Tjønneland A, Overvad K, Grønbæk M. Drinking pattern and mortality in middle-aged men and women. Addiction 2004; 99: 323–30.
26. McElduff P, Dobson AJ. How much alcohol and how often? Population based case-control study af alcohol consumption and risk of a major coronary event. Brit Med J 1997; 317: 1159–1160.
27. Naimi TS, Brewer RD, Mokdad A, Denney C, Serdula MK, Marks JS. Binge drinking among US adults. J Am Med Assoc 2003; 289: 70–75.
28. Grønbæk M, Deis A, Becker U, Hein HO, Schnohr P, Jensen G, Borch-Johnsen K, Sørensen TIA. Alcohol and mortality: is there a U-shaped relation in elderly people? Age Ageing 1998; 27: 739–744.
29. Ruitenberg A, van Swieten JC, Witteman JCM, Mehta KM, van Duin CM. Alcohol consumption and risk of dementia: the Rotterdam study. Lancet, 2002; 359: 281–6.
30. Levnedsmiddelstyrelsen. Kost og kræft – rapport fra en arbejdsgruppe. Publikation 132. Søborg: Levnedsmiddelstyrelsen, 1986.
31. Schatzkin A, Longnecker MP. Alcohol and breast cancer. Where are we now and where do we go from here? Cancer 1994; 74 (Suppl 3): 1101–10.
32. Tjonneland A, Thomsen BL, Stripp C, Christensen J, Overvad K, Mellemkaer L, Gronbaek M, Olsen JH. Alcohol intake, drinking patterns and risk of postmenopausal breast cancer in Denmark: a prospective cohort study. Cancer Causes Control. 2003; 14: 277–284.
33. Abel EL. Fetal alcohol syndrome in families. Neurotoxicol Teratol 1988; 10: 1–2.
34. Graham JM Jr, Hanson JW, Darby BL, Barr HM, Streissguth AP. Independent dysmorphology evaluations at birth and 4 years of age for children exposed to varying amounts of alcohol in utero. Pediatrics 1988; 81: 772–8.

35. Larroque B. Alcohol and the fetus. Int J Epidemiol 1992; 21 (Suppl 1): S8–16.
36. Little RE, Anderson KW, Ervin CH, Worthingon-Roberts B, Clarren SK. Alcohol use during breastfeeding and infant mental and motor development at one year. N Engl J Med. 1989; 321: 425–430.
37. Little RE, Northstone K, Golding J; and the ALSPAC Study Team. Alcohol, breastfeeding, and development at 18 months. Pediatrics 2002; 109: 1–6.
38. Mennella JA. Short-term effect of maternal alcohol consumption on lactational performance. 1998; 22: 1389–92.
39. Mennella JA. Regulation of milk intake after exposure to alcohol in mother's milk. Alcohol Clin Exp Res 2001; 25: 590–3.
40. Mennella JA, Garcia-Gomez PL. Sleep disturbances after acute exposure to alcohol in mother's milk. Alcohol 2001; 25: 153–8.
41. Grønbæk MN, Iversen L, Olsen J, Becker PU, Hardt F, Sørensen TIA. Genstandsgrænser. Ugeskr Laeger 1997; 159: 5939–5945.

CHAPTER 15

Dietary antioxidants

Free radicals and other reactive oxygen and nitrogen species (ROS and RNS) are formed as a result of normal cellular oxidative metabolic reactions. Such molecules are also formed as a consequence of diseases (*e.g.* inflammations) and of tobacco smoke, environmental pollutants, natural food constituents, drugs, ethanol and radiation. If not quenched by antioxidants, these highly reactive compounds react with and potentially alter the structure and function of several cellular components, such as lipid-containing cell membranes, lipoproteins, proteins, carbohydrates, RNA and DNA.

Oxidative stress can result when the critical balance between generation of ROS or RNS and antioxidant defences is unfavourable. Compelling evidence has emerged in the past two decades demonstrating that such oxidative damage or oxidative stress is intimately involved in the pathophysiology of many seemingly unrelated types of disease. Thus, oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematosus, adult respiratory distress syndrome), ischaemic diseases (heart disease, stroke, intestinal ischaemia), cancer, haemochromatosis, acquired immunodeficiency syndrome (AIDS), emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological diseases (multiple sclerosis, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, muscular dystrophy), alcoholism, smoking-related diseases and many others (see references 1–5 for reviews).

A complex endogenous antioxidant defence system has been developed to counteract oxidative damage and oxidative stress. Such an antioxidant defence is essential for all aerobic cells. The antioxidant defence has both enzymatic and non-enzymatic components that prevent radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules and prevent mutations (4–6).

In addition to the endogenous antioxidants, diet may also contribute to antioxidant defence. For discussions of the roles of vitamin C, E and

selenium, see separate chapters. It has recently been appreciated, however, that plants also contain numerous additional compounds with antioxidant properties. The contribution of these dietary antioxidants is not well understood but they are potentially important modulators of several oxidative stress related diseases.

It is now globally accepted that a diet rich in fruits and vegetables reduces the risk of major oxidative stress related diseases. Thus, most countries in the world recommend an increased intake of fruit and vegetables, see the Swedish SBU Report 'Preventing Disease with Antioxidants' (7), the Danish (8) and Norwegian (9) report on fruit and vegetables and the report 'Food, Nutrition and the Prevention of Cancer: A Global Perspective' by the World Cancer Research Fund (10). There are several candidates for the protective compounds in fruits and vegetables, but antioxidants that are abundant in many fruits and vegetables have been regarded as the most likely candidate.

The dietary antioxidants present in fruits and vegetables may contribute to two important components of the antioxidant defence system: 1) the ability to scavenge or neutralize free radicals directly, and 2) the ability to induce endogenous antioxidants.

Compounds with ability to scavenge or neutralize free radicals

Carotenoids are ubiquitous in the plant kingdom, and as many as 1,000 naturally occurring variants have been identified. At least 60 carotenoids occur in fruit and vegetables commonly consumed by humans (6). Besides the provitamin A carotenoids, α- and β-carotene and β-cryptoxanthin, lycopene and the hydroxy-carotenoids (xanthophylls) lutein and zeaxanthin are the main carotenoids present in the diet. Their major role in plants is light harvesting as auxiliary components and quenching of excited molecules that may be formed during photosynthesis (4–6).

Phenolic compounds are also ubiquitous in dietary plants (6). They are synthesized in a large variety of forms belonging to several molecular families such as benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. Over 8,000 plant phenols have been isolated. Plant phenols are antioxidants by virtue of the hydrogen-donating properties of the phenolic hydroxyl groups (6).

Glutathione, which is the major cellular antioxidant, is present in abundant amounts in the diet, although it is not absorbed as such from the diet but broken down to its constituent amino acids on digestion. The dietary availability of sulphur amino acids can, however, modulate cellular glutathione production (4–6, 11–12).

It is likely that antioxidants from the molecular families described above with different chemical and biochemical properties recharge each other in an integrated manner. Such interaction has been proven *in vitro* for α-tocopherol, α-tocotrienol, ascorbic acid, lipoic acid and thiols by Packer and colleagues (11), but the concept could have much broader validity as suggested by Buettner (12). This raises the prospect that a variety of antioxidants are necessary to maintain the proper antioxidant defence system. Thus, the total amount or balance of antioxidants (*i.e.* electron- or hydrogen-donating reductants) in the diet may be a better concept than individual dietary antioxidants.

Different methods have been used to assess total antioxidant capacity of dietary plants (13–17). The results from such studies differ somewhat since the methods are built on different chemical properties of the antioxidants, and their ability to pick up both water- and fat-soluble antioxidants may differ. Foods identified as containing high levels of total antioxidants include several berries (such as blueberries, blackberries, strawberries and raspberries), fruits (pomegranates, grapes and oranges), nuts and seeds (walnuts and sunflower seeds), vegetables (kale, red cabbage and pepper) and drinks (green tea and red wine). It remains to be proven whether individuals eating high amounts of such antioxidant-rich dietary foods have a reduced, unchanged or increased risk of oxidative stress related diseases.

Compounds with ability to induce antioxidant enzymes

An additional antioxidant defence mechanism involves the induction of detoxification enzymes, including members of the glutathione S-transferase family, γ-glutamyl cysteine synthetase and NAD(P)H: quinone reductase (quinone reductase) (18–19). These enzymes are generally referred to as phase 2-enzymes because they catalyse conversion of xenobiotics, mutagenic metabolites or their precursors to compounds that are more readily excreted. It is believed that if benign or non-damaging plant compounds induce the phase 2 enzymes, cells are more readily able to 'neutralize' toxic agents such as free radicals and other toxic electrophiles when they appear.

The major plant compounds believed to be able to support antioxidant defence via this mechanism include the glucosinolates and several other sulphur containing plant compounds. Glucosinolates are widespread plant constituents, and it is believed that glucosinolate breakdown products (such as the isothiocyanate sulphoraphane) induce phase 2 enzymes and are therefore responsible for the protective effects shown by Brassica vegetables (6, 18–19). Allium vegetables contain a

number of other sulphur containing compounds that may also induce phase 2 enzymes. These compounds include the cysteine sulphoxides and the dithiolthiones. Like the glucosinolates, the active components found in Allium vegetables result from enzymatic degradation of the plant compounds (6, 18–19).

Dietary plants rich in compounds that induce phase 2 detoxification enzymes include broccoli, Brussels sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach and garlic. However, the evidence for phase 2 enzyme inductions at ordinary intake levels of plant foods in humans is limited, and the importance of this defence mechanism in the overall protection against oxidative damage is still uncertain.

Protective effects of antioxidant-rich diets in experimental animal and human studies

The suggested protective role of dietary antioxidants against oxidative damage is often based on extensive studies in cell culture systems. However, it is not always clear whether the effects in cell cultures, often observed with high doses of single compounds, can be readily extrapolated to humans. For example, studies in cell cultures most often do not reflect how the phytochemicals are processed *in vivo*, how they are absorbed and metabolised in the body, or whether they are available to the tissues of interest.

Some initial experimental dietary studies in animals and humans confirm the beneficial effects of dietary plants rich in either phytochemicals with ability to scavenge free radicals, or phytochemicals with ability to induce phase 2-enzymes. For example, Joseph and colleagues (20–21) have demonstrated that feeding of strawberries, spinach and blueberries to rats retards and reverses age-related neurodegeneration. Antioxidant-rich berries such as raspberries and strawberries (22–23) also efficiently inhibit carcinogenesis in experimental animals. Walnuts (24–25) and pomegranates (26–27), which are exceptionally rich in scavenging antioxidants, reduce LDL oxidation and atherosclerosis related processes in animals and humans.

It has also been observed that Brussels sprouts, onions and tomatoes are able to reduce the excretion of 8-oxo-deoxyguanosine, a biomarker for oxidative free radical DNA damage, into urine and to reduce the level of DNA damage in lymphocytes in animals and humans (28–32). However, examples also exist of rigidly controlled studies where little effect was observed after intervention with plant food items rich in antioxidants (33–34). Low bio-availability and extensive metabolism of

some plant-derived antioxidants may in part explain some of these discrepancies.

Human dietary intervention studies using disease endpoints provide the strongest evidence for an effect of antioxidant-rich dietary plants on disease risk. Such types of studies typically need to be large to have adequate statistical power. They should be monitored for many years and include a number of dietary permutations, and are consequently too expensive to be conducted by most scientists. Many phytochemicals also have overlapping mechanisms of action, and may have synergistic, additive, or inhibitory effects on each other. Additionally, it is quite difficult in practice to perform classical placebo-controlled studies, which are the hallmark for proving the efficiency of pharmaceutical drugs. Despite these challenges, human experimental dietary studies should be designed and executed to explore the relationship between dietary antioxidants and oxidative stress related diseases.

Intervention trials with antioxidant supplements

Results from intervention trials with antioxidant supplements such as vitamins E, vitamin C or β-carotene have not been conclusive with respect to their protective effect. Indeed, supplementation with antioxidants has often resulted in no effect or even adverse disease outcomes in clinical trials.

Albanes *et al.* (35) in the ATBC Study randomly assigned 29,133 Finnish men aged 50–69 years who smoked five or more cigarettes daily to receive α-tocopherol (50 mg), β-carotene (20 mg), α-tocopherol and β-carotene, or a placebo daily for 5–8 years (median 6.1 years). Disappointingly, however, the results showed that β-carotene supplementation was associated with about a 20% increase in lung cancer risk. Rapola *et al.* (36) studied the frequency of major coronary events in 1,862 men enrolled in the ATBC Study who had a previous myocardial infarction, and observed that there were significantly more deaths from fatal coronary heart disease in the beta-carotene groups than in the placebo group.

Similar findings were observed in the CARET study by Omenn *et al.* (37), who tested a combination of 30 mg β-carotene and 25,000 IU retinyl palmitate taken daily against a placebo in 18,314 men and women at high risk of developing lung cancer. The CARET intervention was stopped 21 months early because of clear evidence of no benefit and substantial evidence of possible harm; there were 28% more lung cancers and 17% more deaths in the active intervention group that was administered the combination of beta-carotene and retinyl palmitate.

The CARET study also observed that the active treatment group had a 26% increase in relative risk of death from cardiovascular disease (38).

Adverse effects have also been observed in three additional studies. In a study which included 160 patients with coronary disease, Brown *et al.* (39) observed that antioxidants (100 mg selenium, 1 g vitamin C, 800 IU vitamin E and 25 mg β-carotene) had no effect alone, but attenuated the protective effects of simvastatin on both lipid markers and clinical endpoints. Potential adverse effects of antioxidants (800 IU vitamin E and 1 g vitamin C per day) were also observed by Water *et al.* (40) studying coronary atherosclerosis in 423 postmenopausal women with coronary stenosis. Finally, Graat *et al.* (41) studied the effect of antioxidant supplement on immune response in 652 non-institutionalised individuals aged 60 years or older. They observed that the individuals treated with 200 mg vitamin E per day had an increased severity of infections compared to the controls.

Several studies have observed no clinical effects of antioxidant treatment. For example, the MRC/BHF Heart Protection Study (42) which included 20,536 adults with coronary disease, observed that an antioxidant mixture (600 mg vitamin E, 250 mg vitamin C and 20 mg β-carotene) improved plasma biomarkers, but had no effects on clinical endpoints. Furthermore, the HOPE study (43) in which a total of 9,541 men and women 55 years of age and older who were at high risk for cardiovascular events were enrolled, observed no significant effects of 400 IU vitamin E per day for a mean of 4.5 years.

Positive effects on clinical endpoints have been observed in two studies. The CHAOS study (44) (1,035 patients with coronary atherosclerosis who either received 800 IU vitamin E or placebo) observed that vitamin E reduced the rate of non-fatal myocardial infarction after 1 year of treatment. In addition, in the ASAP study (45) (N = 520) retarded progression of carotid atherosclerosis was observed in men, but not in women, after treatment with 182 mg α-tocopherol and 500 mg vitamin C per day for three years.

Recommendations

Antioxidant supplements

In summary, although experimental studies in cell cultures and animals have indicated that antioxidants such as β-carotene, ascorbic acid or α-tocopherol may reduce oxidative stress, and oxidative stress related diseases, the human studies published so far do not support a beneficial effect of antioxidant supplements. Therefore Governmental and non-governmental organizations such as The Swedish Council on Technol-

ogy Assessment in Health Care (SBU) (7), the US Preventive Task Force (46), the US Institute of Medicine (Dietary Reference Intake) (47), the Danish Veterinary and Food Administration (48), the American Heart Association (49) and the report 'Food, Nutrition and the Prevention of Cancer: A Global Perspective' by the World Cancer Research Fund (10) do not recommend intakes of single or combinations of supplemental antioxidants.

Antioxidant-rich foods

It is generally recommended that one should 'eat 400–800 grams or five or more portions a day of a variety of vegetable and fruits (pulses, tubers and starchy roots not included) all year round' (7–10). However, there is insufficient scientific evidence to show that antioxidative mechanisms are involved in the protective effects of fruits and vegetables. Recommendations for specific antioxidant-rich fruits and vegetables beyond the ordinary dietary recommendations can therefore not be given at this point.

REFERENCES

1. McCord JM. The evolution of free radicals and oxidative stress. Am. J. Med. 2000; 108: 652–59.
2. Beckman KB Ames BN. The free radical theory of aging matures. Physiol. Rev. 1998; 78: 547–81.
3. Sies H. Oxidative stress: Oxidants and antioxidants. Exp. Physiol. 1997; 82: 291–95.
4. Gutteridge JM, Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann. N.Y. Acad. Sci. 2000; 899: 136–47.
5. Halliwell B. Antioxidants in human health and disease. Annu. Rev. Nutr. 1996; 16: 33–50.
6. Lindsay DG, Astley SB. European research on the functional effects of dietary antioxidants – EUROFEDA. Mol. Aspects Med. 2002; 23: 1–38.
7. Isaksson B, Andersson C, Asplund K, von Bahr C, Borgström B, Boström H, Dahlgren H, Hallerby N, Marké LÅ, Ribohn M, Werkö L, Engström-Laurent A, Lingnert H, Åkerlund B. Preventing Disease with Antioxidants. SBU-reports 135/1, 135/2: 1, 135/2: 2, Stockholm 1997.
8. Ovesen L, Lyhne Andersen N, Dragsted L, Godtfredsen J, Haraldsdottir J, Stender, S, Sølling K, Tjønneland A, Trolle, E. Frugt, grønt og helbred, Fødevarerapport 22, Fødevaredirektoratet, Søborg, 2002.
9. Blomhoff R, Lande G, Ose T. Nye anbefalinger for inntak av frukt og grønnsaker. Statens Ernæringsråd, Oslo, 1995.
10. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: A Global Perspective. Washington DC: American Institute for Cancer Research, 1997.
11. Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. J. Nutr. 2001; 131: 369S–373S.
12. Buettner GR. The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol and

ascorbate. Arch. Biochem. Biophys. 1993; 300: 535–543.
13. Miller NJ, Rice-Evans CA. Spectrophotometric determination of antioxidant activitry. Redox. Rep. 1996; 2: 161–68.
14. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Anal. Biochem. 1996; 239: 70–76.
15. DeLange RJ, Glazer AN. Phycoerythrin fluorescence-based assay for peroxyl radicals: A screen for biologically relevant protective agents. Anal. Biochem. 1989; 177: 300–306.
16. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Rad. Biol. Med. 1993; 14: 303–311.
17. Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerod H, Andersen LF, Moskaug OJ, Jacobs DR Jr, Blomhoff R. A systematic screening of total antioxidants in dietary plants. J Nutr. 2002; 132: 461–71.
18. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. USA 1997; 94: 10367–72.
19. Talalay P. Chemoprotection against cancer by induction of phase 2 enzymes. Biofactors 2000; 12: 5–11.
20. Youdim KA, Joseph JA. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions; a multiplicity of effects. Free Radic. Biol. Med. 2001; 30: 583–94.
21. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC. Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with diets supplemented with blueberry, spinach or strawberry dietary supplementation. J. Neurosci. 1999; 19: 8114–21.
22. Kretsty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, Blackwood M, Stoner, GD. Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. Cancer Res. 2001: 61: 6112–19.
23. Xue H, Aziz RM, Sun N, Cassady JM, Kamendulis LM, Xu Y, Stoner GD, Klaunig JE. Inhibition of cellular transformation by berry extracts. Carcinogenesis 2001; 22: 351–56.
24. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit *in vitro* human plasma and LDL oxidation. J. Nutr. 2001; 131: 2837–42.
25. Sabate J. Nut consumption, vegetarian diets, ischemic heart diseas risk and all-cause mortality: Evidence from epidemiological studies. J. Nutr. 1999; 70: 500–3.
26. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, Hayek T, Presser D, Fuhrman B. Pomegranate juice consumption reduces oxidative stress, atherogenic modification to LDL, and platelet aggregation: Studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am. J. Clin. Nutr. 2000; 71: 1062–76.
27. Kaplan M, Hayek T, Raz A, Coleman R, Dornfeld L, Vaya J, Aviram M. Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. J. Nutr. 2001; 131: 2082–89.
28. Verhagen H, de Vries A, Nijhoff WA, Schouten A, van Poppel G, Peters WH, van den Berg H. Effect of Brussels sprouts on oxidative DNA-damage in man. Cancer Lett. 1997; 114: 127–30.
29. Verhagen H, Poulsen HE, Loft, S, van Poppel G, Willems MI, van Bladeren PJ. Reduction of oxidative DNA-damage in humans by brussels sprouts. Carcinogenesis 1995; 16: 969–70.
30. Lean MEJ, Noroozi M, Kelly I, Burns J, Talwar D, Sattar N, Crozier A. Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNS. Diabetes 1999; 48: 176–81.

31. Boyle SP, Dobson VL, Duthie SJ, Kyle JAM, Collins AR. Absorption and DNA protective effects of flavonoid glycosides from an onion meal. Eur. J. Nutr. 2000; 39: 213–23.
32. Lampe JW Health effects of vegetables and fruits: Assessing mechanisms of action in human experimentsl studies Am. J. Clin. Nutr. 1999; 70: 475–90.
33. van het Hof KH, de Boer HSM, Wiseman SA, Lien N, Weststrate JA, Tijburg LBM. Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. American Journal of Clinical Nutrition 1997; 66, 1125–32.
34. Young JF, Dragsted LO, Haraldsdóttir J, Daneshvar B, Kall MA, Loft S, Nilsson L, Nielsen SE, Mayer B, Hyunh-Ba T, Hermetter A, Sandström B. Green tea extract only affects markers of oxidative status postprandially: Lasting antioxidant effect of flavonoid-free diet, Br. J. Nutr. 2002; 87, 343–55.
35. Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea, JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: Effects of base-line characteristics and study compliance. J. Natl. Cancer Inst. 1996; 88: 1560–70.
36. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infraction. Lancet 1997; 349: 1715–20.
37. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Cherniack MG, Brodkin CA, Hammar S. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J. Natl. Cancer Inst. 1996; 88: 1550–59.
38. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N. Engl. J. Med. 1996; 334: 1150–55.
39. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. New Engl. J. Med. 2001; 345: 1583–92.
40. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, Ouyang P, Thompson P, Tardif JC, Higginson L, Bittner V, Steffes M, Gordon DJ, Proschan M, Younes N, Verter JI. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women. JAMA 2002; 288: 2432–40
41. Graat JM, Schouten EG, Kok FJ. Effects of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons. JAMA 2002; 228: 715–21.
42. Heart Protection Study Collaborative Group. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002; 360: 23–33
43. The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. N. Engl. J. Med. 2000; 342; 154–60.
44. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge heart antioxidant study (CHAOS). Lancet 1996; 347: 781–86.

45. Salonen RM, Nyyssonen K, Kaikkonen J, Porkkala-Sarataho E, Voutilainen S, Rissanen TH, Tuomainen TP, Valkonen VP, Ristonmaa U, Lakka HM, Vanharanta M, Salonen JT, Poulsen HE. Antioxidant Supplementation in Atherosclerosis Prevention Study. Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression: The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study. Circulation 2003; 107: 947–53.
46. US Preventive Task Force. Routine vitamin supplementation to prevent cancer and cardiovascular disease. Nutrition in Clinical Care 2003; 6: 102–7. www.ahrq.gov/clinic/3rduspstf/vitamins/vitaminswh.htm.
47. Food and Nutrition Board. Dietary Reference Intakes. Proposed Definitions and Plan for Review of Dietary Antioxidants and Related Compounds. Institute of Medicine National Academy Press, Washington DC, 1998.
48. Dragsted L. Antioxidants in Fruits and Vegetables. Veterinær- og Fødevaredirektoratet, Søborg, 1998. www.foedevarestyrelsen.dk/FDir/Publications/1999002/Rapport.htm.
49. Tribble DL. Antioxidant consumption and risk of coronary heart disease: Emphasis on vitamin C, vitamin E and beta-carotene. Circulation 1999; 99: 591–95.

CHAPTER 16

Vitamin A

Vitamin A, RE/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	700	900	350	400	600
Average requirement	AR	500	600			
Lower level of intake	LI	400	500			

Vitamin A is a term reserved to designate any compound possessing the biological activity of retinol (1). The term retinoids include both the naturally occurring forms of vitamin A as well as the many synthetic analogues of retinol, with or without biological activity (2).

All-trans retinol, the parent retinoid compound, is a primary alcohol. In most animal tissues, the predominant retinoid is retinyl palmitate, but other fatty acid esters, such as retinyl oleate and retinyl stearate, are also found. Most of these metabolites occur in the all-trans configuration. Furthermore, the 11-cis aldehyde form, 11-cis retinal, is present in the retina of the eye, whereas several acid forms such as the all-trans retinoic acid, 13-cis retinoic acid and 9-cis retinoic acid, may be present in many tissues (3–4).

Vitamin A exists in the plant world only in the form of precursor compounds such as β-carotene. β-Carotene is one of 50–60 members with vitamin A activity of a large class of naturally occurring compounds called carotenoids. In all cases, a requirement for vitamin A activity is that at least one intact molecule of retinol or retinoic acid can be obtained from the carotenoid.

Originally, international units (IUs) (1 IU = 0.3 µg of all-trans retinol) were used for nutritional recommendations of vitamin A. Then 'retinol equivalents' (RE) were used to convert all sources of preformed retinol and provitamin A carotenoids in the diet into a single unit. When defining RE it was assumed that the efficiency of absorption of provitamin A carotenoids was relatively efficient (5). However, recent studies report that absorption of carotenoids is much lower and appears to be quite variable. Percentage absorption of a single dose of 45 µg to 39 mg

β-carotene has been reported to range from 9 to 22% (6). In addition, a number of factors such as protein-energy malnutrition, zinc-deficiency, dietary fat, alcohol, infections and degree of food processing/food matrix affect the bioavailability and bioconversion of retinol and carotenoids (3–4, 6). Based on these and similar studies, which are limited and not conclusive as yet, the US FNB (6) have introduced the concept 'retinol activity equivalents' (RAE). 1 RAE is equal to:

- 1 µg of dietary or supplemental preformed vitamin A (*i.e.* retinol)
- 2 µg of supplemental β-carotene
- 12 µg of dietary β-carotene
- 24 µg of other dietary provitamin A carotenoids (*e.g.* α-carotene and β-cryptoxanthin)

In NNR the same factors are used, but the term retinol equivalents (RE) is maintained.

Physiology and metabolism

Vitamin A is essential for the life of all vertebrates. The vitamin has numerous important functions including a role in vision, maintenance of epithelial surfaces, immune competence, growth, development and reproduction (3–4, 7). When intake of vitamin A is inadequate to meet the body's needs, clinical vitamin A deficiency characterised by several ocular features (xerophthalmia) and a generalised impaired resistance to infection occurs. A series of epidemiological and intervention studies in children living under poor conditions have documented a relationship between poor vitamin A supply and increased rates and severity of infections, as well as mortality related to infectious diseases such as measles (8). Vitamin A deficiency is a public health problem in over 120 countries (9). The problem is probably uncommon in developed countries but may be under-recognised since simple screening tests to measure sub-clinical deficiency are lacking. Vitamin A, may however, be a double-edged sword since it was recently suggested that intake marginally above the recommended dietary intake is associated with embryonic malformations (7, 10), reduced bone mineral density and increased risk for hip fracture (11).

The major dietary sources of vitamin A are provitamin A carotenoids from vegetables and preformed retinyl esters from animal tissues (3, 4, 12, 13). Carotenoids such as α- and β-carotene and β-cryptoxanthin are absorbed by passive diffusion. After entry into the enterocytes, provitamin A carotenoids are cleaved yielding either one or two molecules of retinol. Absorption of retinyl esters includes enzymatic conversion to

retinol in the intestinal lumen prior to entry into enterocytes. Retinol is then esterified to long chain fatty acids before incorporation into chylomicrons. Generally 70–90% of ingested preformed vitamin A (*e.g.* retinol) is absorbed.

Most of the chylomicron retinyl esters are transported to the liver. In vitamin A sufficient states, most of the retinyl esters taken up by hepatocytes are transferred to perisinusoidal stellate cells in the liver for storage. Normally, 50–80% of the body's total retinol is stored in the hepatic stellate cells as retinyl esters. The normal reserve of stellate cell retinyl esters is adequate to last for several months (14).

Retinol bound to retinol-binding protein is released from the liver and circulates in plasma, ensuring an ample supply of retinol to target cells. Inside target cells, retinol is oxidized to retinal and retinoic acid which are the active retinol metabolites. These metabolites are usually synthesised in target cells by a complex metabolic system involving numerous enzymes and binding proteins (3, 4, 12, 13). Retinal functions as a chromophore in the visual process while retinoic acid activates specific nuclear retinoic acid receptors and thereby modulates gene transcription (15).

Requirement and recommended intake

Earlier recommendations have mainly been based on studies aimed at eliminating symptoms of vitamin A deficiency. In the Sheffield study (16), symptoms of vitamin A deficiency (reduced plasma retinol, reduced dark adaptation, dryness of the skin, eye discomfort) developed in several of 16 healthy men following intake of a diet essentially free of vitamin A for 8 months. Of the 16 subjects studied, only 3 had changes in dark adaptation of sufficient magnitude to serve as a criterion to investigate the curative ability of varying amounts of retinol and β-carotene. Addition of 390 µg retinol per day to one of the individuals with vitamin A deficiency eventually improved dark adaptation and also improved somewhat the plasma retinol levels. Supplementation with 780 mg retinol per day for 45 days had little further effect on the subject's plasma retinol level. However, retinol supplement of 7,200 µg retinol per day increased his plasma retinol above his initial level of 1.2 µmol/L. Furthermore, it was demonstrated in the other vitamin A-deficient individuals that daily intake of 1,500 µg β-carotene in oil, but not 768 µg β-carotene in oil, improved dark adaptation and plasma retinol levels. Hume and Krebs (16) concluded that daily retinol intake of 390 µg represented the minimum protective dose. This figure should

be raised to 470 µg to correct for an error in the conversion factor used in the analytical measurements (17).

Similar observations were obtained in the Iowa study (18) where vitamin A deficiency developed in 8 healthy men after several months on a vitamin A-deficient diet. Abnormal electroretinograms occurred at plasma retinol levels of 0.1–0.4 µmol/L and impaired dark adaptation was observed at plasma retinol levels of 0.1–0.9 µmol/L, whereas follicular hyperkeratosis was found at plasma levels of 0.3–1.3 µmol/L. Plasma levels below 1.1 µmol/L were associated with a mild degree of anaemia that responded to retinol supplementation. The Iowa study also observed that daily intake of 300 µg retinol partially corrected the abnormal electroretinograms, whereas supplements of 600 µg/d were needed to prevent eye changes in adult men. By using isotope-labelled retinol it was calculated that the average rate of utilization of retinol during the state of vitamin A depletion was about 910 µg retinol/day. The study (17) concluded that a daily retinol intake of 900 µg per day would maintain a plasma level of 1.1 µM in most adult men. For women, the requirement would be reduced in proportion to body weight.

NNR 1996 recommended intakes of 900 and 800 RE per day for men and women, respectively, primarily based on the Sheffield and Iowa studies (16, 17, 18). The recent US DRIs (6) for vitamin A are based on estimated requirements that assure adequate body stores of retinol where no clinical signs of deficiency are observed, adequate plasma retinol levels are maintained and there is protection against vitamin A deficiency for approximately 4 months on a vitamin A-deficient diet. The underlying evaluation assumes that the body turn-over of retinol is 0.5%, the minimal liver reserve is 20 µg/g, the liver weight : body weight ratio is 1:33, the total body : liver vitamin A reserve is 10:9, and that the efficiency of storage (*i.e.* retention of absorbed vitamin A in liver) is 40%. Based on these assumptions (6), and using reference weights for US adults, the estimated average requirement of preformed vitamin A required to assure an adequate body reserve in an adult male is 627 mg/day. The corresponding value for women was estimated to 503 µg/day. Using a factor of 1.4 to cover the variation, a recommended daily allowance was set to 900 µg/d for men and 700 µg/d for women above 19 years of age (6). These estimations are in general agreement with a large number of recent studies using functional criteria for vitamin A status, such as dark adaptation, papillary response test, conjunctival impression cytology and markers of immune function (see (6) for a review of these studies).

Using the above factorial method for the Nordic reference subjects, the estimated average requirement for vitamin A would be very similar

as for the US reference subjects, *i.e.* close to 600 and 500 µg/d for men and women respectively. In the present NNR, the recommended intakes for adults are based on these considerations and thereby set to 900 RE/d for men and 700 RE/d for women. This means a reduced RI for women compared to the previous edition. Average requirements are set to 600 and 500 RE/d for men and women, respectively. The lower intake level is estimated to be 500 RE/d for men and 400 RE/d for women.

In infants, no functional criteria of vitamin A status have been published that reflect the response to dietary intake. Breast milk from well-nourished mothers in the Nordic countries usually contains sufficient amounts of vitamin A. Infants are generally given vitamin A supplements during the first year. For non-breast fed infants, vitamin A content of formula is sufficient. No specific recommended intake of vitamin A for infants aged 0–6 months is therefore given. Any contribution by carotenoids was not considered since the bioconversion of carotenoids in infants is not known.

Direct studies on the requirement for vitamin A are not available to estimate an average requirement for infants, children and adolescents aged 1–17 years. Thus, the RIs for children and adolescents are extrapolated from those for adults by using metabolic body weight and growth factors ($BW^{0.75}$, see (6)).

Experimental data to estimate an average requirement during pregnancy are lacking. Using the retinol accumulation in foetal liver as a criterion, about 50 µg vitamin A per day would be needed in addition to the AR for non-pregnant women (6). The RI for pregnancy is set to 800 RE/d to cover the individual variation.

The vitamin A content of breast milk varies with the dietary vitamin A intake. Reported values for Western countries are 450–600 RE/L. With an average milk production of 750 mL/d, this corresponds to 350–450 RE/day. An additional intake of 400 RE/d is therefore recommended during lactation.

In elderly subjects, intakes of 800–900 RE/d vitamin A seem more than adequate (19). Some early studies (20) have found an age-related trend toward higher serum retinol values with advancing age, but recent studies have found trends towards a slight decrease (21). None of these elderly subjects had retinol values below a cut-off value of 0.35 µmol/L. Using a cut-off value of 0.7 µmol/L as proposed by NHANES data from 18–74-year-old subjects only resulted in very few subjects at risk (21). In a Danish cross-sectional study of 80-year-old men and women, 10% had a dietary intake of vitamin A below the lower level, but only one subject had a retinol value below 0.7 µmol/L (22). Use of the same vitamin A-containing supplements has been linked to higher

circulating retinyl ester values in elderly subjects compared to younger (23), due perhaps to delayed plasma clearance in the elderly (24). A recent intervention study found an altered postprandial plasma retinol concentration in older subjects compared to younger, while the intestinal absorption and esterification were the same in the elderly compared to the younger subjects (25).

Serum retinol levels are generally considered to be a relatively poor reflection of vitamin A status, unless liver stores are either very depleted or highly saturated, while plasma β-carotene seems to be a possible biomarker of the β-carotene status (26). Several studies (21, 27, 28) have found a positive relationship between plasma levels and the intake of β-carotene in elderly subjects. Consumption of carotene-containing fruits and vegetables is inversely related to overall mortality and cardiovascular mortality, even in the elderly (29, 30). However, the role of β-carotene in the prevention of age-related diseases is still too weak to use as a basis for vitamin A recommendations.

The RI for elderly subjects > 60 years of age is the same as for younger adults.

Upper intake levels and toxicity

Several studies have shown that doses up to 180 mg β-carotene per day as supplements may be used for many years with no evidence of vitamin A toxicity and without the development of abnormally elevated blood retinol concentrations. Recently, serious adverse effects of β-carotene in the form of supplements have, however, been reported but these are not related to its conversion to retinol (see discussion in *Chapter 15*).

Retinol toxicity is an important issue for the Nordic countries where the dietary intake of retinol is relatively high, especially in Iceland. The issue of retinol toxicity is complicated by the fact that genetic retinol intolerance is known to exist in a handful of individuals. In addition, intake of ethanol, various drugs and xenobiotics, and diseases of the liver and kidney also affect retinol toxicity (31, 32).

Vitamin D antagonism

Several studies have provided evidence of an antagonism between retinol and vitamin D (33, 34, 35). Recently, animal studies have shown that retinol serves as an antagonist to vitamin D action, not only in toxic amounts but also at the physiological level (36). In a recent meta-analysis, which included all cases of retinol intoxication published in scientific literature (37), it was observed that the mean dose of retinol causing

hypervitaminosis A was higher when the dose originated from a formula containing vitamin D. Although these results are not conclusive as yet, they raise some important questions : Since high levels of vitamin D are associated with reduced toxicity of retinol and vice versa, could vitamin D deficiency increase the sensitivity for retinol toxicity? Conversely, does excess retinol intake exacerbate vitamin D insufficiency? Addressing these questions may have important clinical implications as low vitamin D status is known to be common in the elderly and is an important risk factor for fractures.

Risk of acute and chronic hypervitaminosis A

Retinol toxicity related to osteoporosis and teratogenicity is discussed in separate sections below. There have been no reports in the Nordic countries describing either classical chronic or acute hypervitaminosis A due to intake of foods such as liver, except a few cases of early Arctic explorers eating Polar Bear liver (38). Although adults in the Nordic countries have a generous intake of retinol, very few if any healthy individuals are likely to ingest amounts that may lead to classical hypervitaminosis A. Thus, the risk of hypervitaminosis A due to retinol-rich foods is very low.

A major issue when evaluating the potential toxicity of retinol is the observation that intake of retinol in various physical forms appears to have different thresholds for toxicity (31, 37). Retinol in water-soluble, emulsified or solid (*i.e.* tablets) preparations generally seems to have more acute toxic effects than retinol in foods or oils (37). This may be relevant for potential hypervitaminosis A from supplements and foods fortified with retinol. Several foods commonly used in the Nordic countries are fortified with retinol. If the diet consists of large amounts of retinol-fortified foods, the daily intake may approach the upper safe levels. Therefore, oil-based retinol preparations should preferably be used in supplements and fortification of foods. Supplements and fortification with water miscible/emulsified preparations should be kept to a minimum.

A total of 17 suspected cases of supplement-induced chronic hypervitaminosis A, but no acute cases, have been reported in scientific literature in the Nordic countries (31). Chronic hypervitaminosis A is induced after daily doses of 2 mg/kg/d of retinol in oil-based preparations for many months or years (37). In contrast, only a few weeks of intake of doses as low as 0.2 mg/kg/d of retinol in emulsified/water-miscible and solid preparations caused hypervitaminosis A (31). Thus, emulsified/water-miscible and solid preparations of retinol are about 10 times more toxic than oil-based preparations of retinol. The safe upper single dose of retinol in oil or liver seems to be about 4–6 mg/kg bodyweight (37). These thresholds do not vary considerably with age.

Hepatotoxicity is a manifestation of hypervitaminosis A and toxic symptoms seem to depend on both the amount and duration of exposure. Mechanisms of hepatic effects are linked to overload of the storage capacity of the liver for vitamin A which may cause cellular toxicity, production of collagen and eventually fibrosis and cirrhosis. The lowest dose reported to cause cirrhosis was a consumption of 7,500 RE/d for 6 years, and it can be hypothesized that this value might be the upper threshold of the storage capability of the liver (32).

Risk of retinol-induced teratogenicity

Animal studies demonstrate that both retinol deficiency and retinol excess may give rise to embryonic malformations, and that a single high dose of retinol or retinoic acid may be teratogenic if given at a susceptible stage of early embryonic development (see discussion in (31) and references therein). In humans, several cases of teratogenicity have been reported due to retinoic acid medication, but no cases due to preformed retinol in foodstuffs. Epidemiological data suggest that intakes of retinol supplements up to 3 mg vitamin A per day during pregnancy are not associated with an increased risk of giving birth to a malformed child, and since epidemiological data indicate that the threshold for teratogenicity is higher than 3 mg retinol/d it is assumed that this level offers adequate protection against teratogenic effects (32). Thus, it is recommended that the intake of retinol supplements during pregnancy should be limited to no more than 3 mg per day unless other medical aspects argue for a higher intake. As the possible adverse effects of excess intake of retinol appear very early during pregnancy, this advice is expanded to all women of childbearing age. Furthermore, it is recommended that pregnant women should avoid eating liver as the main course of a meal.

Risk of retinol-induced osteoporosis

There is abundant documentation from animal experiments, *in vitro* studies, pharmacological studies and clinical observations that retinol intoxication is associated with severe detrimental effects on the skeleton (see discussion in (31) and references therein). Recently, retinol intake about twice the recommended dietary intake has been found to be associated with a doubled risk of hip fracture in two large epidemiological studies, while other studies did not find such effects. The available evidence for an increased risk of osteoporosis at doses down to 1.5 mg/d is therefore limited and not consistent and further studies addressing the issue are warranted (32).

Setting an upper intake level for retinol or retinyl esters

Toxic effects have primarily been linked to preformed vitamin A, *i.e.* retinol or retinyl esters. It is clear that the hazards and their associated doses are different for different groups of the population and the severity of the adverse effect varies from minor to irreversible.

Taking into account the low margin between the recommended intake value and doses that might pose a risk to different groups of the population setting an upper level of intake is not easy. The recommended maximum intake of 3 mg/d of retinol supplements for women of childbearing age is chosen as the upper level for the whole population. This level is 2.5 times below the level which may cause hepatotoxicity. With regard to risk of osteoporosis this upper level may not adequately address the possible risk of bone fracture in vulnerable groups. Postmenopausal women who are at greater risk for osteoporosis and bone fractures should therefore restrict their intake to 1,500 µg/day.

Dietary sources and intake

Vitamin A is present in the diet either as preformed vitamin A (*i.e.* retinol and its fatty acyl esters) in animal sources such as milk, eggs, butter and fish liver oils or as provitamin A carotenoids in dark green leafy vegetables and in red or orange coloured fruits and vegetables such as carrots. In addition, preformed vitamin A is also contained in a number of mono and multivitamin supplements (31).

Mean intake of preformed retinol in the Nordic countries varies from 740 to 1,200 µg/10 MJ. In general, Icelanders have the highest intake followed by Norwegians. The main sources of retinol are liver and liver products, edible fat, milk and milk products, including retinol fortified margarine, spreads and milk. Cod liver oil is an important source of retinol in Iceland and Norway (31).

The 10% of the adult population with the highest intake (the 90th percentile) have daily intakes of preformed retinol from foods that are up to 2–3 times higher than the RI for vitamin A. The 90th percentile intakes were the following among men: in Denmark 1,600 µg, in Finland 1,600, Norway 2,800 and in Sweden 1,900 µg retinol per day. For women the corresponding intakes were: Denmark 950, Finland 1,200, Norway 2,500 and in Sweden 1,200 µg retinol per day (31, 39, 40).

REFERENCES

1. International Union of Pure and Applied Chemistry – International Union of Biochemistry (IUPAC-IUB) Joint Commission on Biochemical Nomenclature. Eur J Biochem 1982; 129: 1–5.
2. Sporn MB, Dunlop NM, Newton DL *et al.* Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1976; 35: 1332–8
3. Blomhoff R. Vitamin A in Health and Disease. New York: Marcel Dekker, 1994.
4. Sporn MB, Roberts AB, Goodman DS. The retinoids. Orlando: Academic Press, 1984.
5. Sivakumar, B. and Reddy, V. Absorption of labelled vitamin A in children during infection. Br. J Nutr. 1972; 27: 299–304.
6. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Institute of Medicine, National Academy of Sciences, Washington, DC: 2001
7. Ross SA, McCaffery PJ, Drager UC, De Luca LM. Retinoids in embryonal development. Physiol Rev 2000; 80: 1021–54.
8. D'Souza RM, D'Souza R. Cochrane database syst rev CD00/479, 2002.
9. World Health Organization. Global prevalence of vitamin A deficiency. WHO/NUT/95-3. Geneva, 1995.
10. Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. Teratogenicity of high vitamin A intake. N. Engl. J. Med. 1996; 333: 1369–73.
11. Melhus H, Michaelsson K, Kindmark A, Bergström R, Holmberg L, Mallmin H *et al.* Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. Ann Intern Med 1998; 129: 770–78.
12. Blomhoff R, Green MH, Berg T, Norum KR. Transport and storage of vitamin A. Science 1990; 250: 399–404.
13. Blomhoff R, Helgerud P, Rasmussen M, Berg T, Norum KR. *In vivo* uptake of chylomicron [3H]retinyl ester by rat liver: evidence for retinol transfer from parenchymal to nonparenchymal cells. Proc Natl Acad Sci 1982; 79: 7326–30.
14. Blomhoff R Wake K. Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J 1991; 5: 271–77.
15. Gudas LJ, Sporn MB, Roberts AB. Cellular biology and biochemistry of the retinoids. In The Retinoids: Biology, Chemistry and Medicine. Sporn, Roberts, and Goodman, D.S. (eds), pp 443–520. Raven Press, New York, 1994.
16. Hume EM, Krebs HA. Vitamin A requirement of human adults. An experimental study of vitamin A deprivation in man. Medical Research Council Special Series No. 264, London: His Majesty Stationery Office,1949.
17. Leitner ZA, Moore T, Sharman IM. Vitamin A and vitamin E in human blood – levels of vitamin A and carotenoids in British men and women, 1948–57. Br J Nutr 1960; 14: 157–69.
18. Sauberlich HE, Hodges HE, Wallac DL, Kolder H, Canham JE, Hood J, Raica N, Lowry LK. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam Horm 1974; 32: 251–75.
19. Russel RM, Suter PM. Vitamin requirements of elderly people: an update. Am J Clin Nutr 1993; 58: 4–14.
20. Garry PJ, Hunt WC, Bandrofchak JL, Vanderjagt D, Goodwin JS. Vitamin A intake and plasma retinol levels in healthy elderly men and women. Am J Clin Nutr 1987; 46: 989–994.

21. Haller J, Weggemans RM, Lammi-Keefe CJ, Ferry M. Changes in the vitamin status of elderly Europeans: plasma vitamins A, E, B-6, B-12, folic acid and carotenoids. Eur J Clin Nutr 1996; 50 (Suppl 2): S32–S46.
22. Pedersen AN. Nutritional status of 80-year old people – relations to functional capacity (80-åriges ernæringsstatus og relationen till fysisk funktionsevne. 80-årsundersøgelsen 1994/95). PhD-avhandling. Københavns Universitet 2001. Fødevaredirektoratet og Københavns Amts Center for Sygdomsforebyggelse.
23. Krasinski SD, Russel RM, Otradovec CL, Sadowski JA, Hartz SC, Jacob RA, McGandy RB. Relationship of vitamin A and vitamin E intake to fasting plama retinol, retinol-binding protein, retinyl esters, carotene, a-tocopherol, and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A-supplement users. Am J Clin Nutr 1989; 49: 112–20.
24. Krasinski SD, Cohn JS, Schaefer EJ, Russell RM. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. J Clin Invest 1990; 85: 883–92.
25. Borel P, Mekki N, Boirie Y, Partier A, Alexandre-Gouabau MC, Grolier P, Beaufrere B, Portugal H, Lairon D, Azais-Braesco V. Comparison of the postprandial plasma vitamin A response in young and older adults. J Gerontol 1998; 53: B133–B140.
26. Nielsen F. Vitamin status in Danes – a population study. Ph. D. Thesis. Faculty of Health Sciences, Odense University. 1998.
27. Heseker H, Schneider R. Requirement and supply of vitamin C, E and beta-carotene for elderly men and women. Eur J Clin Nutr 1994; 48: 118–27.
28. Bates CJ, Prentice A, Cole TJ, van der Pols JC, Doyle W, Finch S, Smithers G, Clarke PC. Micronutrients: highlights and research challenges from the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. Brit J Nutr 1999; 82: 7–15.
29. Gaziano JM, Manson JE, Branch LG, Colditz GA, Willet WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol 1995; 5: 255–60.
30. Sahyoun NR, Jacques PF, Russel RM. Carotenoids, vitamin C and E, and mortality in an elderly population. Am J Epidemiol 1996; 144: 501–11.
31. Blomhoff R, Beckman-Sundh U, Brot C, Solvoll K., Steingrimsdóttir L, Hauger Carlsen M (2003). Health risks related to high intake of preformed retinol (vitamin A) in the Nordic countries. Nordiska ministerrådet. TemaNord 2003:502.
32. SCF Opinion on Tolerable Upper Intake Level of preformed vitamin A (retinal and retinyl esters). SCF/CS/NUT/UPPLEV/24 Final 7 October 2002.
33. Grant AB, O'Hara PB. The rachitogenic effect of vitamin A. New Zeal J Sci Tech 1957; 38: 576.
34. Aburto A, Edwards HM Jr, Britton WM. The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25 dihydroxycholecalciferol in young broiler chickens. Poult Sci 1998; 77: 585–93.
35. Metz AL, Walser MM, Olson WG. The interaction of dietary vitamin A and vitamin D related to skeletal development in the turkey poult. J Nutr 1985; 115: 929–35.
36. Rohde CM, Manatt M, Clagett-Dame M, DeLuca HF. Vitamin A antagonizes the action of vitamin D in rats. J Nutr 1999; 129: 2246–50.
37. Myhre A, Carlsen MH, Bøhn SK, Wold HL, Laake P, Blomhoff R. Water-miscible, emulsified and solid forms of vitamin A-supplements are more toxic than oil-based preparations. Am J Clin Nutr. 2003; 78: 1152–9.
38. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and Energy Intakes for the European

Community. (Thirty first series of Food Science and Techniques.) Luxembourg: Office for Official Publications of the European Communities, 1993.

39. Danskernes kostvaner 2000–2002. Hovedresultater. Danmarks Fødevareforskning, 2005. In press.
40. FINDIET 2002 Study, unpublished data. Written communication Hannila M-L. 12.11.2004.

CHAPTER 17

Vitamin D

Vitamin D, µg/d		Women	Men
Recommended intake	RI		
age 2–60 years		7.5	7.5
age ≥ 61 years		10	10
Average requirement	AR	–	–
Lower level of intake	LI	2.5	2.5

Vitamin D_3 or cholecalciferol is a steroid-like molecule that can be synthesised from 7-dehydrocholesterol in the skin under the influence of ultraviolet B light (wavelength between 290 nm and 315 nm) (1). It may also be derived from the diet. Vitamin D_2 or ergocalciferol formed by UV irradiation of ergosterol from yeast is used as a pharmacological preparation of vitamin D. The requirement for vitamin D_3 can be completely satisfied by solar exposure of the skin. Experience demonstrates, however, that under our living conditions and at the latitude of the Nordic countries vitamin D deficiency may occur if the diet is devoid of the vitamin. Infants may develop rickets and elderly people osteomalacia. For this reason vitamin D has to be considered a micronutrient. Vitamin D is also a prohormone because it is converted to a hormone, 1,25-dihydroxyvitamin D, in the body.

One IU (international unit) corresponds to 0.025 µg vitamin D.

Physiology and metabolism

A rather modest exposure to sunlight is sufficient to produce a satisfactory amount of vitamin D_3 in the skin (1). Exposure of the face, arms, hands and legs to sunshine for 6–8 minutes 2–3 times a week is more than adequate to satisfy the requirement (2). Dermal production of vitamin D is reduced by pigmentation of the skin and with age. It is generally considered that regular outdoor activity exposing just face and hands is sufficient to cover the needs. Vitamin D may be stored in the body, in particular in the adipose tissue. Studies from the UK have shown that children who have been outside frequently during the summer may have an acceptable vitamin D status during the rest of the year (3).

The liver rapidly takes up Vitamin D formed in the skin or absorbed from the gut where it is hydroxylated to 25-hydroxyvitamin D (25-OH-D). This metabolite is transported in plasma bound to the vitamin D binding protein (also known as the group specific protein, Gc).

The circulating concentration of 25-OH-D is a good marker of vitamin D status. Reliable methods are now available for determination of 25-hydroxyvitamin D in plasma. Variations between laboratories may be of the order of 30%, however (4). Serum concentration of 25-OH-D < 40 nmol/L may indicate moderate hypovitaminosis (5) while values > 50 nmol/L may be considered desirable (6, 7)

25-OH-D is further converted into 1,25-dihydroxyvitamin D in the kidneys. This is a calcium-regulating hormone that becomes active after binding to a nuclear receptor, (the vitamin D receptor). Together with parathyroid hormone and calcitonin 1,25-dihydroxyvitamin D ensures that the concentration of calcium and phosphate in plasma is kept within narrow limits. Its main function is to stimulate the absorption of calcium from the intestine. In concert with parathyroid hormone it also stimulates release of calcium from bone, thereby increasing the concentration of calcium in the plasma. By contributing to maintenance of normal concentration of calcium (and phosphate) in the blood and the extracellular fluid, vitamin D is essential for normal mineralisation of the skeleton. Deficiency of vitamin D results in defective mineralisation with development of rickets in children and osteomalacia in adults. The presence of vitamin D receptor in a number of tissues, epidemiological and experimental data indicate that in addition to the functions mentioned above, vitamin D may play a role in cancer, autoimmune diseases, infections and muscle strength. Epidemiological evidence suggests that lack of vitamin D during infancy may increase the risk of type 1 diabetes (8). However, in a large population-based case-control study, use of cod liver oil but not other vitamin D supplements during the first year of life was found to be associated with lower risk of type 1 diabetes (9).

As a consequence of a good public health service and because most infants receive vitamin D supplement, rickets has become very rare in the Nordic countries during the past 3–4 decades. More recently an increased number of infants with rickets has been admitted to hospitals. Almost all are children of Muslim immigrants (10–12).

In Finland 90 cases of rickets were diagnosed during 1981–85 and 245 cases during 1986–90 (13). The cause of this increased incidence was probably that the infants did not receive vitamin D supplements and that milk formula and baby foods were not enriched with vitamin D during that time.

Requirement and recommended intake

Based on a large number of studies where 25-OH-D has been used as a criterion for vitamin D status the following conclusions can be drawn:

New-borns

New-borns have a store of vitamin D that depends on the vitamin D status of the mother. During the first six weeks of life there is a rapid fall of 25-OH-D to a level seen in rickets (14). Human milk does not contain sufficient vitamin D to prevent rickets even if the mother takes vitamin D supplement (15).

Sun exposure has a marked effect on vitamin D status in infants and vitamin D supplementation may not be required provided exposure is sufficient. At northern latitudes, however, as in the Nordic countries, vitamin D supplementation is required in order to ensure that no infant develops rickets. During the first 6 weeks after birth, serum 25-OH-D concentration falls to a range where there is a high risk of rickets (< 27.5 nmol/L). Supplementation therefore should start from the age of 2–4 weeks. An intake of 2.5 µg vitamin D/d may prevent rickets but a study from northern China showed that this amount does not ensure that 25-OH-D is raised to a satisfactory level in the majority of the infants (16). Even supplementation with 5 µg failed to raise the concentration above 27.5 nmol/L in about 30% of the infants, while with a supplementation of 10 µg all but 2 out of 33 attained a concentration above this level (16). In the study by Markestad (14), a group of 8 infants were fed exclusively on milk formula containing 10 µg/L. At 6 weeks of age they all had serum 25-OH-D within the normal range. It thus appears that a mean intake of 7.5 µg (corresponding to an intake of 750 ml of formula) ensures satisfactory vitamin D status. This is supported by a Chinese study where 150 bottle-fed infants received amounts of about 5 to 8.5 µg/d up to the age of 18 months. At that age they all had 25-OH-D concentrations above 50 nmol/L (17). The amount needed to cure rickets is 2.5–7.5 µg, while intakes between 8.5 and 15 µg/d would have the maximal effect on linear growth (references cited in 16).

Based on these considerations, 10 µg/d is recommended for newborns from 2–4 weeks of age to 2 years.

There is a marked increase in 1,25-dihydroxyvitamin D in plasma during pregnancy. A close correlation has also been found between vitamin D status of the mother and the new-born (14). Levels of 25-OH-D have been found to be particularly low during winter in unsupplemented pregnant woman living under our climatic conditions, suggesting that vitamin D supplementation may be warranted (19). A supple-

ment of 10 mg/d to pregnant women results in an 25-OH-D level in the upper normal range (20). For these reasons 10 µg/d is recommended during pregnancy and lactation.

Children and adolescents

Large variations in vitamin D status have been found among children and adolescents. In the northern parts of Scandinavia after rainy summers, a large fraction has unsatisfactory vitamin D status (21). In a recent study from Finland it was found that 13.4% of girls aged 9–15 years had 25-OH-D levels < 20 nmol/L during winter (22). Supplementation with 10 µg of vitamin D_2 per day did not restore serum 25-OH-D to within the normal range. It should be noted, however, that vitamin D_2 is less efficient than vitamin D_3 in increasing 25-OH-D level (23). In a similar study from Sweden it was found that 15% of young girls (mean age 15.7 yrs) had serum 25-OH-D concentrations below 20 nmol/L and 75% between 20 and 38 nmol/L (24). An intake of 7.5 µg/d is recommended for this group.

Adults

Vitamin D deficiency is rare among native adults (20–60 years) in the Nordic countries. Under our climatic conditions there are indications that exposure to sunlight is insufficient for enough vitamin D to be formed in the skin and for vitamin D status to be maintained during the winter months. A study from northern Finland in 1980 showed that vitamin D status was satisfactory during the summer but that a large number had unsatisfactory vitamin D status during the winter months (25). On the other hand satisfactory serum levels of 25-OH-D and seasonal variation were found among adults in a similar study from Tromsø in northern Norway, indicating that light intensity is sufficient at 70 degrees north to stimulate vitamin D formation in the skin (26). A reasonable explanation for the difference in vitamin D status between these two population groups during the winter months is that at the time of these studies the consumption of fish and margarine fortified with vitamin D was higher in Norway than in Finland. These findings indicate that dietary vitamin D is essential to ensure satisfactory vitamin D status at northern latitudes.

Practically all adults have been found to have satisfactory vitamin D status during the summer months, but during the winter months low serum concentrations of 25-OH-D have been found in a small number of young adults (21). A high prevalence of hypovitaminosis D was found among both male and female 42–46 year-old ambulatory patients in Finland (27) as well as among middle-aged Danish women during the win-

ter-spring period (28). Vitamin D deficiency appears to be prevalent among Muslim immigrants. Thus, low serum levels of 25-OH-D and secondary hyperparathyroidism have been found among pregnant Pakistani women in Oslo (11). Similar findings have been reported from Denmark (12).

The lowest amount of oral vitamin D to prevent or cure osteomalacia is about 2.5 µg/d (29–32). However, no effect of this amount on serum 25-OH-D is observed in non-sun exposed individuals (33). A significant increase is seen after intake of 3–4 µg of vitamin D_2 (34). 4–5 µg of dietary vitamin D was found to be associated with plasma 25-OH-D levels of around 50 nmol/L during winter and 75 nmol/L during summer in independently living British elderly (35). However, in a more recent study on the effect of oral dosing of vitamin D on 25-OH-D, it was estimated that 12.5 µg is necessary to maintain a stable level through the winter (36). It may thus be assumed that the previously recommended dietary intake of 5 µg/d may be insufficient to maintain an acceptable plasma concentration of 25-OH-D during the Nordic winter. In order to diminish the seasonal drop in 25-OH-D, the recommended intake is therefore set at 7.5 µg/d for the age groups 2 to 60 years.

Elderly

A number of studies from the Nordic and other countries have shown that a large proportion of the elderly population has an unsatisfactorily low vitamin D status (37–42). Elderly people living in institutions are at particularly high risk of vitamin D deficiency, as are elderly patients with dementia living in their own homes (6). There may be several reasons for the low vitamin D status in the elderly. Time spent outdoors is limited, the amount of 7-dehydrocholesterol in the skin epidermis diminishes with age and the efficiency of conversion of this precursor into vitamin D is less effective than in younger individuals (1). The cut-off limit for a satisfactory serum concentration of 25-OH-D in the elderly is, however, controversial. Proposed cut-off limits range from 30 nmol/L (43) to 100 nmol/L (44), mainly based on the association to PTH status. Furthermore it has been found that the level of PTH increases between the ages of 70 to 95 at similar 25-OH-D levels, corresponding to a difference of 20% in PTH levels (45). Mean serum concentrations of 25-OH-D corresponding to 85–105 nmol/L have been found in Norwegian elderly people receiving a total of at least 10 µg/d (6, 39). Only a marginal increase in serum 25-OH-D and a decrease in PTH is seen when the intake of vitamin D_3 is increased from 10 to 20 µg/d (46).

A high incidence of osteoporotic hip fracture is seen in all Nordic countries. During the past 50 years, there has been an age-adjusted in-

crease in fracture incidence by a factor of about 2–3. The incidence is higher in urban areas than in rural. This indicates that exogenous factors are of importance. The more rapid bone loss and higher fracture rate in elderly women than in men is related to diminished oestrogen production in post-menopausal women. The extent to which vitamin D deficiency and osteomalacia contribute to the risk of fracture in the elderly is unclear. Some studies indicate that vitamin D deficiency may contribute to osteoporotic fractures in the highest age groups. Three intervention studies where both vitamin D and calcium were given to the elderly showed a reduction in incidence of osteoporotic fractures (47–49). Studies in Finland and England in which only vitamin D was given also resulted in a reduction in total number of fractures (50, 51), while studies in the Netherlands and Norway showed no effect of supplementary vitamin D (52, 53). In a large prospective study among elderly women, an inverse relationship was also found between vitamin D intake and risk of hip fracture (54).

A total intake of 10 µg/d is recommended for all individuals above the age of 60 years. Elderly people with little or no sun exposure should receive a supplement of 10 µg vitamin D_3/d added to the dietary intake.

Upper intake levels and toxicity

Large amounts of vitamin D are toxic and may lead to hypercalcaemia, nephrocalcinosis and kidney failure. Infants and children are more sensitive than adults. Adults can tolerate a single yearly megadose of 7.5 mg (50) while children given such a dose once a year developed hypercalcaemia (55). There are several reports of hypercalcaemia in connection with uncritical supplementation of infant foods with vitamin D (56) and in connection with incidental over-enrichment of milk (57, 58). No clear toxic level has been defined, but based on the effects on serum 25-OH-D level and the risk of hypercalcaemia, the following upper levels of intake have been proposed by the EU Scientific Committee on Food: for infants and children up to age 10 years 25 µg/d, for adolescents and adults 50 µg/d (59).

Sources and intake

Oily fish, margarine and milk enriched with vitamin D are the only dietary sources of importance. Certain freshwater fish may contain appreciable quantities of vitamin D (60, 61). Eggs contribute a small amount of vitamin D but contain some 25-OH-D_3 (62). Dietary surveys

disclose a low intake (2 to 6 µg/d) in all the Nordic countries. A relatively large proportion of the population has an intake below 2.5 µg/day.

REFERENCES

1. Holick M.F. Vitamin D: Photobiology, metabolism and clinical application. In: The Liver: Biology and Pathobiology, 3d Edition, ed. by I.M. Arias, J.L. Boyer, N. Fausto, W.B. Jakoby, D.A. Schachter, and D.A. Shafritz. Raven Press, Ltd., New York, 1994 pp 543–562.
2. Holick MF. Sunlight and vitamin D. Both good for cardiovascular health. J Gen Intern med 2002; 17: 733–5.
3. Poskitt EME, Cole TJ, Lawson DEM. Diet, sunlight, and 25-hydroxy vitamin D in healthy children and adults. Brit. Med. J. 1979; 1: 221–223.
4. Lips P, Chapuy MC, Dawson-Hughes B, Pols HAP, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporos Int 1999; 9: 394–7.
5. Thomas MK, Lloyd DJ, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT *et al.* Hypovitaminosis D in medical inpatients N Engl J Med 1998; 338:777–783.
6. Nes M, Sem SW, Rousseau B, Bjørneboe G-EA, Engedal K, Trygg K, Pedersen JI. Dietary intakes and nutritional status of old people with dementia living at home in Oslo. Europ. J. Clin. Nutr. 1988; 42: 581–93.
7. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998; 351: 805–806.
8. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. lancet 2001; 358: 1500–3.
9. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large population-based, case-control study. Amer J clin Nutr 2003; 78: 1128–34.
10. Brunvand L, Nordshus T. Nutritional rickets. An old disease with new relevance. Tidsskr Nor Laegeforen 1996; 116: 15582–4.
11. Brunvand L, Haug E. Vitamin D deficiency amongst Pakistani women in Oslo. Acta Obstet. Gynecol. Scand. 1993; 72: 264–8.
12. Glerup H. Vitamin D deficiency among immigrants. Ugeskr Laeger 2000; 162: 6196–9.
13. Ala-Houhala M, Sorva R, Pelkonen A, Johansson C, Stålberg M-R, Hakulinen A, *et al.* Riisitaudin uusi tuleminen – esiintyvyys, diagnostiikka ja hoito (Rakitens återkomst – förekomst, diagnostik och behandling). Duodecim 1995; 111: 337–44.
14. Markestad T. Effect of season and vitamin D supplementation on plasma concentration of 25-hydroxyvitamin D in Norwegian infants. Acta Pædiatr Scand 1983; 72: 817–21.
15. Olafsdottir AO, Wagner K-H, Thorsdottir I, Elmadfa I. Fat-soluble vitamins in the maternal diet, influence of cod liver oil supplementation and impact of the maternal diet on human milk composition. Ann Nutr Metab 2001; 45: 265–72.
16. Specker BL, Ho ML, Oestreich A, Yin T, Shui Q, Chen X, Tsang RC. Prospective study of vitamin D supplementation and rickets in China. J. Pediatr. 1992; 120: 733–739.
17. Leung SSF, Lui S, Swaminathan R. Vitamin D status of Hong Kong Chinese infants. Acta Pædiatr Scand 1989; 78: 303–306.
18. Recommended Dietary Allowances, 7th ed. 1968, National Academy of Sciences, Washington DC
19. Kokkonen J, Koivisto M, Kirkinien P. Seasonal variation in serum 25-OH-D_3 in mothers and newborn infants in northern Finland. Acta Pædiatr Scand 1983; 72: 93–6.
20. Markestad T, Ulstein M, Aksnes L, Aarskog D. Serum concentrations of

vitamin D metabolites in vitamin D supplemented pregnant women. Acta Obstet. Gynecol. Scand. 1986; 65: 63–67.
21. Rapport om vitamin D inntak og vitamin D status i den norske befolkning, Statens ernæringsråd, Oslo, 1989.
22. Lehtonen-Veromaa M, Möttönen T, Irjala K, Kärkkäinen M, Lamberg-Allardt C, Hakola P, Viikari J. Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15 year-old Finnish girls. Europ J Clin Nutr 1999; 53: 746–751.
23. Trang HM, Cole DEC, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D_3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D_2. Am J clin Nutr 1998; 69: 854–858.
24. Hulthén LR 2003. Personal communication.
25. Savolainen K, Mäenpää PH, Alhava EM, Kettunen K. A seasonal difference in serum 25-hydroxyvitamin D_3 in a Finnish population. Med Biol 1980: 58: 49–52.
26. Vik T, Try K, Strømme JH. The vitamin D status of man at 70o north. Scand J Clin Lab Invest. 1980; 40: 227–32.
27. Kauppinen-makelin R, Tahtela R, Loyttyniemi E, Valimaki MJ. A high prevalence of hypovitaminosis D in Finnish medical in- and outpatients. J Intern Med 2001; 249: 559–63.
28. Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann AP, Sørensen OH. Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationship to dietary intake, sun exposure and serum parathyroid hormone. Brit J Nutr 2001; 86 Suppl. 1: S97–S103.
29. Morgan DB, Paterson CR, Woods CG, Pulvertaft CN, Fourman P. Osteomalacia after gastrectomy. A response to very small doses of vitamin D. Lancet, 1965; 2, 1089–1091.
30. Dent CE, Smith R. Nutritional osteomalacia. Quart J Med. 1969; 38: 195–209.
31. Fourman P, Morgan DB. Effects of vitamin D in the human. Nutritio et Dieta. 1969; 13: 30–43.
32. Smith R, Dent CE. Vitamin D requirements in adults. Nutritio et Dieta. 1969; 13: 44–5.
33. Fraser DR. The physiological economy of vitamin D. Lancet, 1983; 1: 969–72.
34. Newton HMV, Sheltawy M, Hay AWM, Morgan B. The relationship between vitamin D_2 and D_3 in the diet and plasma $25OHD_2$ and $25OHD_3$ in elderly woman in Great Britain. Am J Clin Nutr 1985; 41: 760–4.
35. Bates CJ, Carter GD, Mishra GD, O'Shea D, Jones J, Prentice A. In a population study, can parathyroid aid the definition of adequate vitamin D status? A study of people aged 65 years and over from the British National Diet and Nutrition Survey. Osteoporos Int 2003; 14: 152–9.
36. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to external oral dosing with cholechalciferol. Am J Clin Nutr 2003; 77: 204–10.
37. Toss GS, Almqvist S, Larsson L, Zetterqvist H. Vitamin D deficiency in welfare institutions for the aged. Acta med Scand 1980; 208: 87–9.
38. Lamberg-Allardt C. The relationship between serum 25-hydroxy-vitamin D levels and other variables related to calcium and phosphorus metabolism in the elderly. Acta Endocrinol. 1984; 105: 139–44.
39. Sem SW, Sjøen R, Trygg K, Pedersen JI. Vitamin D status of two groups of elderly in Oslo: living in old people's homes and living in own homes. Compr Gerontol A. 1987; 1: 126–30.
40. Russel RM, Suter PM. Vitamin requirement of elderly people: an update. Am J Clin Nutr. 1993; 58: 4–14.
41. Melin AL, Wilske J, Ringertz H, Saaf M. Vitamin D status, parathyroid function and femoral bone density in an elderly Swedish population living at home. Aging (Milano) 1999; 11: 200-7.

42. Pedersen AN. Nutritional status of 80-year old people – relations to functional capacity [80-åriges ernæringsstatus – og relationen till fysisk funktionsevne. 80-årsundersøgelsen 1994/95]. Ph.d.-thesis. Københavns Universitet 2001. Fødevaredirektoratet og Københavns Amts Center for Sygdomsforebyggelse.
43. Ooms ME, Lips P, Roos JC, van der Eijgh WJF, Popp-Snijders C, Bezemer PD, Bouter LM. Vitamin D Status and Sex Hormone Binding Globulin: Determinants of Bone Turnover and Bone Mineral Density in Elderly Women. J Bone Miner Res 1995; 10: 1177–1184.
44. McKenna MJ, Freaney R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. Osteoporos Int 1998; suppl 8: S3–S6.
45. Reginster J-Y, Frederick I, Deroisy R, Dewe W, Taquet A-N, Albert A, Collette J, Pirenne H, Zheng SX, Gosset C. Parathyroid hormone plasma concentrations in response to low 25-OH vitamin D circulation levels increases with age in elderly women. Osteporos Int 1998; 8: 390–392.
46. Lips P, Wiersinga A, van Ginkel FC, Jongen MJM, Netelenbos JC, Hackeng WHL, Delmas PD, van der Vijgh WJF. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. J Clin Endocrinol Metab 1988; 67: 644–50.
47. Chapuy MC, Arlot ME, Duboeuf F, Brun J, *et al.* Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med 1992; 327, 1637–42.
48. Dawson-Hughes B, Haris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age and older. N Engl J Med 1997; 337: 670–7.
49. Larsen ER. Falls and fractures among elderly community residents: Risk factors and prevention. Ph.D. Thesis, University of Aarhus 2002.
50. Heikinheimo RJ, Harju EJ, Haavisto MV, Karela RH, *et al.* Annual injection of vitamin D and fractures of aged bones. Calcif Tissue Int. 1992; 51: 105–10.
51. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. Brit Med J 2003; 326: 469–474.
52. Lips P, Graafmans WC, Ooms ME, Bezemer PD, Bouter LM. Vitamin D supplementation and fracture incidence in elderly persons. A randomized, placebo-controlled clinical trial. Ann Intern Med 1996; 124: 400–6.
53. Meyer HE, Smedshaug GB, Kvaavik E, Falch JA, Tverdal Aa, Pedersen JI. Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomised controlled trial. J Bone Min Res 2002; 17: 709–15.
54. Feskanich D, Willett WC, Colditz GA. Calcium, Vitamin D, Milk Consumption and Hip Fractures: A Prospectie Study among Postmenopausal Women. Amer J clin Nutr 2003; 77: 504–11.
55. Hesse von V, Siebenhüner M, Plenert W, Markestad T, Aksnes L, Aarskog D. Überprüfung der Vitamin D-'Stossprophylaxe' der Rachitis im Säuglingsalter. Kiderärtztl Raxis 1985; 53: 3383–94.
56. Seelig, MS. Vitamin D and cardiovascular, renal, and brain damage in infancy and childhood. Ann NY Acad Sci. 1969; 147: 539–82.
57. Jacobus CH, Holick MF, Shao Q, *et al.* Hypervitaminosis D associated with drinking milk. N Engl J Med 1992; 326: 1173–7.
58. Blank S, Scanlon KS, Sinks TH, *et al.* An outbreak of hypervitaminosis A associated with the overfortification of milk from a home-delivery dairy. Am J Publ Health 1995; 85: 656–9.
59. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable upper intake level of vitamin D. European Commission. SCF/CS/NUT/UPPLEV/38

Final, Brussels 2002. http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html.
60. Mattila P, Piironen V, Uusi-Rauva E, Koivistoinen P. Cholecalciferol and 25-hydroxycholecalciferol contents in fish and fish products. J Food Comp Anal 1995; 8: 232–43.
61. Becker W, Brabencová D. Vitamin D content of selected foods in Sweden. Abstract. 4th International Food Data Conference, 24–26 August, 2001, Bratislava.
62. Ovesen L, Brot C, Jacobsen J. Food content and biological activity of 25-hydroxyvitamin D: A vitamin D metabolite to be reckoned with? Ann Nutr Metab 2003; 47: 107–113.

CHAPTER 18

Vitamin E

Vitamin E, α-TE/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	8	10	5	6	7/8
Average requirement	AR	5	6			
Lower level of intake	LI	3	4			

Vitamin E has traditionally been used as a common term for two groups of components with biological vitamin E activity in mammals, tocopherols and tocotrienols, which are synthesised in plants (1). Vitamin E is essential for neurological function and acts as a lipid soluble antioxidant (2). Tocopherols (α, β, δ, γ) have a ring structure and a saturated side chain. Tocotrienols have an unsaturated side chain. The natural tocopherol isomer is RRR-α-tocopherol. In human tissues α-tocopherol is the most common form and contributes about 90% of the vitamin E content in plasma (3) and 50–80% in other tissues (4).

The biological vitamin E activity of the various tocopherols and tocotrienols used up till now was primarily established on the basis of the rat foetal resorption assay (5–7). Based on these, the activity of 1 mg RRR-α-tocopherol was set to 1 tocopherol equivalent (α-TE), and relative activity factors applied to the other components: 0.5 for β-tocopherol, 0.1 for γ-tocopherol, and less than 0.1 for other tocopherols and tocotrienols. The activity factor used for all-rac-α-tocopherol was 0.74 and for all-rac-α-tocopherol acetate 0.67, vitamin E compounds used in foods and supplements. In older literature 1 α-TE corresponds to 1.49 IU.

The appropriateness of the above activity factors for the various tocopherols and tocotrienols other than 2R-α-tocopherols has been questioned (9). Recent studies have shown low affinity of tocopherols and tocotrienols, other than the 2R-stereoisomeric forms to hepatic α-tocopherol transfer protein (α-TPP), which transfers α-tocopherol in the liver to plasma lipoproteins. In the US DRIs (9), only the 2R-stereoisomeric forms (RRR-, RSR-, RRS- and RSS-α-tocopherol) are used for establishing reference values for intake. However, these affinity data are derived from *in vitro* experiments and no information is currently avail-

able for stereoisomers other than RRR-α-tocopherol. This means that only α-tocopherol in foods and 2R-α-tocopherols in vitamin E preparations contribute vitamin E activity. For commercially available vitamin E preparations, the following factors have been suggested: 0.5 for all-rac-α-tocopherol, 0.455 for all-rac-α-tocopheryl acetate and 0.91 for RRR-α-tocopheryl acetate 0.91 (4, 8).

Physiology and metabolism

The absorption of α-tocopherol and other vitamin E forms generally varies between 51% and 86% of the ingested amount. The absorption of α-tocopherol is related to the efficiency of fat absorption and requires a normal pancreas and bile secretion (9). Lower values (21–29%) have been found in patients with fat malabsorption, cystic fibrosis, gastric carcinoma and lymphatic leukaemia (9). The relative absorption tends to decrease with increasing intakes. Absorbed vitamin E is transported in chylomicrons and taken up mainly by the liver, and some is redistributed to other lipoproteins and tissues (9). The liver vitamin E is secreted in lipoproteins bound to α-TPP, which preferentially carries α-tocopherol. α-Tocopherol and the other vitamin E components are found in the cell membranes. High concentrations occur in liver, adrenal glands and myocardium. Compared to α-tocopherol, lower levels of other vitamin E forms are generally found in tissues, especially in plasma. A recent study, however, showed that γ-tocopherol constituted 31–53% of total tocopherols in adipose tissue, muscles, skin and vein (4). This indicates that other vitamin E forms, *e.g.* γ-tocopherol, may have important biological roles, despite the low plasma concentrations (10, 11).

All tocopherols and tocotrienols are fat-soluble and act as a membrane-bound defence against free radical formation, *e.g.* peroxidation of fatty acids, primarily polyunsaturated fatty acids (1, 8). Vitamin E prevents peroxidation by stopping the peroxyl radical-initiated chain-reaction. During this reaction a radical is formed which, at least partially, can be regenerated by vitamin C. Vitamin E protects to a lesser degree against peroxidation initiated by Fe^{2+}-containing complexes and inhibits, together with vitamin C, the formation of nitrosamines, components that have been associated with an elevated risk for gastric cancer (12). Through its antioxidative capacity, vitamin E protects erythrocytes from haemolysis. At a plasma concentration of α-tocopherol below 12 µmol/L (5 mg/L) the haemolytic tendency of the erythrocytes increases among adults, a property that can be used as a criterion of vitamin E adequacy.

α-Tocopherol can also affect the vascular function and has been shown to influence *e.g.* prostacyclin formation. High vitamin E intakes have been associated with prolonged bleeding in humans (13) and animals.

Vitamin E has been associated with a number of degenerative diseases, *e.g.* cardiovascular disease, cataracts and cancer, and several epidemiological studies have suggested a protective effect of either high serum concentrations (> 30 µmol/L) or large dietary intakes, generally well above current recommended daily intakes. Most well controlled intervention studies using large intakes (50–800 mg/d) of supplemental vitamin E (α-tocopherol) have, however, failed to show a protective effect in healthy subjects (see *Chapter 15*, Dietary Antioxidants).

Requirement and recommended intake

Vitamin E deficiency due to low dietary intake has not been described in normal, healthy individuals. Clinical symptoms have only been documented among premature very low birth weight infants receiving infant formula with a high iron and polyunsaturated fatty acid (PUFA) content (14), as part of protein-energy malnutrition syndrome (15), among adults with prolonged fat malabsorption (9), or in connection with genetic abnormalities in vitamin E transport, *i.e.* abnormal α-TTP (9, 16). In premature children, symptoms such as haemolytic anaemia, trombocytosis and oedema have been reported (14). Clinical symptoms in adults include peripheral neuropathy, ataxia and skeletal myopathy. In adults, prolonged low intakes of vitamin E have been shown to increase haemolytic tendency *in vitro* (17) without any clinical symptoms. In the elderly, reduced plasma levels of α-tocopherol (15 µmol/L compared to 20 µmmol/L) have been associated with impaired cognitive function although a cause-effect relationship remains to be proven (18).

The plasma concentration of α-tocopherol is regarded as the most adequate indicator of vitamin E status (8, 19). However, since the plasma lipid level influences the α-tocopherol concentration, correction for plasma lipids may be warranted in subjects with high lipid levels when assessing vitamin E status in populations.

The vitamin E requirement is partly related to the PUFA intake, which is generally not a practical problem since most foods rich in PUFA also are rich in vitamin E.

Adults

Among adults, criteria for establishing requirement and recommended intake are the plasma concentration of α-tocopherol or the relationship

to PUFA intake. Data from studies by Horwitt *et al.* (17) showed an increased haemolytic tendency in subjects with a plasma α-tocopherol concentration below 12 µmol/L, corresponding to a tocopherol: total cholesterol ratio of 2.25 µmol/mmol (20). However, the *in vitro* haemolytic response was dependent on the PUFA content of the diet and the limited number of subjects makes this level uncertain. A plasma level above 16.2 µmol/L has been suggested as an indicator of acceptable vitamin E status (19).

Among adults consuming a diet with the lower level of PUFA, *i.e.* 3 E%, an α-tocopherol intake of 3–4 mg/d probably covers the basic needs. This is supported by a study in which plasma α-tocopherol levels of 17–18 µmol/L (4 µmol/mmol cholesterol) were seen in young subjects who were fed diets with about 1–2 mg α-tocopherol per day for three weeks (21). Data from Nordic populations show that α-tocopherol intakes of on average 6–8 mg/d (8–10 α-TE) are associated with mean plasma α-tocopherol concentrations of 20–40 µmol/L among adults (3, 22–31). Low vitamin E status has been observed in high consumers of alcohol (32) and occasional cases of neurological symptoms, which have responded to vitamin E supplementation (33), have been reported in the Nordic countries. Otherwise available data indicate that vitamin E status is sufficient in the Nordic populations at current vitamin E intakes. In a controlled clinical study, α-tocopherol levels were about 40 µmol/L (6 µmol/mmol cholesterol) after intakes of less than 8 mg/d of α-tocopherol for six months (25). It is thus not possible to establish an intake level at which plasma levels exceed 30 µmol/L, which has been associated with *e.g.* prevention of cardiovascular disease. The relationship between vitamin E and PUFA intake could also be used as a criterion for the recommended intake. This ratio is related to the general antioxidant effect of vitamin E. Using a ratio of 0.6 α-TE/g PUFA (34) and an average PUFA level of 5% of energy intake (E%), an intake of 7 and 9 α-TE/d for women and men, respectively, would be sufficient. The Scientific Committee on Food considered a ratio of 0.4 α-TE/g total PUFA to be adequate (20) both for adults and infants. This ratio could be used as a basis for the average requirement. The estimated average requirement would thus be 5 and 6 α-TE/d for women and men, respectively.

In the absence of signs of vitamin E inadequacy in the general Nordic population, the recommended intake of vitamin E is set to 8 α-tocopherol/d for women and 10 α-tocopherol/d for men. As no human data are available on the biopotency, apart from antioxidative activity, of tocopherols and tocotrienols other than the 2R-isomers of α-tocopherol, the reference values only apply to the 2R-isomers. Compared to NNR 1996, this means an increase of approximately 20%, which is the esti-

mated average contribution from the other vitamin E forms in a mixed diet using the old activity factors. A number of studies suggest that other vitamin E forms, *e.g.* γ-tocopherol, may have important functions and beneficial health effects. Although the evidence is insufficient to give numerical recommendations, it is recommended that the diet should contain various vitamin E compounds in addition to α-tocopherol.

Children

The recommended intakes for infants and children are generally based on the vitamin E content in breast milk and the relationship between α-tocopherol and linoleic acid or total PUFA (35). The Scientific Committee on Food considered a ratio of 0.4 α-TE/g total PUFA to be adequate (20). In NNR 2004, the recommended intakes are based on a ratio of at least 0.6 and a mean intake of PUFA corresponding to 5E%.

Pregnancy and lactation

The recommended intake value for pregnancy is set to 10 α-TE, which is applicable in the last two trimesters. The recommended intake during lactation includes the extra need to cover secretion in breast milk.

Upper intake levels and toxicity

Vitamin E is less toxic than other fat-soluble vitamins. In most studies no signs of adverse effects have been documented at intakes of 100–800 mg/d among adults. In the US DRIs the upper safe limit of intake is set to 1,000 mg/d of α-tocopherol (9). It is stated that this level might be re-evaluated if other studies in addition to the Finnish ATBC study show risks associated with high intakes. The Scientific Committee on Food (36) has proposed an upper level of α-tocopherol of 300 mg/d for adults. This level is mainly based on effects of increased intakes of vitamin E supplementation on blood clotting and includes an uncertainty factor.

In the ATBC study a weak, but non-significant, increase in total mortality was observed among smoking middle-aged men consuming 50 mg supplemental α-tocopherol for about 6 years (37). Mortality due to haemorrhagic stroke and cancer was increased, while mortality in ischaemic stroke was decreased (37). Further analysis (38, 39) showed that the risk of haemorrhagic stroke was increased, while the risk of ischaemic stroke was reduced only among hypertensives. The net effect on stroke incidence and mortality was, however, non-significant. The authors conclude that vitamin E supplements might increase the

risk of haemorrhagic stroke and decrease the risk of ischaemic stroke among high-risk individuals with hypertension. No benefits of vitamin E supplementation among subjects with a high risk for cardiovascular disease have been observed (40–42). Supplementation with 200 mg vitamin E to elderly subjects did not alleviate acute symptoms of respiratory tract infections, instead adverse effects on illness severity were observed (43).

Supplemental of α-tocopherol in the order of 30–50 mg/d may affect bleeding tendency in combination with acetylsalicylic acid and in subjects treated with anticoagulants (13, 44).

Taken together, available scientific data suggest that there are no benefits of high intakes of vitamin E. Although the risks associated with high supplemental intakes of vitamin E remain unclear, available data suggest some potential risks, especially among long-term smokers and subjects with increased risk of cardiovascular disease. In the absence of clear health benefits, prolonged intake of supplemental vitamin E does not seem to be justified for the general population.

Dietary sources and intakes

Vegetable oils, vegetable oil-based spreads, nuts and seeds, certain fatty fish, egg yolk and whole grain cereals are the main dietary sources of vitamin E. The content of the various vitamin E forms varies considerably between the vegetable oils commonly available on the market. The α-tocopherol content is high in sunflower seed and corn oil, but moderate to low in olive, rapeseed and soybean oil. Rapeseed oil is high in γ-tocopherol. Recent national dietary surveys in the Nordic countries have found intakes of 8–13 α-TE per 10 MJ (calculated with previous conversion factors), corresponding to about 7–11 mg α-tocopherol (see *Chapter 43*).

REFERENCES

1. Traber M. Vitamin E. In: Shils ME, Olson JA, Shike M, editors. Modern nutrition in health and disease, 9th ed. Philadelphia: Lippincott Williams & Wilkins, 1999.
2. Brigelius-Flohe R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM, Azzi A. The European perspective on vitamin E: current knowledge and future research. Am J Clin Nutr 2002; 76: 703–16.
3. Piironen V, Varo P, Syväoja EL, Salminen K, Koivistoinen P, Arvilommi H. High-performance liquid chromatographic determination of tocopherols and tocotrienols and its application to diets and plasma of Finnish men. II. Applications. Int J Vitam Nutr Res 1984; 54: 41–6.

4. Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L, Ingold KU. Human plasma and tissue α-tocpherol concentrations in response to supplementation with deutereated natural and synthetic vitamin E. Am J Clin Nutr 1998; 67: 669–84.
5. Bieri JG, Evarts RP. Gamma tocopherol: metabolism, biological activity and significance in human vitamin E nutrition. Am J Clin Nutr 1974; 27: 980–86.
6. Bieri JG, McKenna MC. Expressing dietary values for fat-soluble vitamins: changes in concepts and terminology Am J Clin Nutr 1981; 34: 289–95.
7. Weiser H, Vecchi M. Stereoisomers of α-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. Internat J Vit Nutr Res 1982; 52: 351–70.
8. Horwitt MK. My valedictory on the differences in biological potency between RRR-alpha-tocopheryl and all-rac-alpha-tocopheryl acetate. Am J Clin Nutr 1999; 69: 341–2.
9. Food and Nutrition Board. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Institute of Medicine, National Academy of Sciences, Washington, DC: 2000.
10. Jiang Q, Christen S, Shigenaga MK, Ames BA. γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention.
11. Devaraj S, Traber MG. G-Tocopherol, the new vitamin E? Am J Clin Nutr 2003; 77: 530–1.
12. Weisburger JH. Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants, and carotenoids. Am J Clin Nutr 1991; 53: 226S–37S.
13. Liede KE, Haukka JK, Saxen LM, Heinonen OP. Increased tendency towards gingival bleeding caused by joint effect of alpha-tocopherol supplementation and acetylsalicylic acid. Ann Med 1998; 30: 542–6.
14. Hassan H, Hashim SA, Van Itallie TB, Sebrell WH. Syndrome in premature infants associated with low plasma vitamin E levels and high polyunsaturated fatty acid diet. Am J Clin Nutr 1966; 19: 147–57.
15. Kalra V, Grover JK, Ahuja GK, Rathi S, Gulati S, Kalra N. Vitamin E administration and reversal of neurological deficits in protein-energy malnutrition. J Trop Pediatr 2001; 47: 39–45.
16. Aparicio JM, Belanger-Quintana A, Suarez L, Mayo D, Benitez J, Diaz M, Escobar H. Ataxia with isolated vitamin E deficiency: case report and review of the literature. J Pediatr Gastroenterol Nutr 2001; 33: 206–10.
17. Horwitt MK, Century B, Zeman AA. Erythrocyte survival time and reticulocyte levels after tocopherol depletion in man. Am J Clin Nutr 1963; 12: 99–106.
18. Ortega RM, Requejo AM, Lopez-Sobaler AM, Andres P, Navia B, Perea JM, Robles F. Cognitive function in elderly people is influenced by vitamin E status. J Nutr 2002; 132: 2065–8.
19. Morrissey PA, Sheehy PJ. Optimal nutrition: vitamin E. Proc Nutr Soc 1999; 58: 459–68.
20. Commission of the European Communities. Reports of the Scientific Committe on Food (Thirty-first series). Nutrient and energy intakes for the European Community. Luxembourg, 1993.
21. Sandström B, Bügel S, Lauridsen C, Nielsen F, Jensen C, Skibsted LH. Cholesterol-lowering potential in human subjects of fat from pigs fed rapeseed oil. Br J Nutr 2000; 84: 143–50.
22. Parviainen MT, Kumpusalo E, Halonen P, Neittaanmaki L, Pekkarinen H. Epidemiology of vitamins A, E, D and C in rural villages in Finland: biochemical, nutritional and socio-economical aspects. Int J Vitam Nutr Res 1992; 62: 238–4.
23. Rautalahti M, Albanes D, Haukka J, Roos E, Gref C-G, Virtamo J. Seasonal variation of serum concentrations of β-carotene and α-tocopherol. Am J Clin Nutr 1993; 57; 551–6.

24. Stegmayr B, Johansson I, Huhtasaari F, Moser U, Asplund K. Use of smokeless tobacco and cigarettes – effects on plasma levels of antioxidant vitamins. Int J Vitam Nutr Res 1993; 63: 195–200.
25. Sarkkinen ES, Uusitupa MI, Nyyssonen K, Parviainen M, Penttila I, Salonen JT. Effects of two low-fat diets, high and low in polyunsaturated fatty acids, on plasma lipid peroxides and serum vitamin E levels in free-living hypercholesterolaemic men. Eur J Clin Nutr 1993; 47: 623–30.
26. Kristenson M, Zieden B, Kucinskiene Z, Elinder LS, Bergdahl B, Elwing B, Abaravicius A, Razinkoviene L, Calkauskas H, Olsson AG. Antioxidant state and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross sectional study of men aged 50. BMJ 1997; 314: 629–33.
27. Anttolainen M, Valsta LM, Alfthan G, Kleemola P, Salminen I, Tamminen M. Effect of extreme fish consumption on dietary and plasma antioxidant levels and fatty acid composition. Eur J Clin Nutr 1996; 50: 741–6.
28. Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 2000; 54: 715–25.
29. Wallström P, Wirfalt E, Lahmann PH, Gullberg B, Janzon L, Berglund G. Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. Am J Clin Nutr 2001; 73: 777–85.
30. Nes M, Lund-Larsen K, Trygg K, Høivik HO, Pedersen JI. Nutrition and the Elderly in Europe: Low Prevalence of Obesity and Biochemical Deficiences in Norwegian Subjects. Age & Nutrition 1993; 4: 72–81.
31. Pedersen AN. Nutritional status of 80-year old people – relations to functional capacity (80-åriges ernæringsstatus – og relationen till fysisk funktionsevne. 80-årsundersøgelsen 1994/95). Ph.d.-avhandling. Københavns Universitet 2001. Fødevaredirektoratet og Københavns Amts Center for Sygdomsforebyggelse.
32. Bjørneboe G-EA, Johnsen J, Bjørneboe A, Bache-Wiig JE, Mørland J, Drevon CA. Diminished serum concentration of vitamin E and selenium in alcoholics. Annals Nutr Metab 1988; 32: 56–61.
33. Gjerde IO, Storstein A, Skeie GO, Wester K, Hegrestad S, Houge G. Ataxi på grunn av vitamin E-mangel. Tidskr Nor Lægeforen 1998; 118: 3126–8.
34. Valk EE, Hornstra G. Relationship between vitamin E requirement and polyunsaturated fatty acid intake in man: a review. Int J Vitam Nutr Res 2000; 70: 31–42.
35. Aggett P, Bresson JL, Hernell O, Koletzko B, Lafeber H, Michaelson KF, Micheli JL, Ormisson A, de Sousa JS, Weaver L. Comment on the vitamin E content in infant formulas, follow-on formulas, and formulas for low birth weight infants. ESPGHAN Committee on Nutrition. European Society of Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 1998; 26: 351–2.
36. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of vitamin E (expressed on 4 April 2003). SCF/CS/NUT/UPPLEV/31 Final. 23 April 2003. European Commission. Health and Consumer Protection Directorate General.
37. ATBC Cancer Prevention Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994; 330: 1029–35.
38. Leppälä JM, Virtamo J, Fogelholm R, Albanes D, Taylor PR, Heinonen OP. Vitamin E and beta-carotene supplementation in high risk for stroke: a subgroup analysis of the Alpha-Tocopherol, Beta-carotene Cancer Prevention Study. Arch Neurl 2000; 57: 1503–9.

39. Leppälä JM, Virtamo J, Fogelholm R, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers. Arterioscler Thromb Vasc Biol 2000; 20: 230–5.
40. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet 1997; 349: 1715–20.
41. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. New Engl. J. Med. 2001; 345: 1583–1592.
42. Heart Protection Study Collaborative Group. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002; 360: 23–33.
43. Graat JM, Schouten EG, Kok FJ. Effects of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons. JAMA 2002; 228: 715–721.
44. Swedish Medical Products Agency (Läkemedelsverket). Naturläkemedlet Curcubin och risk för antikoagulationseffekt – möjligen relaterat till E-vitamininnehållet. Info från Läkemedelsverket 2000: 11 (3): 77–78.

CHAPTER 19

Vitamin K

Vitamin K is the collective term for compounds with vitamin K activity and having the common 2-methyl-1,4-naphtoquinone ring structure.

Vitamin K occurs naturally in two forms. Phylloquinone or vitamin K_1 (2-methyl-3-phytyl-1,4-naphtoquinone) is synthesised by plants. Menaquinones or vitamin K_2 (multi-isoprenylquinones, several species) are produced by bacteria. Both forms are found in animal tissues.

Physiology and metabolism

Compounds with vitamin K activity are required as cofactors for the carboxylation of glutamic acid to γ-carboxyglutamic acid (Gla) needed for the synthesis of factors II (prothrombin), VII, IX, and X, and proteins C, S, and Z, all involved in the coagulation of blood (1, 2). The presence of Gla in these proteins enables them to bind calcium. Three Gla-containing proteins have been identified in bone, *i.e.* osteocalcin, matrix Gla protein and protein S. Osteocalcin may be involved in the regulation of bone mineralisation, but otherwise the exact function of these proteins in bone is not known. Any role of vitamin K in osteoporosis is currently under intense investigation (3). A number of other Gla-containing proteins with unknown functions have been identified in several tissues, such as Gas6 which is presumably involved in cell cycle regulation, nephrocalcin, atherocalcin and the prolin-rich Gla proteins 1 and 2.

Under normal conditions 40–70% of vitamin K is absorbed in the jejunum and ileum but depending on source and the vehicle in which it is administered, absorption may be as low as 10% or as high as 80% (2). Absorption from the colon is very low. Absorption requires bile and pancreatic juice and is increased by fat in the diet. Fat malabsorption decreases the absorption of vitamin K significantly and bleeding is often an early sign of this condition. Poorly absorbable fat substances or fat substitutes may also reduce absorption.

Absorbed vitamin K is transported by lipoproteins in the lymph and is mainly taken up by the liver, considered to be the main storage organ.

Some is also stored in other organs, in particular relatively high contents have been detected in bone tissue. Compared to other fat soluble vitamins, the total body pool is small. Turnover of phylloquinone is rapid, but somewhat slower for menaquinones. Hepatic reserves are rapidly depleted when dietary vitamin K is restricted. A more or less continuous supply is thus required to maintain satisfactory body stores.

Because of poor placental transport of vitamin K and consequent deficiency in the newborn, haemorrhage, sometimes also intracranial, may occur during the neonatal period.

Vitamin K and osteoporosis

High concentrations of undercarboxylated osteocalcin and low concentrations of vitamin K have been reported to be associated with low bone mineral density and increased risk of hip fracture. Low vitamin K intake was found to been associated with increased risk of hip fracture in 2 large prospective studies (4, 5). Low dietary vitamin K has also been found to be associated with low bone mineral density in women, but not in men (6). A possible confounder in these studies may be that high dietary intake of vitamin K is associated with high intake of vegetables, which may be suggestive of a healthy lifestyle. In a meta-analysis of the effect of long-term treatment by oral anticoagulant on bone density, no differences were found any site apart from lower bone density in the ultradistal radius (7). It may thus be premature to conclude that low vitamin K status is a risk factor for osteoporosis.

In connection with bone formation, the interplay between vitamin D and vitamin K should be mentioned. Vitamin D is involved in transcriptional regulation of proteins subsequently post-translationally gamma carboxylated by vitamin K.

Requirement and recommended intake

Clinical deficiency is normally not detected after the first few months of life. Deficiency has been seen in connection with malabsorption, antibiotic treatment and parenteral nutrition without vitamin K supplementation.

Determination of the requirement for vitamin K has been difficult since it is not possible to observe clinical deficiency symptoms on a vitamin K-free diet. Bacterial synthesis in the intestine is not sufficient, however, to maintain normal serum levels of vitamin K. The traditional method to evaluate vitamin K status has been to determine the concentration of coagulation factors, most often measured as prothrombin

time. This is an insensitive method and more recently serum concentration of under-γ-carboxylated prothrombin and percentage under-γ-carboxylated osteocalcin and urinary GLA excretion have been introduced as measures of vitamin K status (8). The Food and Nutrition Board did not find these methods reliable to be used in the assessment of requirement (9) because of uncertainty surrounding their true physiological significance and the lack of sufficient dose-response data. Therefore, the recent US DRIs, 120 and 90 µg/d for men and women respectively, are based on reported vitamin K dietary intake in apparently healthy population groups (8, 9). A depletion-repletion study on 10 young men showed that a reduction of phylloquinone in the diet from the normal level of 80 µg/d to about half that level resulted in reduced plasma phylloquinone after 3 weeks, increase in undercarboxylated prothrombin in plasma and reduced urinary excretion of Gla (10). Supplementation by 50 µg/d reversed these changes. Healthy young individuals on intakes of about 60 to 80 µg/d have shown no signs of deficiency, indicating that this intake is adequate for the majority of individuals (10–12). The findings are in accordance with older data indicating a requirement of the order of 1 µg/kg/d (13). In the absence of more specific data, a recommendation of 1 µg/kg/d is provisionally proposed for both children and adults. It cannot be excluded, however, that this amount might be insufficient to fully support maximal osteocalcin γ-carboxylation in the elderly (14).

Breast fed newborns are at risk of haemorrhage. Vitamin K concentrations in human milk have ranged from 0.85 to 9.2 µg/L with a mean of 2.5 µg/L (9). Using the highest concentration as a basis would transform to a recommended intake of about 2 µg/kg/day. All newborns should routinely be given vitamin K (as a 1 mg intramuscular dose, or as daily oral doses) to avoid haemorrhage during the neonatal period and oral prophylaxis should be continued for the 3 first months (15).

Upper intake levels and toxicity

No evidence of toxicity associated with high intakes of either form of natural vitamin K has been reported. The Scientific Committee on Food of the European Commission concludes in their report that there is no evidence of adverse effects associated with supplementary intakes of vitamin K in the form of phylloquinone of up to 10 mg/d for limited periods of time (16). Synthetic analogues such as menadione have been associated with liver damage and haemolytic anaemia and should not be used therapeutically.

Dietary sources and intake

Leafy green vegetables, vegetable oils and vegetable margarines are the main sources of phylloquinone (8, 17). Menaquinones are found in liver and certain cheeses. Natto, a fermented soybean preparation, is particularly rich in menaquinone-7. The best estimates of intake levels in the Nordic countries are probably those by Koivu-Tikkanen from Finland (17, 18). Based on HPLC analyses of vitamin K in a large number of food products and food intake data from various sources, an average intake of 120 µg/d was calculated.

REFERENCES

1. Ferland G. Vitamin K. In: Present Knowledge in Nutrition, 8th ed. (Bowman BA and Russell RM, eds). ILSI Press, Washington, DC, 2001: 164–172.
2. Olson RE. Vitamin K. In: Modern Nutrition in Health and Disease, 9th ed. Shils ME, Olson JA, Shike M and Ross AC, eds). Williams & Wilkins, Baltimore, 1999: 363–380.
3. Olson RE. Osteoporosis and vitamin K intake. Am J Clin Nutr 2000; 71: 1031–1032.
4. Weber FD, Willett WC, Booth RH, Colditz GA. Vitamin K intake and hip fracture in women: a prospective study. Am J Clin Nutr 1999; 69: 74–79.
5. Booth SL, tucker KL, Chen H, Hannan MT, *et al.* Dietary vitamin K intakes are associated with hip fracture but not with bone density in elderly men and women. Am J Clin Nutr 2000; 71: 1201–1208.
6. Booth SL, Broe KE, Gagnon DR, Tucker KL, *et al.* Vitamin K intake and bone density mineral in women and men. Am J Clin Nutr 2003; 77: 512–516.
7. Caraballo PJ, Gabriel SE, Castro MR, Atkinson EJ, Melton LJ. Changes in bone density after exposure to oral anticoagulants: A meta-analysis. Osteoporos Int 1999; 9: 441–448.
8. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J Nutr 1998; 128: 785–788.
9. Food and Nutrition Board. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001: 162–196.
10. Suttie JW, Mummah-Schendel LL, Shah DV, Lyle BJ, Greger JL. Vitamin K deficiency from dietary vitamin K restriction in humans. Am J Clin Nutr 1988; 47: 475–480.
11. Jones DY, Koonsvitsky BP, Ebert ML, Jones MB, *et al.* Vitamin K status of free-living subjects consuming olestra. Am J Clin Nutr 191; 53: 943–946.
12. Bach AU, Anderson SA, Foley AL, Williams EC, Suttie JW. Assessment of vitamin K status in human subjects administered 'minidose' warfarin. Am J Clin Nutr 1996; 64: 894–902.
13. National Research Council. Vitamin K in: Recommended Dietary Allowances, 10th. Ed. Washington DC: National Academy Press, 1998, pp 107–14.
14. Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ.

Dietary phylloquinone depletion and repletion in older women. J Nutr 2003; 133: 2565–9.
15. Hansen KN, Ebbesen F. Neonatal vitamin prophylaxis in Denmark: three years' experience with oral administration during the first three months of life compared to one oral administration at birth. Acta Paediatr 1996; 85: 1137–1139.
16. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin K (expressed on 4 April 2003). http://europa.eu.int/comm/food/fs/sc/scf/index_en.html
17. Koivu-Tikkanen J. Determination of phylloquinone and menaquinones in foods. Academic dissertation, University of Helsinki 2001.
18. Piironen V, Koivu T, Tammisalo O, Mattila P. Determination of phylloquinone in oils, margarines and butter by high-perfomance liquid chromatography with electrochemical detection. Food Chem 1997; 59: 473–480.

CHAPTER 20

Thiamin

Thiamin, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	1.1	1.4	0.6	0.9	1.0/1.2
Average requirement	AR	0.9	1.2			
Lower level of intake	LI	0.5	0.5			

Thiamin is essential for the utilisation of carbohydrates in the body. In the metabolism thiamin participates in the form of thiamin pyrophosphate (diphosphate) as a coenzyme for pyruvate dehydrogenase, transketolase and a ketoglutarate dehydrogenase in the oxidative decarboxylation of keto acids to aldehydes and in the utilisation of pentoses (1, 2). Thiamin pyrophosphate is also a coenzyme for keto acid dehydrogenase in the metabolism of branched chain amino acids (2). Thiamin triphosphate (TTP) is involved in nerve and possibly muscle function (2).

Physiology and metabolism

In vegetable foods, thiamin occurs mainly in the free form and in animal foods mainly in phosphorylated forms (3). Absorption takes place in the small intestine, generally via an active, carrier mediated system involving phosphorylation. At high intakes passive diffusion also takes place. Studies with ^{14}C labelled thiamin in young men (4) showed that more than 95% of the vitamin was absorbed at intakes of 1–2 mg/day. At intakes above 5 mg/d the relative absorption rapidly decreases.

In the liver, thiamin is converted to its biologically active form, thiamin pyrophosphate (TPP). The main proportion of the total body pool of about 30 mg in an adult is found in the muscles and liver (1). The metabolism of thiamin in the body is relatively fast, and the half life of ^{14}C labelled thiamin is estimated to be 9–18 days.

Thiamin deficiency causes beri-beri. In adults, symptoms include disturbances in the peripheral nervous system and heart function. Early deficiency symptoms may include anorexia, weight loss, mental

changes and muscle weakness. In alcoholics, conditions such as Wernicke's encephalopathy and Korsakoff's psychosis occur, which are strongly related to insufficient thiamin intake and/or malabsorption. Among children symptoms appear more instantly and are generally more severe, *e.g.* heart failure.

Commonly used indicators of thiamin status include the activity of the enzyme transketolase in the erythrocytes and urinary thiamin excretion. The activity coefficient represents the degree of enzyme activity stimulation *in vitro*. An activity coefficient below 1.15 is regarded as an indicator of sufficient status, while a coefficient of 1.15–1.25 indicates marginal status (5). The concentration of free thiamin and its phosphate esters in blood or erythrocytes has been shown to be a good indicator of thiamin status (6), especially among subjects at risk for thiamin deficiency (6–8). The usefulness of the activity coefficient as an indicator of thiamin status in population surveys has been questioned, mainly due to its low correlation with *e.g.* erythrocyte thiamin (9).

Requirement and recommended intake

The requirement of thiamin has generally been related to the energy and carbohydrate intake (10–14). A clear relationship was shown by Sauberlich *et al.* (11). The recent US dietary reference values are, however, based on absolute intakes (15). Generally, thiamin intakes are related to energy and protein intakes at normal intake ranges of populations such as those of the Nordic countries.

Clinical signs of deficiency have been observed at intakes below 0.5 mg/d, corresponding to 0.05 mg/MJ (0.2 mg/1,000 kcal) (10–15). In other studies thiamin excretion in urine and erythrocyte transketolase activity coefficients were normalised at intakes of 0.07–0.08 mg/MJ (0.30–0.33 mg/1,000 kcal).

In the absence of new data, the reference intakes set in NNR 1996 are kept unchanged. The average requirement for adults and children is thus set at 0.10 mg/MJ and the recommended intake at 0.12 mg/MJ. However, when planning diets with energy levels below 8 MJ/d, the thiamin content should be at least 0.8 mg/day. The recommended intake for infants 0–12 months is set to 0.10 mg/MJ. The lower level of intake is estimated at 0.05 mg/MJ.

Studies on pregnant and lactating women indicate a higher requirement as assessed with biochemical parameters. An additional intake of 0.4 mg/d during pregnancy and 0.5 mg/d during lactation is recommended.

A few studies indicate that thiamin utilisation is impaired among elderly subjects. Therefore, when planning diets with energy levels below 8 MJ/d, the thiamin content should be at least 1.0 mg/day.

Upper intake levels and toxicity

There are no data on toxicity at oral intakes up to 500 mg/d for periods up to one month (16).

Dietary sources and intakes

Major food sources of thiamin in the Nordic diet are cereals and cereal products, meat and meat products and milk products. The dietary supply of thiamin in the Nordic countries is 1.3–1.6 mg/10 MJ (see *Chapter 43*).

REFERENCES

1. Tanphaichitr, V. Thiamin. In: M.E. Shils, J.A. Olson & M. Shike (editors). Modern Nutrition in Health and Disease, 9th ed. Philadelphia: Lipincott Williams & Wilkins, 1999.
2. Bender DA. Optimum nutrition: thiamin, biotin and pantothenate. Proc. Nutr. Soc. 1999; 58: 427–33.
3. Gregory, JF. III. Bioavailability of thiamin. Eur J Clin Nutr ; 1997; 51 (Suppl. 1): S34 S37.
4. Ariaey Nejad MR, Balaghi M, Baker EM, Sauberlich HE. Thiamin metabolism in man. Am J Clin Nutr 1970; 23: 764–78.
5. Finglas PM. Thiamin. Int J Vitam Nutr Res. 1993; 63: 270–4.
6. Talwar D, Davidson H, Cooney J, St JO'Reilly D. Vitamin B1 status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: Comparison with erythrocyte transketolase activation assay. 2000; Clin. Chem. 46: 704–10.
7. Tallaksen CME, Bøhmer T, Bell H. Blood and serum thiamin and thiamin phosphate esters concentrations in patients with alcohol dependence syndrome before and after thiamin treatment. Alcoholism: Clin Exp Res 1992; 16: 320–5.
8. Tallaksen CME, Bell H, Bøhmer T. Thiamin and thiamin phosphate esters deficiency assessed by high performance liquid chromatography in four clinical cases of Wernicke encephalopathy. Alcoholism: Clin Exp Res 1992; 17: 712–6.
9. Baily AL, Finglas PM, Wright AJA, Southon S. Thiamin intake, erythrocyte transketolase (EC 2.2.1.1) activity and total erythrocyte thiamin in adolescents. Br. J Nutr 1994; 72: 111–25.
10. FAO/WHO Expert Group. Requirements of Vitamin A, Thiamin, Riboflavin and Niacin. FAO Nutr. Meetings. Report Series No. 41, WHO Technical Report Series No. 362. Rome: FAO, 1967.
11. Sauberlich HE, Herman YF, Stevens CO, Herman RH. Thiamin requirement of the adult human. Am J Clin Nutr 1979; 32: 2237–48.
12. FAO. Handbook on Nutritional Requirements. Rome: FAO, 1974.
13. National Research Council. Recommended Dietary Allowances. 10th ed. Washington DC: National Academy Press, 1989.

14. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and Energy Intakes for the European Community. (Thirty first series of Food Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1993.
15. Food and Nutrition Board. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Institute of Medicine, National Academic Press, Washington, 1998.
16. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin B1 (expressed on 11 July 2001). SCF/CS/NUT/UPPLEV/46 Final. 16 July 2001 European Commission. Health and Consumer Protection Directorate General.

CHAPTER 21

Riboflavin

Riboflavin, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	1.3	1.7	0.7	1.1	1.2/1.4
Average requirement	AR	1.1	1.4			
Lower level of intake	LI	0.8	0.8			

Riboflavin has its function as precursors for coenzymes, FMN (flavin mononucleotide), FAD (flavin adenin dinucleotide) and covalently bound flavin. These are necessary components of a number of oxidative enzyme systems and participate in the electron transport (1).

Physiology and metabolism

Riboflavin occurs in foods free or as FAD or FMN as a complex with protein. Protein bound riboflavin is hydrolysed to free riboflavin in the gastrointestinal tract and absorbed via a specific transport mechanism rather than by passive diffusion (2–4). This mechanism is saturated at intakes of about 30–50 mg, but at habitual intakes urine excretion is proportional to the intake, since the body has a small body pool. Absorption rates of free riboflavin are reported to be 50–60% at doses of 2–25 mg (4).

Riboflavin is mainly stored in the body as flavoproteins and to a lesser degree as free riboflavin. As a consequence, urinary excretion may be affected by changes in nitrogen balance. The urinary excretion of riboflavin may increase in conditions of negative nitrogen balance and infections, while the opposite may be seen in rapid growth (5). No consistent relation has been found between riboflavin requirement, measured by urine excretion or retention, and protein intake in situations of positive protein balance. Riboflavin is also involved in the folate metabolism in that FAD is a co-enzyme for methylenetetrahydrofolate reductase (MTHFR), which influences the metabolism of homocysteine.

Low riboflavin status, assessed by erythrocyte gluthathion reductase (EGRAC), has been associated with increased plasma homocysteine levels in subjects with a specific genotype of the MTHFR (6).

Indicators used to assess riboflavin status include the activity coefficient of EGRAC and urinary excretion of the vitamin (7). Depletion-repletion studies show that the urinary excretion of riboflavin increases gradually with increasing intakes, with a sharp increase at intakes about 1 mg/d, indicating tissue saturation. The EGRAC represents the degree of stimulation of the enzyme activity *in vitro* after addition of FAD. There is generally a relation between the activity coefficient and the riboflavin intake, which is most clear at intakes up to about 1 mg/day. Different criteria for normal EGR-activity have been suggested, which may complicate the interpretation of results from studies on riboflavin status (1, 7, 8).

In setting reference values most previous expert groups have related the riboflavin intake to either energy or protein intake (9, 11, 12). The recent US dietary reference intakes are, however, based on absolute intakes (1). Generally, riboflavin metabolism and intake are related to energy and protein intake at normal intake ranges of populations such as the Nordic. However, at energy intakes below 8 MJ/d the requirement expressed per MJ may be higher, while the opposite may be the case at energy intakes well above 12 MJ/day.

Although the metabolic effects of riboflavin deficiency are profound, there are only a few clear-cut clinical symptoms. These include various skin changes (angular stomatitis, seborrhoeic dermatitis) and glossitis. Severe riboflavin depletion has been associated with impaired iron status, anemia and mental disturbances (13). Isolated dietary riboflavin deficiency does not usually occur, but deficiency is normally seen in association with other nutritional deficiencies.

Clinical signs of riboflavin deficiency have been observed in men at intakes of 0.6 mg/d or less, corresponding to 0.06 mg/MJ (0.25 mg/1,000 kcal) (1, 9–12). In a long-term study consumption of a diet containing 0.75–0.85 mg/d equal to 0.3–0.4 mg/1,000 kcal (4.2 MJ) during up to two years certain clinical symptoms were observed in one man.

Requirement and recommended intake

In the absence of new scientific data the reference values for riboflavin given in NNR 1996 are retained. The average requirement is estimated to 0.12 mg/MJ based on studies in which riboflavin status has been assessed using urinary excretion of riboflavin or EGRAC (11). The recommended intake is set to 0.14 mg/MJ, *and applies to both children and adults.*

This corresponds to an intake of about 1.6 mg/d for adult men and 1.3 mg/d for adult women. However, when planning diets the riboflavin content should not be lower than 1.2 mg/d, even at energy intake below 8 MJ/d (9). For pregnant and lactating women an extra 0.3 and 0.4 mg/d, respectively, is recommended. The lower intake level is estimated to 0.8 mg/day.

Upper intake levels and toxicity

There are no reports of adverse effects of high riboflavin intakes from dietary sources. The limited studies in which large doses (100–400 mg/d) of supplemental riboflavin have been administered do not indicate any adverse effects (4).

Dietary sources and intake

Major sources of riboflavin in the Nordic diets are milk and milk products, meat and meat products. The average dietary intake according to national dietary surveys is in the range 1.8–2.3 mg/10 MJ (see *Chapter 43*).

REFERENCES

1. Food and Nutrition Board. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. Institute of Medicine, National Academic Press, Washington. 1998.
2. Rivlin RS. Riboflavin metabolism. New Engl J Med 1970; 283: 463–72.
3. Bates CJ. Bioavailability of riboflavin. Eur J Clin Nutr 1997; 51 (Suppl 1): S38–S42.
4. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of vitamin B2 (expressed on 22 November 2000). SCF/CS/NUT/UPPLEV/33 Final. 7 December 2000. European Commission. Health and Consumer Protection Directorate General.
5. Sauberlich HE. Vitamin metabolism and requirements. Some aspects reviewed. SA Med J 1975; 48: 2235–44.
6. McNulty H, McKinley MC, Wilson B, McPartlin J, Strain JJ, Weir DG, Scott JM. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr 2002; 76: 436–41.
7. Powers HJ. Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. Proc Nutr Soc 1999; 58: 435–40.
8. Toh SY, Thompson GW, Basu TK. Riboflavin status of the elderly: dietary intake and FAD-stimulating effect on erythrocyte glutathione reductase coefficients. Eur J Clin Nutr 1994; 48: 654–9.
9. FAO/WHO Expert Group. Requirements of vitamin A, thiamine, riboflavine and niacin. FAO Nutr. Meetings. Report Series No. 41, WHO

Technical Report Series No. 362. Rome: FAO, 1967.
10. Horwitt MK, Harvey CC, Hills OW, Liebert E. Correlation of urinary excretion of riboflavin with dietary intake and symtoms or ariboflavinosis. J Nutr 1950; 11: 247–64.
11. National Research Council. Recommended Dietary Allowances, 10th ed. Washington DC: National Academy Press, 1989.
12. Commission of the European Communities. Reports of the Scientific Committee on Food (Thirty-first series). Nutrient and energy intakes for the European Community. Luxembourg, 1993.
13. Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr 2003; 77: 1352–60.

CHAPTER 22

Niacin

Niacin, NE/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	15	19	9	12	14/16
Average requirement	AR	12	15			
Lower level of intake	LI	9	12			

Niacin is the common term for nicotinic acid and nicotinamide and derivates that exhibit the biological activity of nicotinamide. Niacin has its main function in the form of the coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate), which are involved in a number of redox reactions in the metabolism of glucose, amino acids and fatty acids.

Physiology and metabolism

In foods, niacin mainly occurs as NAD and NADP, which are effectively hydrolysed and absorbed in the gut (1). Data from human studies indicate near complete absorption of up to 3 grams of nicotinic acid. In cereals, *e.g.* maize, niacin can be present in a form considered to be less available, *e.g.* esterified to polysaccharides (2). Alkaline treatment during preparation releases much of the niacin.

In the body niacin is formed from tryptophan. On average, 60 mg of dietary tryptophan is estimated to give rise to 1 mg niacin (= niacin equivalent, NE). The body has a limited capacity for storing niacin nucleotides and deficiency symptoms can occur after 50–60 days of consumption of a low-niacin corn-based diet (1).

Niacin status can be measured by urinary excretion of certain metabolites, *e.g.* N'-methylnicotinamide and methyl pyridone carboxamides.

Niacin deficiency results in pellagra, mainly observed in populations consuming a diet predominantly based on maize or other cereals with a low protein content and low bioavailablity of niacin. In a controlled study, pellagra developed at an intake of 8.8 NE/d (2). In another study

no clinical symptoms were seen in subjects with an intake of 9.2–12.3 NE per day, equivalent to 1 NE/MJ (3–5).

Requirement and recommended intake

In the absence of new scientific data the reference values for niacin given in NNR 1996 are retained. The average requirement is set to 1.3 NE/MJ based on studies in which niacin status has been assessed using urinary excretion of niacin metabolites, which is considered to be an appropriate marker (5, 6). The recommended intake is set to 1.6/MJ. This corresponds to an intake of 17–19 NE/d for adult men and 14–15 NE/d for adult women. However, when planning diets the niacin content should not be lower than 13 NE/d, even at energy intake below 8 MJ/day. For pregnant women an extra 1–2 NE/d, and for lactating women an extra intake of 4–5 NE/d is recommended, which is based on the niacin content of breast milk and the increased energy requirement.

For infants and children over 6 months of age, the recommended intake for adults is applied.

The lower level of intake is estimated to be 1 NE/MJ. At energy intakes below 8 MJ/d the lower level is estimated to be 8 NE/day.

Upper intake levels and toxicity

There are no studies indicating adverse effects of consumption of naturally occurring niacin in foods. Intakes of nicotinic acid, but not nicotinamide, as a supplement or fortificant in the range 30–1,000 mg/d can result in mild symptoms such as flushing. Higher intakes have been reported to induce liver damage. The US Food and Nutrition Board (5) set an upper limit of 30–35 mg/d for adolescents and adults, based on the risk of flushing. For children 1–3 years, FNB set the UL to 10 mg/d, for 4–8 years 15 mg/d and for 9–13 years 20 mg/day.

The EU Scientific Committee on Food has proposed an upper limit for nicotinic acid of 10 mg/d and for nicotinamide of 900 mg/d for adults (7). These levels are also used in NNR.

Dietary sources and intake

Preformed niacin occurs in foods such as meat, fish, and pulses. Protein-rich foods also contribute to the niacin intake through conversion from tryptophan. The diet in the Nordic countries provides 30–40 NE/10 MJ (see *Chapter 43*).

REFERENCES

1. Cervantes-Laurean D, McElvaney NG, Moss J. Niacin. I: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease, 9th ed. Philadelphia: Lipincott Williams & Wilkins, 1999.
2. van den Bergh H. Bioavailability of niacin. Eur J Clin Nutr 1997; 51 (Suppl 1): S64–S65.
3. Food and Nutrition Board. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. Institute of Medicine, National Academic Press, Washington DC: 1998.
4. National Research Council. Recommended Dietary Allowances, 10th ed. Washington DC: National Academy Press, 1989.
5. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community. (Thirty-first series of Food – Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1993.
6. Powers HJ. Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. Proc Nutr Soc 1999; 58: 435–40.
7. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of niacin (expressed on 17 April 2002). SCF/CS/NUT/UPPLEV/39 Final. 6 May 2002. European Commission. Health and Consumer Protection Directorate General.

CHAPTER 23

Vitamin B_6

Vitamin B_6, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	1.2	1.6	0.7	1.0	1.1/1.3
Average requirement	AR	1.0	1.3			
Lower level of intake	LI	0.8	1.0			

Vitamin B_6 is a common term for pyridoxine, pyridoxal and pyridoxamine. Pyridoxal phosphate (PLP) and pyridoxamine phosphate function as coenzymes for a number of enzymes that participate in amino acid metabolism (1). PLP is a co-enzyme for glycogen phosphorylase.

Physiology and metabolism

The absorption of the different vitamers takes place via a passive process in the gut. The bioavailability of vitamin B_6 in foods varies and depends on the chemical form of the vitamin (2). Studies indicate that pyridoxal and pyridoxamine raise PLP-concentration somewhat less (10%) than pyridoxine. In most fruits, vegetables and grains, a part of the pyridoxine occurs as a glucoside, which is considered to be less bioavailable than other non-glucoside forms (2, 4). The content of pyridoxine-glucoside in a mixed American diet has been estimated to be about 15% of the total vitamin B_6 content (5). The bioavailability (as assessed by plasma PLP levels and urinary pyridoxine excretion) of vitamin B_6 in a mixed American diet was estimated to be 71–79% of pyridoxine (as hydrochloride), but the design of the study was not optimal (3).

The body stores of vitamin B_6 have been estimated to be approximately 1,000 µmol (170 mg), of which 80–90% is found in muscles. The turnover of the vitamin is relatively fast, with a half-life of 25–33 days for PLP in plasma (1).

Dietary deficiency of vitamin B_6 is rare and is usually seen in combination with a lack of other B-vitamins. Clinical symptoms seen in infants and children include epileptiform convulsions, weight loss, gastrointestinal problems and anaemia (6). Experimentally induced deficiency

among adults has induced various mental disturbances, abnormal EEG and various types of skin changes in the face. Among adults, clinical symptoms have generally only been seen with diets containing 0.1–0.2 mg/d or less (7).

Insufficient intake of vitamin B_6 also induces certain biochemical disturbances, *e.g.* increased excretion of xanthurenic acid in urine and decreased transaminase activity in the erythrocytes.

The vitamin B_6 status can be assessed using a variety of biochemical indicators, of which the plasma PLP level is considered one of the most reliable (7, 8). PLP is the predominant vitamer in plasma, representing 70–90% of the total vitamin B_6 in plasma, and the level reflects the tissue stores and intake of vitamin B_6. PLP levels may, however, be affected by factors independent of the dietary supply, such as age, pregnancy, physical exercise.

The PLP levels among adult subjects with clinical symptoms of vitamin B_6 deficiency have been reported to be less than 10 nmol/L. PLP levels reflecting adequate tissue stores and enzyme functionality have been suggested to be 20 or 30 nmol/L (7,8). Available studies show a direct relationship between vitamin B_6 intake and PLP, but the data are less consistent with respect to the relationship between measured vitamin B_6 intake, PLP and other biochemical indicators of adequacy, *e.g.* urinary pyridoxine excretion or xanthurenic acid excretion after a tryptophan load.

The vitamin B_6 status is to a certain extent influenced by the protein intake. In two controlled studies in adult men and women in which a constant vitamin B_6 intake was administered, protein intakes of 1.5 g/kg BW resulted in approximately 40% lower PLP levels than a protein intake of 0.5 g/kg (9, 10). However, in another study (11) the PLP levels did not vary systematically with the protein intake.

Some studies (11, 12) indicate that the PLP level decreases with age, suggesting an increased vitamin B_6 requirement among elderly subjects, while some recent longitudinal studies (2–5 years) found a weak increase in the PLP status of elderly Europeans (13, 14) despite an observed apparent decreased intake of vitamin B_6 (15). In a study by Pannemans *et al.* (11), the PLP levels were 30–40% lower among a group of elderly subjects (27–32 nmol/L) than among young subjects (45–47 nmol/L) after consuming a controlled diet with a similar vitamin B_6 content (1.5–1.8 mg/d). At these intakes of vitamin B_6 both young and elderly participants had PLP levels above 20 nmol/L. However, other studies have failed to detect any major difference in vitamin B_6 metabolism due to age (16, 17), although these were of short duration. Thus, concerning the elderly, the relationship between protein and vitamin B_6 intake has

so far been difficult to estimate and, likewise, the data to establish a vitamin B_6 requirement are conflicting.

The intake of riboflavin may also influence the vitamin B_6 status, as flavin enzymes are involved in the formation of *e.g.* PLP. Severe riboflavin deficiency may therefore affect PLP-levels.

Via the trans-sulphuration pathway, vitamin B_6 intake influences the plasma homocysteine level, which has been proposed to be a risk factor for coronary heart disease (18). However, homocysteine levels are more influenced by folate status (18). Recent case-control (19) and prospective studies (20) among middle-aged and elderly subjects have found an inverse relationship between the risk of coronary heart disease and PLP-levels, independent of the homocysteine levels, indicating that vitamin B_6 offers independent protection. In a randomised, double-blind, placebo-controlled intervention study (21) 22 healthy subjects over 60 years of age (mean age 70 years), of whom 10 had PLP-levels below 20 nmol/L, had their folate and riboflavin status repleted for 12 weeks. This resulted in a 20% decrease in the homocysteine levels. Thereafter, an intervention group of 11 subjects was supplemented with 1.6 mg vitamin B_6 per day for 12 weeks, resulting in a significant reduction in plasma homocysteine of 7.5% compared to the placebo group. The study suggests that impaired vitamin B_6 status may influence plasma homocysteine status independent of folate and riboflavin status.

Requirement and recommended intake

In infants, symptoms such as convulsions have been seen at intakes of formula containing 0.06 mg/L (6). Among adults clinical symptoms have not been observed at intakes above 0.5 mg/day. Available controlled studies suggest that the PLP levels are related to the protein intake, both in men and women. The effect is estimated to be rather limited within the usual range of protein intakes (1.0–1.5 g/kg BW) seen in the Nordic countries.

Results from available depletion-repletion studies with controlled intakes of vitamin B_6 (expressed as free pyridoxine) indicate that PLP-levels above 20 nmol/L can be reached at intakes of 0.6–1.0 mg/d or around 0.01 mg/g dietary protein (22–27). The estimated average requirement (AR) of vitamin B_6 for adult men and women is set at 0.013 mg/g dietary protein, *i.e.* the same level as in NNR 1996. The recommended daily intake (RI) is also kept at the same level, *i.e.* 0.015 mg/g protein. However, when planning diets, the daily intake of vitamin B_6 should not be lower than 1 mg/day. The values for RI for each sex and

age group are calculated based on the reference value for energy intake and assuming a protein content of the diet of 15 E%.

According to the conflicting data concerning the vitamin B_6 requirements of elderly subjects and the possibility of a protective role in the prevention of coronary heart disease, the recommended daily intake for subjects over 60 years of age is set to the same level as for younger adults, *i.e.* 1.6 mg per day for men and 1.2 mg per day for women, without an age-related decrease as suggested in the NNR 1996.

For pregnant women the basic requirement is increased, especially during the last trimester, to cover the extra need of the foetus. For lactating women an increased intake is necessary to cover the needs for vitamin B_6 in breast milk. Assuming an increased energy requirement during the last two trimesters of pregnancy and during lactation, an additional intake of 0.2 mg/d and 0.3 mg/d, respectively, is warranted.

For infants and older children the reference intakes are based on the same value as for adults, *i.e.* 0.015 mg/g protein, due to lack of scientific data.

The lower level of intake (LI) for adults is set to 0.01 mg/g protein. However, the scientific basis for LI is weak.

In earlier studies, consumption of high-dose oral contraceptives was found to influence biochemical vitamin B_6 status of some women, *e.g.* increased excretion of xanthurenic acid after a tryptophan load (7). The clinical relevance of this effect is uncertain and the observation has not been included when setting the reference values. There is no need for general supplementation.

Some epidemiological studies have suggested an association between low vitamin B_6 status and/or intake and increased risk of cardiovascular disease (28, 29), while another shows no such relationship (30). The present data are considered insufficient as a basis for any detailed recommendation.

There is limited information on the vitamin B_6 status in Nordic populations (31). Studies on the elderly show on average good status but up to 30% of 80-year old Danes had plasma PLP levels below 20 nmol/L, indicating insufficient intake, despite an acceptable calculated dietary intake.

Upper intake levels and toxicity

Adverse effects of high vitamin B_6 intakes have been observed at intakes above 50 mg/d consumed for prolonged periods (months to years). Symptoms include minor neurological symptoms and, at higher levels, 500 mg/d or more, neurotoxicity (32). The EU Scientific Committee on

Food concluded that adverse effects are unlikely to occur at doses below 100 mg/d and proposed an upper safe intake level (UL) for adults of 25 mg/d (32). This level is also adopted in NNR.

Dietary sources and intake

Important sources of vitamin B_6 are fish, offal, meat and potatoes, and in some countries fortified flour and bread. The dietary content in the Nordic diet is on average 1.5–2.3 mg/10 MJ (33–35, see *Chapter 43*).

REFERENCES

1. Leklem JE. Vitamin B_6. In: Shils ME, Olson JA, Shike M, editors. Modern nutrition in health and disease, 9th ed. Philadelphia: Lippincott Williams & Wilkins, 1999.
2. Gregory JF. Bioavailability of vitamin B_6. Eur J Clin Nutr 1997; 51 (Suppl 1): S43–S48.
3. Tarr JB, Tamura T, Stokstad EL. Availability of vitamin B_6 and pantothenate in an average American diet in man. Am J Clin Nutr 1981; 34: 1328–37.
4. Nakano H, McMahon LG, Gregory JF 3rd. Pyridoxine-5'-beta-glucoside exhibits incomplete bioavailability as a source of vitamin B-6 and partially inhibits the utilization of co-ingested pyridoxine in humans. J Nutr 1997; 127: 1508–13.
5. Andon MB, Reynolds RD, Moser-Veillon PB, Howard MP. Dietary intake of total and glycosylated vitamin B-6 and the vitamin B-6 nutritional status of unsupplemented lactating women and their infants. Am J Clin Nutr 1989; 50: 1050–8.
6. Coursin DB. Vitamin B_6 metabolism in infants and children. Vit Hormones 1964; 22: 755–86.
7. Food and Nutrition Board. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline. Institute of Medicine, National Academic Press, Washington: 1998.
8. Bitsch R. Vitamin B_6. Int J Vit Nutr Res 1993; 247: 278–82.
9. Miller LT, Leklem JE, Shultz TD. The effect of dietary protein on the metabolism of vitamin B-6 in humans. J Nutr 1985; 115: 1663–72.
10. Hansen CM, Leklem JE, Miller LT. Vitamin B-6 status of women with a constant intake of vitamin B-6 changes with three levels of dietary protein. J Nutr 1996; 126: 1891–901.
11. Pannemans DLE, van den Berg H, Westerterp KR. The influence of protein intake on vitamin B_6 metabolism differs in young and elderly humans. J Nutr 1994; 124: 1207–14.
12. Lee CM, Leklem JE. Differences in vitamin B_6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B_6. Am J Clin Nutr 1985; 42: 226–34.
13. Bailey AL, Maisley S, Southon S, Wright AJA, Finglas PM, Fulcher RA. Relationship between micronutrient intake and biochemical indicators of nutrient adequacy in a 'free-living' elderly UK population. Brit J Nutr 1997; 77: 225–242.
14. Haller J, Weggemans RM, Lammi-Keefe CJ, Ferry M. Changes in the vitamin status of elderly Europeans: plasma vitamins A, E, B-6, B-12, folic acid and carotenoids. Eur J Clin Nutr 1996; 50 (Suppl 2): S32–S46.
15. Amorim Cruz JA, Moreiras O, Brzozowska A. Longitudinal changes in the intake of vitamins and minerals

of elderly Europeans. Eur J Clin Nutr 1996; 50 (Suppl 2): S77–S85.
16. Ferroli CE, Trumbo PR. Bioavailability of vitamin B-6 in young and older men. Am J Clin Nutr 1994; 60: 68–71.
17. Kant AK, Moser-Veillon PB, Reynolds RD. Effect of age on changes in plasma, erythrocyte, and urinary B-6 vitamers after an oral vitamin B-6 load. Am J Clin Nutr 1988; 48 (5): 1284–90.
18. Powers HJ. Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. Proc Nutr Soc 1999; 58: 435–40.
19. Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, Willet WC. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B_6, B_{12}, and folate. Am J Epidemiol 1996; 143: 845–859.
20. Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 1998; 98: 204–210.
21. McKinley MC, McNully H, McPartlin J, Starin JJ, Pentieva K, Ward M, Weir DG, Scott JM. Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine i healthy elderly persons who are folate and riboflavin replete. Am J Clin Nutr 2001; 73: 759–764.
22. Brown RR, Rose DP, Leklem JE, Linkswiler HM. Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B_6. Am J Clin Nutr 1975; 28: 10–19.
23. Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD, Gershoff SN. Vitamin B_6 requirements of elderly men and women. J Nutr 1991; 121: 1062–74.
24. Coburn SP, Ziegler PJ, Costill DL, Mahuren JD, Fink WJ, Schaltenbrand WE, Pauly TA, Pearson DR, Conn PS, Guilarte TR. Response of vitamin B-6 content of muscle to changes in vitamin B-6 intake in men. Am J Clin Nutr 1991; 53: 1436–42.
25. Huang YC, Chen W, Evans MA, Mitchell ME, Shultz TD. Vitamin B-6 requirement and status assessment of young women fed a high-protein diet with various levels of vitamin B-6. Am J Clin Nutr 1998; 67: 208–20.
26. van der Beek EJ, van Dokkum W, Wedel M, Schrijver J, van den Berg H. Thiamin, riboflavin and vitamin B_6: impact of restricted intake on physical performance in man. J Am Coll Nutr 1994; 13: 629–40.
27. Kretsch MJ, Sauberlich HE, Skala JH, Johnson HL. Vitamin B-6 requirement and status assessment: young women fed a depletion diet followed by a plant- or animal-protein diet with graded amounts of vitamin B-6. Am J Clin Nutr 1995; 61: 1091–101.
28. Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P, Rubba P, Palma-Reis R, Meleady R, Daly L, Witteman J, Graham I. Low circulating folate and vitamin B_6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. Circulation. 1998; 97: 437–43. Erratum in: Circulation 1999; 99: 983.
29. Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 1998; 98: 204–10.
30. de Bree A, Verschuren WM, Blom HJ, Nadeau M, Trijbels FJ, Kromhout D. Coronary heart disease mortality, plasma homocysteine, and B-vitamins: a prospective study. Atherosclerosis. 2003; 166: 369–77.
31. Pedersen AN. Nutritional status of 80-year old people – relations to func-

tional capacity (80-åriges ernæringsstatus – og relationen till fysisk funktionsevne. 80-årsundersøgelsen 1994/95). PhD-avhandling. Københavns Universitet 2001. Fødevaredirektoratet og Københavns Amts Center for Sygdomsforebyggelse.

32. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of vitamin B_6 (expressed on 19 October 2000). SCF/CS/NUT/UPPLEV/16 Final. 28 November 2000. European Commission. Health and Consumer Protection Directorate General.

CHAPTER 24

Folate

Folate, µg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	300 (400*)	300	80	130	200
Average requirement	AR	200	200			
Lower level of intake	LI	100	100			

*Women of fertile age are recommended 400 µg/day.

Folate is a generic term for a group of compounds that includes folic acid and derivatives having nutritional properties similar to folic acid. Folacin may be used synonymously with folate. Folic acid (pteroyl monoglutamic acid, PGA) consists of three parts, a pteridine ring, p-aminobenzoic acid and glutamic acid. Folic acid is the synthetic form of the vitamin and is not found naturally in foods. Folates in foods are pteroylpolyglutamates, which contain one to six additional glutamate units.

Physiology and metabolism

The metabolically active forms of the vitamin are reduced folic acid – tetrahydrofolic acid (THF) – coupled to additional glutamate molecules. They serve as coenzymes in transport of one-carbon units in amino acid metabolism and nucleic acid synthesis. The carbon can be carried as *e.g.* a methyl (CH_3-THF) or formyl group (CHO-THF). Folate coenzymes are required for normal cell division, and deficiency appears first in fast growing tissues such as the formation of blood cells in bone marrow. A central folate dependent reaction in amino acid metabolism is the remethylation of homocysteine to methionine. The methyl donor is 5-CH_3-THF, the synthesis of which is catalysed by the enzyme methylenetetrahydrofolate reductase (MTHFR). The reaction requires vitamin B_{12}. Homocysteine is also removed by a reaction that requires vitamin B_6. Elevated levels of homocysteine in serum may indicate low folate status and normal serum homocysteine is thus a measure of adequate folate supply. Other markers of folate status are erythrocyte (or RBC) folate and serum folate. Erythrocyte folate reflects tissue stores and is an indicator of long-term dietary intake. Unlike serum folate, erythrocyte folate is not affected by recent or transient changes in intake, which

may reflect actual intake. However serum folate is strongly correlated to intake in population studies (1).

Bioavailability

Food folates must be hydrolysed by pancreatic and brush border folate conjugase to monoglutamates prior to absorption in the upper part of the small intestine (2). The degree of absorption varies from one food to another and depends on the chemical form of the vitamin and the presence of absorption inhibitors or enhancers in the meal. Another important factor is the food matrix in which the folate is entrapped (3, 4). It is not possible to predict the overall bioavailability of folates from the composition of a diet (2), and there are few studies on absorption of food folate from composite meals. Bioavailability of folates from an average American diet is approximately 50% of PGA taken on an empty stomach (5). Brouwer *et al.* (6) found bioavailability of folate from diets rich in citrus fruits and vegetables to be 60–98% relative to folic acid depending on whether the endpoint used was folate or homocysteine status. This is in line with a high bioavailability of folate from spinach (7) and orange juice (8, 9).

Availability of folic acid – supplement or fortificant – eaten as part of a meal may be reduced relative to folic acid taken on an empty stomach. Pfeiffer *et al.* (10) found a 15% lower bioavailability when folic acid was consumed with a light breakfast meal. Fortification of cereal products has given equivocal results. Studies on maize, rice and bread in South Africa showed absorption rates ranging from 40 to 60% (11), while Pfeiffer *et al.* (10) found no difference between availability of folic acid added to cereal grain products and taken in aqueous solution. RBC folate was lowered in a group of women when they changed from fortified breakfast cereals to non-fortified (12). The same group (13) studied means of optimising folate status and showed a lower efficacy of increased intakes of foods rich in folate compared to folic acid supplements or foods fortified with folic acid.

In conclusion, figures describing absorption of native dietary folates have to be interpreted with care, but are generally in the magnitude of 40–70% (14). A pragmatic estimate of bioavailability of folates from the diet is set to 50%.

Requirement and recommended intake

The minimum requirement to prevent folate deficiency anaemia for adults is estimated to be a dietary intake of 50–100 µg/d (15) or 50 µg/d of

absorbed folate judged from a daily parenteral dose of this amount (16). On a virtually folate-free diet, the daily losses from stores in liver and extrahepatic tissues are on average ~60 µg/d (17). Well-nourished individuals excrete 5–40 µg/d in the urine, and the losses from the enterohepatic circulation are of the same order (17). Based on these criteria, the lower level of intake of dietary folate for adults is set to 100 µg/day.

Assessment of AR and RI is, however, based on a combination of indicators reflecting folate status – serum or plasma folate, RBC folate and serum or plasma homocysteine. Serum and RBC folate concentrations below 6.8 and 317 nmol/L respectively are considered low (18). As deficiency of folate is one of several causes of hyperhomocysteimia, total plasma homocysteine is regarded as a functional index of folate status. There is no consensus considering the definition of normal or elevated plasma homocysteine levels. The range of proposed cut-off values is 9.3–16.3 µmol/L (19, 20). According to a Danish study, the upper limit of reference interval is 12 µmol/L and values between 12 and 30 µmol/L are considered moderate hyperhomocysteimia (21).

Studies that carefully control dietary intake and have adequate duration are important. Unfortunately there are only few studies, which do not use folic acid to control intake instead of controlling folates from foods. Observational studies linking dietary intake and levels of plasma and RBC folate are less precise because of possible underestimation of intake. Some degree of underestimation may result from a combination of underreporting of food intake in dietary surveys and use of food tables, which underestimate folate content of foods (20). However, this is counteracted if calculations do not correct for losses during cooking. Despite these reservations, observations of intake and folate status in an apparently healthy population can give useful additional information on folate requirements.

An average dietary intake of 200 µg folate per day for 6–8 months maintained normal levels of serum and RBC folate in adult men staying in a metabolic unit (22). A (calculated) intake of 200 µg/d was also sufficient to keep all individuals in a group of women aged 17–40 years (n = 45) within the normal reference range of serum and RBC folate concentration (12). In another study the same group found increasing levels of RBC folate as intake of folate from diet, or supplementation was increased. However even the baseline (and in the control group), where intake was 200 µg/d RBC folate, was above cut-off (24). A depletion-repletion study concluded that a daily intake of 200–250 µg from food folates appears to meet the folate requirement for non-pregnant adult females, and an intake of 300 µg/d provides an allowance for storage for this group (5). Rasmussen *et al.* (23) found median intakes of food folate in

two groups of Danish women (25–30 & 60–65 y) to be 283 and 268 µg/d respectively and 316 µg/d in both groups when folic acid from supplements was included. Of the subjects, 37% of the young and 46% of older women were users of supplements containing folic acid. The corresponding median concentrations of RBC folate were 676 and 959 nmol/L respectively. Mean plasma folate was 13.7 and 13.0 nmol/L in Finnish men and women (non-users of supplements). Dietary intake of folates was 240 µg/d in men and 205 µg/d in women. Intakes were corrected for losses in food preparation (24). A Dutch study among 444 adults found mean folate intakes to be 270, 308 and 325 µg/d in women, older and younger men respectively. Mean serum folate levels were 1.3–14.2 nmol/L. Intakes between 100 and 200 µg/d were observed in 10–20% among different age-gender groups and the prevalence of serum folate levels below 7 nmol/L was 6–13% (25). The above-cited studies indicate that intakes around 300 µg/d are sufficient to keep serum and RBC folate well above cut-off values.

Elevated concentrations of homocysteine in the blood are associated with increased risk of cardiovascular diseases (26, 27), but it is not known whether normalising elevated plasma homocysteine reduces the number of diseases. Furthermore, it is not yet clear whether there is a threshold below which the association for risk of cardiovascular disease is eliminated. Ongoing controlled randomised intervention trials that are testing the efficacy of folate supplementation in reducing risk of cardiovascular disease may produce the answers. An adequate supply of folate is one of several conditions for keeping homocysteine levels low.

Mild hyperhomocysteimia can be caused by a combination of low folate intake and disruption of homocysteine metabolism. A common mutation in MTHFR makes the enzyme less stable and thus lowers its activity. Several studies show that homocysteine is elevated in homozygote mutant genotypes (TT) compared to heterozygotes (CT genotypes) and normal genotypes (CC) if folate status is low. If plasma folate is in the upper part of the range, there are little or no differences between the three genotypes (24, 28–31). The frequency of TT-homozygotes in studies of Nordic populations is 5–8.4% (24, 32). These frequencies are below earlier reported figures – average ≈12% (range 5.4–16%) – of the white population (33). In general men have higher concentrations of homocysteine than women and plasma homocysteine tends to increase with age in both sexes (21, 24). The cause and significance of homocysteine increasing with age is not well understood, but the physiological decline in renal function may partly explain the age effect (34).

Ten healthy adult men housed in a metabolic ward for a total of 108 days participated in a depletion-repletion study. During the depletion

period the subjects were fed a diet providing 25 µg/d of dietary folate. Plasma homocysteine increased and did not normalize during the repletion period, when the participants were provided with 25 µg/d of folate and 74 µg/d of folic acid – assumed to be equivalent to 170 µg/d from diet. However, the individual response to changes in folate intake varied from very strong to absent. It is concluded that an intake of 200 µg/d may not provide a large enough margin of safety (35).

A 4-week dietary controlled intervention trial with 66 healthy men and women aged 18–45 years showed a significant change in plasma homocysteine from 11.0 to 9.5 µmol/L on a diet providing approximately 500 µg/d of folate compared to no change in the placebo group on 200 µg/day. Folate intake at baseline was not estimated, but as plasma homocysteine levels were similar in placebo and treatment group intake should have been around 200 µg/d (6).

In the Danish study cited above, median concentrations of plasma homocysteine were 7.5 and 9.3 µmol/L respectively in younger and older women on a folate intake of ~300 µg/d (23). Another Danish study of 234 healthy elderly subjects (80 years) found mean intakes of folate to be 340 and 320 µg/d in males and females respectively (36). The corresponding concentrations of plasma homocysteine were 15.9 and 13.6 µmol/L. About 40% of the participants used a combined vitamin-mineral supplement. In this group homocysteine was 13 µmol/L and among non-users 16 µmol/L. Based on total plasma homocysteine as a functional marker of folate status, 90% of Finnish adults had an optimal folate intake (24). The majority – 87% of males and 83% of females – did not take supplements containing folic acid. In this group the mean intakes corrected for losses in cooking were 240 µg/d and 205 µg/d in men and women respectively. A depletion-repletion study of elderly women found that plasma homocysteine increased from 9.2 to 11.3 µmol/L on a depletion diet providing 118 µg/d for 7 weeks and that level was maintained on 200 µg/d for another 7 weeks. A repletion diet providing 415 µg/d lowered plasma homocysteine to 9.9 µmol/L (8).

In the 1996 edition of NNR, the recommended intake of folate was increased from 200 to 300 µg/d in order to provide a greater margin of safety and allow possibilities to increase stores, especially in fertile women. At the same time it was recognised that a low level of homocysteine is desirable. It could be argued that further increasing RDI would lead to lower homocysteine, but as the health significance of such a step is uncertain no convincing evidence for changing recommendations was found.

Keeping the possible underestimation of folate intake in mind and since a few of the cited studies found individuals below cut-off, the aver-

age requirement with respect to maintaining normal blood levels is assessed to 150–200 µg/day. An intake of 300 µg/d seems to keep folate levels in blood above and homocysteine below accepted cut-off values. In NNR estimated average requirement for adults is set to 200 µg/d and recommended intake to 300 µg/day.

Women in the reproductive age represent a specific problem, since numerous studies have indicated that an adequate supply of folate before and up to 12 weeks after conception reduces the risk of neural tube defects (NTD). Authorities in all the Nordic countries recommend consumption of 400 µg folate per day to women planning a pregnancy in order reduce the number of NTD-affected pregnancies. Since far from all pregnancies are planned, it is recommended that young women capable of becoming pregnant should eat a diet in line with the recommendations for the pregnancy planning group.

Folate requirements increase during pregnancy, especially in the last trimester, as does the risk of deficiencies in women with low stores. When folate intake is inadequate, maternal serum and RBC folate concentrations decrease and megaloblastic anaemia may develop. Caudill and colleagues (37) compared pregnant (second trimester) and non-pregnant women on controlled intake of dietary folate plus folic acid and concluded that 450 µg/d (judged to be equivalent to ~600 µg/d from diet alone) was sufficient to maintain folate status in pregnant women. Both serum and RBC folate concentrations were high at the end of the 12-week study, indicating that a lower intake could be sufficient. In NNR 1996, an intake of 400 µg/d was recommended from the beginning of the pregnancy. As 400 µg/d are now recommended for all women capable of becoming pregnant, women will enter pregnancy with moderate folate stores, and taken together with results from the study of Caudill *et al.* (37) 400–500 µg/d are considered sufficient to meet the increased requirement from fast growing tissues in pregnancy. NNR is set to 500 µg/d for pregnant women.

The concentration of folate in human milk varies through the lactation period, the highest mean being 60 µg/L (38). Smith *et al.* (39) reported average concentration of folate in human milk to be 85 µg/L. Based on a milk production of 0.75 L and a bioavailability of 50%, the diet should contain approximately 100 µg of extra folate. Lactating women are thus recommended 500 µg/day. This amount will allow repletion of stores before a possible new pregnancy.

In NNR 1996 infants were recommended 5 µg per kg body weight. A diet that supplied 3.5–5.0 µg/kg maintained growth, haemopoiesis and clinical well-being in 20 infants aged 2–11 months during a period of 6–9 months (40). Slightly higher concentrations of folate in serum and

RBC were found in the upper end of the interval. Since no data on requirements of children were found, the recommendations for children in the age group 1–14 years are unchanged and based on 5 µg per kg body weight.

Upper intake levels and toxicity

There is no evidence for risk associated with high intakes of folates from natural sources. A high intake of folic acid may mask haematological symptoms caused by deficiency of vitamin B_{12} and a higher intake of supplements is not recommended without medical advice. SCF (43) has set the upper level of intake of folic acid to 1,000 µg/d for adults. UL for children and adolescents is adjusted on the basis of bodyweight: 200, 300, 400, 600 and 800 µg/d of PGA for the 1–3, 4–6, 7–10, 11–14 and 15–17 years age group respectively (41).

Dietary sources and intake

Most important food groups contributing to folate intake are cereal products (including bread) and vegetables, but dairy products and fruits are also significant sources. Folate is present in most foods. High concentrations are found in liver, green vegetables and legumes. Food tables in general may underestimate folate content mainly because common methods of analysis fail to open up the food matrix and liberate all folate (42).

Folates are labile and significant losses in the cooking process are common. On average, the estimated loss is 30%, 30% and 40% in cooking of meat, fish and vegetables respectively, but in each group the loss may be substantially higher (43).

A balanced diet following general dietary advice and recommended energy distribution will contain ~400 µg/10 MJ. The average in the diet in the Nordic countries is 240–340 µg/10 MJ (see *Chapter 43*).

REFERENCES

1. Jacques PF, Sulsky SI, Sadowski JA, Phillips JCC, Rush D, Willett WC. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. Am J Clin Nutr 1993; 57: 182–9.
2. Gregory JF. Case Study: Folate Bioavailability. J Nutr 2001; 131: 1376S–82S.
3. Castenmiller JJM, van de Poll CJ, West CE, Brouwer IA, Thomas CMG, van Dusseldorp M. Bioavailability of folate from processed spinach in humans. Effect of food matrix and interaction with carotenoids. Ann Nutr Met 2000; 44: 163–9.
4. Brouwer IA, van Dusseldorp M, West CE, Steegers-Theunissen RPM. Bioavailability and bioefficacy of folate and folic acid in man. Nutr Res Rev 2001; 14: 267–93.
5. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. Folate requirement and metabolism in nonpregnant women. Am J Clin Nutr 1987; 46: 1016–28.
6. Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CMG, Duran M, van het Hof K, Eskes TKAB, Hautvast JGAJ, Steegers-Theunissen RPM. Dietary Folate from Vegetables and Citrus Fruit Decreases Plasma Homocysteine Concentrations in Humans in a Dietary Controlled Trial. J Nutr 1999; 129: 1135–9.
7. Prinz-Langenohl R, Brönstrup A, Thorand B, Hages M, Pietrzik K. Availability of Food Folate in Humans. J Nutr 1999; 129: 913–6.
8. Kauwell GPA, Lippert BL, Wilsky CE, Herrlinger-Garcia K, Hutson AD, Theriaque DW, Rampersaud GC, Cerda JJ, Bailey LB. Folate status of elderly women following moderate folate depletion responds only to a higher folate intake. J Nutr 2000; 130: 1584–90.
9. Gregory JF. The Bioavailability of Folate. In: Folate in Health and Disease. Bailey LB (Ed). New York: Marcel Dekker, 1995.
10. Pfeiffer CM, Rogers LM, Bailey LB, Gregory JF. Absorption af folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. Am J Clin Nutr 1997; 66: 1388–97.
11. Colman N. Addition of folic acid to staple foods as a selective nutrition intervention strategy. Nutr Rev 1982; 40: 225–33.
12. Cuskelly GJ, McNulty H, Scott JM. Fortification with low amounts of folic acid makes a significant difference in folate status in young women: implications for the prevention of neural tube defects Am J Clin Nutr 1999; 70: 234–9.
13. Cuskelly GJ, McNulty H, Scott JM. Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. Lancet (British edition) 1996; 347: 657–9.
14. Witthöft CM, Forssén K, Johannesson L, Jägerstad M. Folates – food souces, analyses, retension and bioavailability. Scand J Nutr 1999; 43: 138–46.
15. Herbert V. Minimal daily adult folate requirement. Arch Intern Med 1962; 110: 649–52.
16. Zalusky R, Herbert V. Megaloblastic anemia in scurvy with response to 50 microgm. of folic acid daily. N Eng J Med 1961; 21: 1933–8.
17. Herbert V. Recommended dietary intakes (RDI) of folate in humans. Am J Clin Nutr 1987; 45: 661–70.
18. Sauberlich HE. Folate Status of US. Population Groups. In: Folate in Health and Disease. Bailey LB (Ed). New York: Marcel Dekker, 1995.
19. Kihlberg R. Homocystein – en riskfaktor för hjärt-kärlsjukdom. Betydelsen av intaget av folsyra, vitamin B_6 och B_{12}. Scand J Nutr 1995; 39: 66–72.
20. de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH,

Steegers-Theunissen RPM. Folate intake in Europe: recommended, actual and desired intake. Eu J Clin Nutr 1997; 51: 643–60.
21. Rasmussen K, Møller J, Lyngbak M, Holm Pedersen A-M, Dybkjær L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. Clin Chem 1996; 42: 630–6.
22. Milne DB, Johnson LK, Mahalko JR, Sandstead HH. Folate status of adult males living in a metabolic unit: possible relationship with iron nutriture. Am J Clin Nutr 1983; 37: 768–73.
23. Rasmussen LB, Ovesen L, Bülow I, Knudsen N, Laurberg P, Perrild H. Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women. Am J Clin Nutr 2000; 72: 1156–63.
24. Alfthan G, Laurinen MS, Valsta LM, Pastinen T, Aro A. Folate intake, plasma folate, homocysteine status in a random Finnish population. Eu J Clin Nutr 2003; 57: 81–88.
25. Brussaard JH, Löwik MRH, van den Berg H, Brants HAM, Goldbohm RA. Folate intake and status among adults in the Netherlands. Eu J Clin Nutr 1997; 51: S46–S50.
26. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002; 325: 1202–6.
27. Homocysteine Studies Collaboration. Homocysteine and Risk of Ischemic Heart Disease and Stroke. A Meta-analysis. JAMA 2002; 288: 2015–23.
28. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation Between Folate Status, a Common Mutation in Methylenetetrahydrofolate Reductase, and Plasma Homocysteine Concentrations. Circulation 1996; 93: 7–9.
29. Ma J, Stampfer MJ, Hennekens CH, Frostt P, Selhub J, Horsford J, Malinow R, Willett WC, Rozen R. Methylenetetrahydrofolate Reductase Polymorphism, Plasma Folate, Homocysteine, and Risk of Myocardial Infarcation in US Physicians. Circulation 1996; 94: 2410–6.
30. Christensen B, Frosst P, Lussier-Cacan S, Selhub J, Goyette P, Rosenblatt DS, Genest J, Rozen R. Correlation of a Common Mutation in the Methylenetetrahydrofolate Reductase Gene With Plasma Homocysteine in Patients With Premature Coronary Artery Disease. Arterioscler Thromb Vasc Biol 1997; 17: 569–73.
31. de Bree A, Verschuren WMM, Bjørke-Monsen A-L, van der Put NMJ, Heil SG, Treijbels FJM, Blom HJ. Effect of the methylenetetrahydrofolate reductase 677C→T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample. Am J Clin Nutr 2003; 777: 687–93.
32. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland P-M. Folate, vitamin B_{12}, homocysteine, and the MTHFR 677C→T polymorphism in anxiety and depression: The Hordaland homocysteine study. Arch Gen Psychiatry 2003; 60: 618–26.
33. Brattström L, Wilcken DEL, Öhrvik J, Brudin L. Common Methylenetetrahydrofolate Reductase Gene Mutation Leads to Hyperhomocysteinemia but Not to Vascular Disease. The result of a Meta-Analysis. Circulation 1998; 98: 2520–26.
34. Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Medicine 1998; 49: 31–62.
35. Jacob RA, Wu M-M, Henning SM, Swendseid ME. Homocysteine increases as folate decreases in plasma of healthy men during short-term dietary folate and methyl group restriction. J Nutr 1994; 124: 1072–80.
36. Pedersen AN. 80-åriges ernæringsstatus – og relationen til fysisk funktionsevne. 80-årsundersøgelsen 1994/95. Ph.D.-afhandling. Fødevaredirektoratet 2001.
37. Caudill MA, Cruz AC, Gregory III JF, Hutson AD, Bailey LB. Folate status response to controlled folate intake

in pregnant women. J Nutr 1997; 127: 2363–70.
38. Ek J. Plasma, red cell, and breast milk folacin concentrations in lactating women. Am J Clin Nutr 1983; 38: 929–35.
39. Smith AM, Picciano MF, Deering RH. Folate intake and blood concentrations of term infants. Am J Clin Nutr 1985; 41: 590–8.
40. Asfour R, Wahbeh N, Waslien CI, Guindi S, Darby WJ. Folacin requirement of children III. Normal infants. Am J Clin Nutr 1977; 30: 1098–105.
41. SCF. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Folate.European Commission, 2000. (http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).
42. Martin CA. Folate analysis in foods. How accurate is it? Nutr Bull 1995; 20: 8–15.
43. Bognár A. Tables on weight yield of food and retention factors of food constituents for the calculation of nutrient composition of cooked foods (dishes). Karlsruhe: Bundesforschungsanstalt für Ernährung, 2002.

CHAPTER 25

Vitamin B_{12}

Vitamin B_{12}, µ/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	2	2	0.8	1.3	2.0
Average requirement	AR	1.4	1.4			
Lower level of intake	LI	1	1			

Vitamin B_{12} is a common term for a group of cobalt-containing compounds (corrinoids), which are biologically active in humans. Cobalamin may be used synonymously with vitamin B_{12}. Inactive compounds analogous to vitamin B_{12} are found in the diet, especially in plant foods (1).

Physiology and metabolism

Absorption of vitamin B_{12} is a multistep process. Protein-bound vitamin B_{12} from foods must be cleaved from protein, a process that demands the action of hydrochloric acid and pepsin secreted by the gastric mucosa. The absorption requires a glycoprotein – intrinsic factor – secreted by the parietal cells of the stomach. Vitamin B_{12} liberated from the food matrix binds to the intrinsic factor and the resulting complex is absorbed via special receptors in the ileum (2, 3). The ileal receptors are saturated at intakes of 1.5 to 2.0 mg per meal (4). As the intake increases, the percentage of absorbed vitamin decreases. It can be assumed that approximately 50% of dietary vitamin B_{12} is absorbed by healthy adults with normal gastric function (5, 6).

The function of vitamin B_{12} is related to the metabolism of methyl groups. Methylcobalamin is a cofactor for methionine synthase – the enzyme catalysing the conversion of homocysteine to methionine. This reaction is closely related to folate function. Adenosylcobalamin is a cofactor for methylmalonyl-CoA mutase in the isomerization of methylmalonyl-CoA to succinyl-CoA. An adequate supply of vitamin B_{12} is essential for normal blood formation and neurological function.

Most people have ample stores of vitamin B_{12}, which means that deficiency symptoms develop only after several years of low dietary intake (1). This is supported by an effective reabsorption of the vitamin

excreted in the bile. The daily loss of vitamin B_{12} is 0.1% of total body pool regardless of pool size (4, 7). Deficiency caused by inadequate dietary intake is only observed in adults who have been eating vegan diets for many years or in children from families on such a dietary pattern (8–13).

Elderly people frequently have low cobalamin levels (3, 14), which cannot be attributed to poor intake of cobalamin (15).

A major cause of vitamin B_{12} deficiency is food-cobalamin malabsorption, which usually arises from atrophic gastritis and hypochlorhydria. This disorder is defined as the inability to absorb protein-bound cobalamin by a person who is fully capable of absorbing free cobalamin. Pernicious anaemia, which is a disease caused by a low or missing secretion of intrinsic factor, accounts for only a small fraction of people with low cobalamin concentrations (3).

The prevalence of cobalamin deficiency increases with age and may exceed 10% in the elderly Nordic population (14, 16). The prevalence of pernicious anaemia in Sweden is estimated at 2.6‰ (17).

Vitamin B_{12} deficiency results in macrocytic, megaloblastic anaemia, and/or neurological symptoms due to degeneration of spinal cord, brain and optic and peripheral nerves.

Requirement and recommended intake

The requirement for vitamin B_{12} can be estimated from studies of patients with pernicious anaemia. A minimum intramuscular dose of 0.5 µg to slightly over 1.0 µg per day is necessary for normalizing and maintaining haematological status (18). As these patients are unable to reabsorb vitamin B_{12} excreted in the bile, the dietary requirement of healthy individuals must be smaller. Amounts ranging from 0.3 to 0.65 µg were adequate in a study of four patients with vitamin B_{12} deficiency anaemia due to poor vegetarian diets (19).

From these studies an average requirement of 0.7 µg/d of absorbed vitamin B_{12} would be a conservative estimate. With correction for absorption losses (50%) the AR comes to 1.4 mg/day.

This is in line with Herbert (1), who calculates the average requirement to be 1.4 µg/d under the assumptions of adequate body stores being 1,000 µg, a mean half-life of 1,000 day and a mean absorption of 50%.

In NNR the estimated average requirement (AR) is set to 1.4 µg/d for adults. By assuming a coefficient of variation of 15% and adding two standard deviations to allow for individual variation, the recommended

dietary intake is 2 µg/d for adults. The lower level of intake to prevent anaemia is 1 µg/day. The values are similar to estimates in NNR 1996.

The recommended intake for the elderly is the same as for younger adults. Increasing the RI is not likely to overcome malabsorption of food-bound vitamin B_{12} and certainly not lack of intrinsic factor. The recommended intake for children is unchanged from NNR 1996 and is based on 0.05 µg/kg body weight.

Pregnant women usually have adequate stores to cover estimated extra requirements of 0.1–0.2 µg/d (5). As the recommendation for non-pregnant women is already on the large side, no extra vitamin B_{12} is needed during pregnancy.

Lactating women are recommended an extra amount of 0.6 µg/d to compensate for the content of vitamin B_{12} in breast milk.

Upper intake levels and toxicity

There are no clearly defined adverse effects produced by vitamin B_{12} and data are insufficient to establish an UL. There is no evidence that intakes up to 100 µg/d from foods and supplements represent a health risk (20).

Dietary sources and intake

Vitamin B_{12} is only found in foods of animal origin. Plant foods may contain trace amounts from bacterial contamination or as a result of fermentation but the adequacy of these sources is questionable (13). Seaweed provides some biologically active vitamin B_{12}, but consumption of seaweeds in large amounts is not recommended because it would lead to excessive intake of dietary iodine (13). Vegans are advised to rely on supplements. Liver, meat, fish, shellfish, milk and cheese are particularly good sources. Meat and milk products contribute more than two thirds of vitamin B_{12} in the average diet (21, 22). The diet in the Nordic countries has a mean content of 6–8 µg/10 MJ (see *Chapter 43*).

REFERENCES

1. Herbert V. Vitamin B-12: plant sources, requirements, and assay. Am J Clin Nutr 1988; 48: 852–8.
2. Ho C, Kauwell GPA, Bailey LB. Practitioners' guide to meeting the vitamin B-12 Recommended Dietary Allowance for people aged 51 years and older. J Am Diet Assoc 1999; 99: 725–7.
3. Carmel R. Cobalamin, the stomach, and aging. Am J Clin Nutr 1997; 66: 750–9.

4. Scott JM. Bioavailability of vitamin B_{12}. Eur J Clin Nutr 1997; 51, suppl 1: S49–S53.
5. Herbert V. Recommended dietary intakes (RDI) of vitamin B-12 in humans. Am J Clin Nutr 1987; 45: 671–8.
6. Russell RM, Baik H, Kehayias JJ. Older Men and Women Efficiently Absorb Vitamin B-12 from Milk and Fortified Bread. J Nutr 2001; 131: 291–3.
7. Heysell RM, Bozian RC, Darby WJ, Bell MC. Vitamin B_{12} Turnover in Man. Am J Clin Nutr 1966; 18: 176–84.
8. Ellingsen TJ, Sommer S. Kostbetinget makrocytær anæmi i sidste trimester – primært tolket som HELLP-syndrom. Ugeskr Læger 1994; 156: 1967–8.
9. Halvorsen S, Jørgensen J, Skausig OB. Avitaminoser. Medicinsk årbog, p 111–9, København, Munksgaard: 1983.
10. Chanarin I, Malkowska V, O'Hea A-M, Rinsler MG, Price AB. Megaloblastic anaemia in a vegetarian hindu community. Lancet 1985; 2: 1168–72.
11. Jacobs C, Dwyer JT. Vegetarian children: appropriate and inappropriate diets. Am J Clin Nutr 1988; 48: 811–8.
12. Miller DR, Specker BL, Ho ML, Norman EJ. Vitamin B-12 status in a macrobiotic community. Am J Clin Nutr 1991; 53: 524–9.
13. Rauma A-L, Törrönen R, Hänninen O, Mykkänen H. Vitamin B-12 Status of Long-Term Adherents of a Strict Uncooked Vegan Diet ('Living Food Diet') Is Compromised. J Nutr 1995; 125: 2511–5.
14. Nilsson-Ehle H, Jagenburg R, Landahl S, Lindstedt S, Svanborg A, Westin J. Serum cobalamins in the elderly: A longitudinal study of a representative population sample from age 70 to 81. Eur J Haematol 1991; 47: 10–6.
15. Howard JM, Azen C, Jacobsen DW, Green R, Carmel R. Dietary intake of cobalamin in elderly people who have abnormal serum cobalamin, methylmalonic acid and homocysteine levels. Eur J Clin Nutr 1998; 52: 582–7.
16. Pedersen AN. 80-åriges ernæringsstatus – og relationen til fysisk funktionsevne. 80-årsundersøgelsen 1994/95. Ph.d.-afhandling. Fødevaredirektoratet. 2001.
17. Engel A, Dahlström M, Frank A. Perniciös anemi i Sverige – en epidemiologisk studie. Läkartidningen 1986; 83: 3331–2.
18. Darby WJ, Bridgforth J, le Brocquy J, Clark SL, de Oliveira JD, Kevany J, *et al.* Vitamin B_{12} requirement of adult man. Am J Med 1958; 25: 726–32.
19. Baker SJ, Mathan VI. Evidence regarding the minimal daily requirement of dietary vitamin B_{12}. Am J Clin Nutr 1981; 34: 2423–33.
20. SCF. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin B_{12}, 2000. (http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).
21. Andersen NL, Fagt S, Groth MV, Hartkopp HB, Møller A, Ovesen L, Warming DL. Danskernes kostvaner 1995. Hovedresultater. Publikation nr. 235. Søborg: Levnedsmiddelstyrelsen, 1996.
22. Steingrímsdóttir L, Þorgeirsdóttir H, Ólafsdóttir AE. The Diet of Icelanders. Dietary Survey of the Icelandic Nutrition Council 2002. Main findings. Public Health Institute, Reykjavík, Iceland 2003.

CHAPTER 26

Biotin

Biotin is a water-soluble heterocyclic compound formerly known as vitamin H. Biotin is essential to all known organisms and is synthesized by plants and microorganisms, but animals including man lack the ability to synthesize the vitamin (1). It belongs to the group of B-vitamins. Biotin in foods exists in free or protein-bound form.

Physiology and metabolism

Protein-bound biotin has to be digested before absorption. It is thought that the enzyme biotinidase plays a critical role in the cleavage of the covalent bond to protein (2), but the factors controlling bioavailability are poorly understood (1). Bioavailability of biotin in different foods varies from very low to almost complete utilisation. In general, less than half the biotin in foods is available (3). Raw egg white contains the glycoprotein avidin, which binds biotin and prevents its absorption. The biotin binding capacity of egg white is lost on cooking. A potential source of biotin is microbial synthesis by gut flora. However, evidence indicates that most of this substantial amount of biotin is unavailable and does not contribute to overall biotin status. The amount that is absorbed may not be sufficient to meet the requirement (1).

Biotin functions as a cofactor in carboxylation reactions – transfer of one-carbon units in the form of activated carboxyl groups – in intermediary metabolism. These reactions are important in fatty acid synthesis, in conversion of pyruvate to oxaloacetate (an intermediate in the citric acid cycle), and in degradation of branched amino acids and odd-chain fatty acids.

When activity of 3-methylcrotonyl-CoA carboxylase is decreased, its substrate is shunted to an alternate metabolic pathway, producing 3-hydroxyisovaleric acid (3-HIA), which is then excreted in urine. Elevated urinary concentration of 3-HIA is regarded as an early and sensitive indicator of biotin deficiency (4).

Dietary deficiency of biotin is rare and only demonstrated conclusively in individuals on parenteral nutrition without biotin or on chronic

ingestion of raw egg white. Biotin deficiency has also been demonstrated in inherited biotinidase deficiency (5). Increased excretion of 3-HIA as seen frequently in normal pregnancy reflects reduced biotin status (6). However no untoward effects of marginal biotin status in pregnancy have been documented (7).

Requirement and recommendation

Data providing an estimate of biotin requirements are scarce, and no recommendation could be set. In the American recommendations adequate intake (AI) for adults is set to 30 µg/d (8). This reference intake is based upon intake of biotin in breast fed infants extrapolated by body weights to adults.

Upper intake levels and toxicity

Data on adverse effects from high biotin intake are not sufficient to set a tolerable upper intake level. Although no numerical UL can be established, existing evidence from observational studies indicates that current levels of intake of biotin from all sources do not represent a health risk for the general population (9).

Dietary sources and intake

Biotin is found in most foods at low concentrations. Offal meats such as liver and kidney, egg yolk, rolled oats and wheat bran are rich sources (10). Average intake in Danish adults is estimated at 40 µg per day and approximately 70% of this intake is provided by bread and other cereal products, dairy products and eggs (10).

REFERENCES

1. McMahon RJ. Biotin in metabolism and molecular biology. Annu Rev Nutr 2002; 22: 221–39.
2. Hymes J, Wolf B. Human biotinidase isn't just for recycling biotin. J Nutr 1999; 129: 485S–9S.
3. Combs GF. Biotin. In: The Vitamins. Fundamental Aspects in Nutrition and health. San Diego: Academic Press, Inc., 1992.
4. Mock NI, Malik MI, Stumbo PJ, Bishop WP, Mock DM. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased biotin status in experimental biotin deficiency. Am J Clin Nutr 1997; 65: 951–8.
5. Mock DM. Biotin. In: Present Knowledge in Nutrition. Ziegler EE, Filer Jr. LJ, editors. Washington DC: ILSI Press, 1996.
6. Mock DM, Quirk JG, Mock NI. Marginal biotin deficiency during normal

pregnancy. Am J Clin Nutr 2002; 75: 295–9.
7. Said HM. Biotin: the forgotten vitamin. Am J Clin Nutr 2002; 75: 179–80.
8. Food and Nutrition Board, Institute of Medicine. Biotin. In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Washington DC: National Academy Press, 1998.
9. SCF. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Biotin. European Commission, 2001 (http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).
10. Pedersen JC. Folacin og biotin i levnedsmidler. Publikation nr. 157. Søborg: Levnedsmiddelstyrelsen, 1988.

CHAPTER 27

Pantothenic acid

Pantothenic acid belongs to the group of B-vitamins. The vitamin is water-soluble and is built from 2,4-dihydroxy-3,3-dimethyl-butanoic acid and 3-amino-propanoic acid joined together by a peptide bond. Pantothenic acid is widely distributed in nature, as its name implies (from the Greek *pantos* meaning everywhere).

Physiology and metabolism

As part of coenzyme A and acyl-carrier protein, pantothenic acid plays a central role as a carrier of acyl groups in both catabolism and anabolism. The availability of pantothenic acid from foods to humans is 40–60% on the basis of a single study (1). Deficiency of pantothenic acid is rare because of the widespread nature of the vitamin. Deficiency has only been observed in individuals on a diet free of pantothenic acid or given an antagonist to pantothenic acid (2).

Deficiency-induced greying of hair in mice can be reversed by administration of pantothenic acid, but the once popular idea that pantothenic acid might restore hair colour in humans proved fruitless (3).

Requirement and recommended intake

Since there is insufficient information for estimating the requirement of pantothenic acid no recommendation is set. In the American recommendations adequate intake (AI) for adults is set to 5 mg/d (2). This reference intake is mainly based upon estimated usual intakes of pantothenic acid in the American population and there is no evidence to suggest that this level of intake is inadequate.

Upper intake levels and toxicity

The toxicity of pantothenic acid is very low, but due to lack of systematic oral dose-response intake studies no UL can be derived. Evidence available from clinical studies using high doses of pantothenic acid indi-

cates that intakes considerably in excess of current levels of intake from all sources do not represent a health risk for the general population (4).

Dietary sources and intake

Pantothenic acid is found in many foods. The content of pantothenic acid in the average Danish diet is estimated to be approximately 5 mg per 10 MJ. The majority (~75%) of this amount comes from milk and cheese, cereal products including bread, meats and vegetables (5). Rich sources are offal, dried legumes and whole grain products.

REFERENCES

1. Tarr JB, Tamura T, Stokstad ELR. Availability of vitamin B_6 and pantothenate in an average American diet in man. Am J Clin Nutr 1981; 34: 1328–37.
2. Food and Nutrition Board, Institute of Medicine. Biotin. In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Washington DC: National Academy Press, 1998.
3. Plesofsky-Vig N. Pantothenic Acid. In: Present Knowledge in Nutrition. Ziegler EE, Filer, Jr. LJ, Ed. Washington, DC: ILSI Press, 1996.
4. SCF. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Pantothenic Acid. 2002. (http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).
5. Danish Institute for Food and Veterinary Research. Unpublished results from the survey: Dietary Habits in Denmark 1985.

CHAPTER 28

Vitamin C

Vitamin C, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	75	75	30	40	50
Average requirement	AR	50	60			
Lower level of intake	LI	10	10			

The term vitamin C refers to both ascorbic acid and dehydroascorbic acid, since both forms have an anti-scorbutic effect. Ascorbic acid is, however, the primary and functional *in vivo* form of the vitamin. Although the classical vitamin C deficiency, scurvy, is prevented by small daily intakes (about 10 mg/d) (1), current knowledge of the antioxidant functions of vitamin C has recently had a great influence on the paradigm of daily vitamin C allowances.

Physiology and metabolism

Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters (2). In all these functions, the effects of ascorbic acid are based on its ability to be an electron donor. Consequently, ascorbic acid is oxidised to dehydroascorbic acid. The vitamin is also involved in the biosynthesis of corticosteroids and aldosterone and in the microsomal hydroxylation of cholesterol in the conversion of cholesterol to bile acids. Due to its reducing power, ascorbic acid also improves absorption of non-haem iron.

Ascorbic acid is an extremely potent antioxidant. The vitamin readily scavenges reactive oxygen species and reactive nitrogen species, in addition to singlet oxygen and hypochlorite. It is evident that ascorbic acid provides meaningful antioxidant protection in the eye, in neutrophils, in semen and in plasma (*e.g.* against LDL oxidation) (2, 3). Ascorbic acid may also regenerate other antioxidants, such as vitamin E and glutathione. As a reducing agent, ascorbic acid may also inactivate carcinogenic substances, such as nitrosamines.

Ascorbic acid is absorbed from the intestine by a sodium-dependent, active process that is saturable and dose-dependent. The bioavailability

(efficiency of gastrointestinal tract absorption) is at least 80% for doses of 100 mg or less, 60–70% for 200–500 mg doses and less than 50% for doses exceeding 1,000 mg (3). Unabsorbed ascorbate is degraded in the intestine; this process may lead to diarrhoea and intestinal discomfort, sometimes reported by persons ingesting very large doses from supplements (4).

Vitamin C undergoes glomerular filtration and renal reabsorption. When the transport protein reaches saturation, remaining vitamin C is excreted in the urine. Up to 60 mg doses, no ascorbic acid is excreted (5) but at 100 mg dose, about 25% is excreted. About 50% of a 200 mg dose is excreted and about 80–90% of a dose exceeding 500 mg. The precise threshold dose for excretion is not known, but it is estimated to be around 75–80 mg/d (6).

The body pool of ascorbic acid is increased up to a daily intake of approximately 100 mg (7). This point is reflected by saturation of neutrophils, monocytes and lymphocytes (5, 8). At tissue saturation level, plasma ascorbic acid concentration is approximately 50–60 µmol/l, but very large doses (2,500 mg/d) are capable of increasing plasma levels up to 80 mmol/l (5, 8). At saturation, the total body pool in humans is about 20 mg/kg or approximately 1,500 mg (7). Plasma ascorbic acid concentration below 23 µmol/l reflects marginal vitamin C status (9). This level is reached by an estimated daily intake of 41 mg, depending obviously on body size (9). Marginal status may be reflected by *e.g.* decreased antioxidant capacity, fatigue and irritability (5). Symptoms of scurvy are observed when plasma levels are below 11 µmol/l (9) or the total body pool is below 300 mg (10).

Requirement and recommended intake

The previous Nordic recommendations (11), as well as the US RDIs (10), were based on maintenance of an adequate body-pool (1,500 mg) that would give an ample safety margin against scurvy (12). It was estimated that a daily intake of approximately 30–40 mg would provide a body pool of 900 mg and prevent scurvy for 30–40 days after cessation of this daily intake (10). This intake would also lead to plasma ascorbic acid concentration above 23 mmol/L (9). By assuming a large inter-individual variation (50%) *e.g.* to ensure adequate iron absorption, the NNR was set at 60 mg/d for both males and females.

Due to the increased recognition of the antioxidant function of vitamin C, it has been proposed that the daily recommendations should be based on its antioxidant activity rather than on antiscorbutic activity or body pool (2). Moreover, it seems clear that the maximal antioxidant

activity is reached after higher intakes than the levels needed to prevent scurvy (5).

There are two, partly complementary, starting points for calculation of vitamin C recommendations from its antioxidative activity. One approach is based on pharmacokinetics. Since leukocytes reach their saturation at daily intakes of about 100 mg, this could be used as the basis for calculations (3). However, at these intakes urine excretion is already 25% of daily dose, whereas at an intake of 60 mg/d practically no urinary vitamin C can be detected (5). Since the approximate urinary threshold is 75–80 mg/d, and because leukocyte ascorbic acid concentration is still very close to saturation, this is another pharmacokinetic way of estimating vitamin C requirements. An intake of 75 mg/d would lead to plasma vitamin C concentration around 40 µmol/l (5), a level that has already been associated with inhibition of *in vitro* LDL oxidation (13).

Although the paradigm of saturation (assessed by maximal plasma or leukocyte ascorbic acid concentration or by the detection of urinary ascorbic acid) has awakened recent interest, more data are needed to show that saturation really is associated with optimal health. Eight large prospective studies were found with dose-response data on plasma ascorbic acid (AA) concentration and cardiovascular and/or all-cause mortality (14–21). All of these studies showed that the risk was highest in subjects with the lowest plasma concentration.

The vitamin C concentration associated with increased risk (usually first quintile) varied between 11.4 and 51.7 mmol/l (unweighted mean 25 mmol/l). The large variation was mostly a reflection of arbitrary cut-off points when the study population was divided into tertiles, quartiles or quintiles. Studies with cancer mortality as the outcome have also identified the lowest plasma AA category as being clearly associated with increased risk (21, 22). However, in some studies (14, 15, 18, 19, 21), decreased risk for cardiovascular mortality (significantly different from the category with highest risk) was only seen in categories with higher plasma ascorbic acid concentration (*e.g.* third quintile). By using the cut-off points for clearly lowered risk (in relation to the first quintile), the mean cut-off point was AA concentration 32 µmol/l (unweighed mean of the 8 studies). This plasma level was chosen as the basis for the average requirement in the new Nordic recommendation.

Using the pharmacokinetic data of Levine *et al.* (5, 8), a 32 µmol/l concentration in plasma corresponds to a daily vitamin C intake of approximately 60 mg/d in men and 50 mg/d in women. This is very close to the kidney threshold (5) and corresponds to a body pool of approximately 1,000–1,200 mg (12). By giving a conservative 25% allowance for the inter-individual variation, the daily recommendation is set as 75 mg.

The pharmacokinetics of vitamin C in women seem to be very similar to those in men (8). However, at daily intakes below 100 mg, women have slightly higher concentrations of vitamin C in plasma with a given level of intake. These data suggest that the average requirements are slightly lower in women, which may be due to their smaller body size (10). However, to ensure adequate iron absorption, the coefficient of variation for women was assumed to be double that for men, and hence the same recommendation is applied for both sexes.

The recommendation is increased by 10 mg/d during pregnancy, in order to cover the increased needs due to growth of foetus and catabolised vitamin C (10). Breast milk contains approximately 30 mg vitamin C per litre (10). If the average milk production is 750 ml/d, up to 25 mg/d of additional vitamin C would be needed during lactation. This then increases the daily vitamin C recommendations in pregnancy to 85 mg/d and during lactation to 100 mg/day.

The average requirements for children (< 14 years) were extrapolated from the adult values by assuming growth factors 1.3 (< 2 years) and 1.15 (2–13 years). The recommended intake was calculated as 1.25 times estimated average requirement.

Upper intake levels and toxicity

There is no evidence that high intakes (> 1,000 mg/d) of vitamin C are carcinogenic or teratogenic (23). However, high intakes may cause diarrhoea and other gastrointestinal disturbances, increased oxalate formation and kidney stone formation in susceptible individuals. In theory, too high an intake of vitamin C may have pro-oxidative effects.

Dietary sources and intake

The concentration of vitamin C is high in many vegetables, berries and fruits (*e.g.* citrus fruits). Moreover, intake from vitamin C-enriched products (*e.g.* juices) may be considerable. The average intake of vitamin C in the Nordic countries is adequate (100–140 mg/10 MJ) (see *Chapter 43*).

REFERENCES

1. Weber P, Bendich A, Schalch W. Vitamin C and human health – a review of recent data relevant to human requirements. Int J Vitam Nutr Res 1996; 66: 19–30.
2. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999; 69: 1086–107.

3. Levine M, Rumsey SC, Daruwala R, Park JB, Wang Y. Criteria and recommendations for vitamin C intake. JAMA 1999; 281: 1415–23.
4. Hoffer A. Ascorbic acid and toxicity. N Engl J Med 1971; 285: 635–6.
5. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 1996; 93: 3704–9.
6. Blanchard J, Tozer TN, Rowland M. Pharmacokinetic perspectives on megadoses of ascorbic acid. Am J Clin Nutr 1997; 66: 1165–71.
7. Kallner A, Hartmann D, Hornig D. Steady-state turnover and body pool of ascorbic acid in man. Am J Clin Nutr 1979; 32: 530–9.
8. Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci USA 2001; 98: 9842–6.
9. Jacob RA, Skala JH, Omaye ST, Turnlund JR. Effect of varying ascorbic acid intakes on copper absorption and ceruloplasmin levels of young men. J Nutr 1987; 117: 2109–15.
10. Olson JA, Hodges RE. Recommended dietary intakes (RDI) of vitamin C in humans. Am J Clin Nutr 1987; 45: 693–703.
11. Sandström B, Aro A, Becker W *et al.* Nordiska näringsrekommendationer 1996. Nord 1996:28. Köpenhamn: Nordiska ministerrådet 1996.
12. Kallner A. Requirements for vitamin C based on metabolic studies. Ann NY Acad Sci 1987; 498: 418–23.
13. Jialal L, Vega GL, Grundy SM. Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoprotein. Atherosclerosis 1990; 82: 185–91.
14. Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. Lancet 1991; 337: 1–5.
15. Gale CR, Martyn CN, Winter PD, Cooper C. Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. BMJ 1995; 310: 1563–6.
16. Singh RB, Ghosh S, Niaz MA, Sing R, Beegum R, Chibo H, Shoumin Z, Postiglione A. Dietary intake, plasma levels of antioxidant vitamins, and oxidative stress in relation to coronary artery disease in elderly subjects. Am J Cardiol 1995; 76: 1233–8.
17. Eichholzer M, Stähelin HB, Gey KF, Lündin E, Bernasconi F. Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel Study. Int J Cancer 1996; 66: 145–50.
18. Sahyoun NR, Jacques PF, Russel RM. Carotenoids, vitamins C and E, and mortality in an elderly population. Am J Epidemiol 1996; 144: 501–11.
19. Nyyssönen K, Parviainen MT, Salonen R, Tuomilehto J, Salonen JT. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. BMJ 1997; 314: 634–8.
20. Loria CM, Klag MJ, Caulfield LE, Whelton PK. Vitamin C status and mortality in US adults. Am J Clin Nutr 2000; 72: 139–45.
21. Khaw K-T, Bongham S, Welch A, *et al.* relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. Lancet 2001; 357: 657–63.
22. Comstock GW, Alberg AJ, Huang HY, Wu K, Burke AE, Hoffman SC, Norkus EP, Gross M, Cutler RG, Morris JS, Spate VL, Helzlsouer KJ. The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. Cancer Epidemiol Biomarkers Prev 1997; 6: 907–16.
23. Johnston CS. Biomarkers for establishing a tolerable upper intake level for vitamin C. Nutr Rev 1999; 57: 71–7.

CHAPTER 29

Calcium

Calcium, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	800	800	600	700	900
Average requirement	AR	–	–			
Lower level of intake	LI	400	400			

The content of calcium in the body amounts to approximately 1,000 g and 1,200 g in an adult woman and man, respectively. Over 99% is found in teeth and bones. The remainder is present as an easily exchangeable pool in blood, extracellular fluid, and in all cells in the body. This free calcium plays vital roles in signal transduction both within and between cells, neuromuscular transmission, glandular secretion and in a large number of enzymatic reactions. Maintenance of a constant concentration of ionised calcium is therefore of vital importance and calcium homeostasis is probably the most tightly regulated homeostatic mechanism in the body.

In bone calcium is almost exclusively in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$. Adult bone tissue undergoes continuous remodelling caused by resorption by osteoclasts and formation of new bone by osteoblasts. The rate of exchange of calcium between bone and the exchangeable pool has been estimated to be about 700 mg/day. Bone formation exceeds bone resorption in children. The rate of remodelling is higher in children than in adults and it is higher in trabecular bone than in cortical bone.

Physiology and metabolism

Absorption

In the intestine dietary calcium is mixed with calcium in the digestive juices. From this mixture, absorption takes place mostly in the upper part of the ileum by passive diffusion or by an active energy requiring process. The latter is dependent on the action of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$), the hormonal form of vitamin D. Calcium absorption is thus decreased in vitamin D deficiency. The difference between calcium

in the food and that lost in faeces is termed net absorption. True absorption is much higher because of reabsorption from and secretion to the intestinal juices. The percentage net absorption (or fractional absorption) increases with decreasing amount of calcium in the diet and also with increased physiological needs such as in infancy, during puberty and during pregnancy. This adaptation of calcium absorption according to varying intake and varying physiological needs is of primary importance when discussing calcium requirement. Because adaptation depends on the presence of $1,25(OH)_2D$, the requirement for calcium can only be discussed on the condition that vitamin D status is sufficient. Balance studies have shown that when calcium intake is reduced, a period of up to several weeks of negative balance is observed in most individuals before a new steady state is reached (1). The ability to adapt may be reduced by advancing age (2). However, in the large balance study by Malm it was shown that in men adaptation may be efficient at least up to the age of 70 (1). The calcium absorption *per se* seems to be unaffected by ageing (3).

The absorption of calcium may be inhibited by food containing factors such as phytic acid, oxalic acid, or phosphates. Since calcium is derived from a variety of sources and is generally ample and because of adaptation, these factors probably play a minor role on an ordinary diet. This situation may be different in populations with low calcium intake and consuming large amounts of fibre-rich food like unfermented bread.

The net absorption on a mixed diet is highest in infancy, about 60% (4), is also high during puberty (found to be about 34% on an intake of 925 mg/d (5)) and then declines to 25–20% in adulthood and even lower at advanced age. The varying degree of absorption, both because of adaptation and varying dietary composition, makes it impossible to presuppose a certain fractional absorption as a basis for determining requirement.

Loss of calcium

Calcium is lost from the body via faeces, urine and skin. Non-absorbed calcium is lost with faeces. In adults on intakes of about 1,000 mg the loss amounts to about 70 to 80% of the intake. An appreciable amount is recovered as calcium soaps. Loss via skin and sweat is generally small, about 20–50 mg/d (6, 7). Under warm conditions or high physical activity the loss may be appreciably greater.

Loss via urine may vary appreciably from person to person, generally between 100 and 400 mg/d in adults, but is relatively constant within individuals even if the intake varies. In the balance study by Malm (1) the urinary loss decreased from 231 to 201 mg/d (not significant) upon reduction of the intake from 940 mg/d to 450 mg/day. This finding indi-

cates that the urinary loss of calcium is only to a minor degree affected by the intake and can almost be considered an individual constant. Adaptation to low intakes thus does not to any degree involve the kidneys. Similar findings have been observed in children (8, 9).

An acid pH, high sodium and high protein intakes increase loss via urine. This has been discussed as part of the osteoporosis problem in developed countries in spite of a high calcium intake. Inactivity also increases bone resorption and loss of calcium. Conversely, weight-bearing exercise contributes to higher bone mineral density (10–12). Postmenopausal women have a higher bone resorption (12a) with urinary loss of calcium during the night than during the day.

Calcium homeostasis

The concentration of calcium in plasma is kept constant within narrow limits (2.1–2.6 mmol/L). About half of this is in ionised form and the other half is bound to albumin. Parathyroid hormone and $1,25(OH)_2D$ are the most important hormones in the regulation of calcium homeostasis. They contribute to maintenance of constant calcium concentration in plasma by regulating the influx and efflux of calcium in intestine, bone and kidney.

Requirement and recommended intake

It has been difficult to reach agreement on what should be considered the physiological requirement of calcium. This is because we have no clear deficiency criteria at low intakes. The reason is the slow turnover of bone.

Traditionally two methods have been used to estimate requirement and recommended intake. One involves observations on intake in populations related to bone health. The other is to perform balance studies to determine the lowest intake compatible with zero balance. The main problem with interpretation of balance data is the pronounced ability to adapt to different intake levels as described above. Most balance studies have been of too short duration to allow for adaptation to the intake levels used (13). The considerable methodological problems inherent in balance studies should also be taken into account. Finally, calcium balance is highly influenced by physical activity and so far there are no studies on the interaction between intake of calcium and physical activity. In more recent years bone mineral content or bone mineral density related to intake including supplemental calcium has been used in intervention studies, in particular in the recent American DRI 1997 (14) to evaluate recommended intake. The British recommendations from 1991 (15) refuted the use of bone density as a criterion because bone

mineral density is also dependent on many factors other than calcium intake, in particular on physical activity. The DRI also makes use of factorial estimates of requirements as indicators of adequacy. Such estimates are based on a number of assumptions and do not take into account the ability to adaptation. The appropriateness of such an approach may therefore be questioned. As discussed by Kanis (16), the reality is that for the time being we have no reliable method on which to determine the requirement of calcium.

Children and adolescents

During growth, bone formation exceeds bone resorption and calcium retention in the skeleton amounts to a mean of 160–170 mg/day. It has been calculated to be 160 mg/d during the first year of life and between 70 and 150 mg/d during the period 1–10 years. It increases to 250 and 300 mg/d during pubertal growth for girls and boys, respectively. In addition, there is the requirement for losses in faeces, urine, skin and sweat.

Adaptation to low calcium intake is very efficient in children. Balance studies in young children showed high retention (119–140 mg/d) with intakes down to 300 mg/d (17–19). Increased intake increases retention somewhat. Calcium supplementation increases bone density in children (18). It is questionable, however, if the effect endures after cessation of supplementation in that no such effect was observed in one study (21) while a small effect was observed up to 24 months after cessation in another (22).

Estimation of bone mineral content based on DXA has shown a calcium accretion of about 60 to 200 mg/d in girls aged 1–8 years. The accretion rate is probably of the same order of magnitude in boys. The calcium intake needed to satisfy this rate is not known but it is reasonable to assume that the recommendation of 600 mg/d in NNR 1996 for the age group 1–6 years is sufficient. Retention is considerably higher at the age of 10 than at 2, which was reflected in the recommendation of 700 mg/d for the age group 7–10 years. There is no reason to alter this recommendation.

Calcium retention is very high during puberty and the maximum coincides with the maximum growth velocity. Peak bone mass appears to be attained much earlier than previously thought (23). Thus, at age 17 years 94% of peak bone mass is attained in girls and 86% in boys (24). Supplementation of calcium has been associated with increased bone density in children up to puberty while supplementation of 900 mg/d had no effect in postpubertal children (18). This effect on bone density has not been shown to be lasting, however. Adaptation to the increased demand for calcium is very efficient during puberty (25, 26). The effi-

cient absorption makes it probable that the recommendation of 900 mg/d from NNR 1996 covers the needs. Any higher recommendation has to take into account the possible inhibitory effect of calcium on iron absorption (27, 28) even if the effect in the long term may be small (28a). The US DRI is 1,300 mg/d for both boys and girls for the age groups 9–18 years. These values are based primarily on balance studies performed on 35 girls aged 12–15 years (9). Using a non-linear regression model it was found that calcium retention increased with calcium intake up to above 2 g/day. Mean maximum retention was 473 mg/d. Intake of 1,300 mg/d was the smallest intake that allowed some of the adolescent girls to achieve 100% of maximal retention. These balance experiments were only of 2 weeks duration, allowed only one week for adaptation and disclosed a large variability. Since absorption appears to be more efficient up to age 24 than in adults, the recommendation of 900 mg/d in NNR 96 should cover the entire age group of 10 to 20 years.

Adults

The major proportion of world population has a calcium intake below 500 mg/day. It has not been proven that a calcium intake of this magnitude has any deleterious consequences on bone structure. In fact the highest incidence of osteoporotic fractures is in developed countries with the highest calcium intake. It has been reported that in the Gambia despite the low calcium intake (360 mg/d) osteoporotic fractures are rare (29). Calcium deficiency is thus only one factor in the multifactorial context of osteoporosis. This is not to say that calcium is unimportant. In fact, supplementation of calcium and vitamin D to the elderly on low habitual calcium intake has been shown in three studies (in France, USA and Denmark) to reduce the incidence of osteoporotic fractures (30–32, 32a). Two years after withdrawal there were no remaining supplement-related benefits to BMD and bone turnover in the US elderly subjects (33).

The results from two classical balance studies, that of Hegsted (34) and that of Malm (1) have been used as an argument that man can adapt to very low intake levels. Thus, in the study performed by Hegsted in Peru, men adapted to a low habitual intake were in balance at intakes of 300–400 mg/day. In the study by Malm, 39 men aged 20 to 76 years were monitored for several months on an intake of 940 mg/day. All except one were in positive balance. Subsequently the intake was reduced to 460 mg/d in 26 men monitored for several months. All except 3 adapted to this lower level of intake. It should be noted that 20 of the men went through a transient period of more or less marked negative balance before they again reached balance. This indicates that adaptation in most individuals is a slow process. The results of these studies were part

of the arguments for former low recommendations of calcium intake, *e.g.* the first WHO/FAO 1962 recommendation of 400–500 mg/d (35) or the NNR 1989 of 600 mg/d for men. The recent US DRI 1997 of 1,000 mg/d for the age group 31–50 years and 1,200 mg/d for the age group 51–70 years do not take the ability to adapt into account and do not mention the two balance studies above. The highest level was primarily based on clinical trial data demonstrating reduced bone loss upon calcium supplementation.

Some population studies indicate that a life-long high intake of calcium may affect bone density and reduce osteoporotic fractures in both men and women in the high age groups. Thus Matkovic *et al.* in their cross-sectional study (36), Cooper *et al.* (37) and Holbrook *et al.* (38) in their longitudinal studies found that men on an intake of about 800 mg/d had a lower incidence of hip fracture than those with about half that intake. In one study, bone density of the lumbar vertebrae and upper femur was found to be correlated to calcium intake in men (39). A supplementation trial has also provided some evidence that a high intake may reduce fracture incidence in men (40). These were arguments used when calcium recommendations for men in the NNR 1996 were increased to 800 mg/day. There seem to be no strong arguments to alter this recommendation.

Long-term balance studies in women similar to those in men have not been performed. It is not clear if women after the menopause may be at or near balance on the same low levels of calcium intake as in men. Some balance studies indicate that this is not the case (41). However, most of these balance studies have been of short duration and it is not clear if the experimental individuals were adapted to the actual intakes. Physicians working with the osteoporosis problem recommend a liberal intake of calcium for women, the reason being the results of the mentioned population studies (36–38). Another argument has been that supplementation with calcium to osteoporotic patients has resulted in some reduction in bone loss (42, 43). This is true in late postmenopausal women. The oestrogen deficiency-related bone loss in early menopause is not appreciably altered by calcium supplementation. In addition, supplementation experiments are difficult to interpret because in the short term they alter the rate of remodelling. If they are not of long enough duration (≈4 years) any lasting effect on bone density cannot be evaluated. Most of these supplementation studies have been of shorter duration. In an extensive discussion of the results of a large number of such studies, it was concluded that the available data did not support the hypothesis that an increase in calcium intake up to 1,500 mg/d, as has been proposed, will prevent osteoporosis (44). There

are thus no strong arguments to alter the recommendations from NNR 1996, *i.e.* 800 mg/d for women.

Pregnancy and lactation

Adaptation is very efficient during pregnancy. This may be connected to the increased serum levels of 1,25-dihydroxyvitamin D during pregnancy. Studies with Indian women on a low calcium intake (about 400 mg/d/) and American women on a high intake (700–900 mg/d) have shown that both groups increase calcium absorption during pregnancy (45, 46). In both cases the retention corresponded to the amount of calcium laid down in the foetus. Supplementation trials have shown that extra calcium in addition to that in the diet does not influence the retention (47). Considering that the dietary calcium in the Nordic countries is about 800–1,000 mg/d, there is no need to recommend extra intake during pregnancy. Since many young women become pregnant before termination of skeletal growth, it is reasonable to recommend the same amount of calcium as for young women, *i.e.* 900 mg/day.

After birth there is a rapid fall in circulating 1,25-dihydroxyvitamin D in the mother. The efficiency of calcium absorption during lactation does not appear to be increased above normal (48). Upon return of menstruation a compensatory increase in calcium absorption has been observed (48). Calcium supplementation does not alter fractional absorption (49, 50). The extra calcium needed for milk production appears to be provided by increased bone resorption (51) combined with renal conservation of calcium (52). These adaptive changes are not influenced by calcium intake. The bone loss resulting from calcium mobilisation is regained when ovarian function is resumed and menstruation reappears. The available data support the view that no extra calcium above that of pregnant women is needed (53). The recommendation during lactation is thus the same as for pregnant women, *i.e.* 900 mg/day.

Upper intake levels and toxicity

A daily intake of 2.5 g is well tolerated (54). Higher intakes are rare in ordinary diets. Supplementation to above these levels may be deleterious, in particular in combination with high intakes of vitamin D, with risk of hypercalcaemia, kidney stones and kidney damage.

Dietary sources and intake

Milk and milk products are good sources of calcium. Due to the high consumption of milk and milk products in the Nordic countries the

mean intake is above recommendations for practically all age groups, of the order of 900–1,200 for women and 900–1,400 for men. Mean intake in the Nordic countries is 1,000–1,400 mg/10 MJ (see *Chapter 43*). However, there may be groups of people in which the intake does not meet the recommendation. Other sources of calcium are fish and fish products, especially when eaten with the bones intact. Green vegetables have a variable content of calcium which is mostly not very well absorbed – with a few exceptions such as kale.

REFERENCES

1. Malm OJ. Calcium requirements and adaptation in adult men. Oslo: Oslo University Press, 1958. (Also published as Suppl. to vol. 10, 1958 of Scand J Clin & Lab Invest).
2. Fleet J. How well you absorb calcium is important for limiting hip fracture risk. Nutr Rev 2001; 59: 338–341.
3. Somerville PJ, Lien JWK, Kaye M. The calcium and vitamin D status in an elderly female population and their response to administered supplemental vitamin D_3. J Gerontol 1977; 32: 659–663.
4. Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc and iron from breast milk by 5- to 7-month-old infants. Pediatr Res 1997; 41: 384–90.
5. Abrams SA, Stuff JE Calcium metabolism in girls: Current dietary intakes lead to low rates of calcium absorption and retention during puberty. Am J Clin Nutr 1994; 60: 739–43.
6. Lentner C, Lauffenburger T, Guncaga J, Dambacher MA, Haas HG. The metabolic balance technique: A critical reappraisal. Metabolism 1974; 24: 461–71.
7. Charles P, Eriksen EF, Hasling C, Søndergård K, Mosekilde L. Dermal, intestinal, and renal obligatory losses of calcium: relation to skeletal calcium loss. Am J Clin Nutr 1991; 54: 266S–73S.
8. Matkovic V. Calcium metabolism and calcium requirement during skeletal modeling and consolidation of bone mass. Am J Clin Nutr 1991; 54: 245S–60S.
9. Jackman LA, Millane SS, Martin BR, Wood OB, McCabe GP, Peacock M, Weaver CM. Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females. Am J clin Nutr 1997; 66: 327–33.
10. Need AG, Wishart JM, Scopacasa F, Horowitz M, Morris HA, Nordin BEC. Effect of physical activity on femoral bone density in men. BMJ 1995; 310: 1501–2.
11. Anderson JJB. The important role of physical activity in skeletal development: how exercise may counter low calcium intake. Am J clin Nutr 2000; 71: 1384–6.
12. Mosekilde L. Mekanismer ved osteoporose. Ugeskr Læger 2001 ; 163: 1243–1246.

12a. Eastell R, Calvo MS, Burritt MF, Offord KP, Russell RG, Riggs BL. Abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis. J Clin Endocrinol Metab. 1992; 74: 487–494.

13. Isaksson B, Sjögren B. A critical evaluation of the calcium balance technic. I. Variation in fecal output. Metabolism 1967; 16: 295–302.
14. Food and Nutrition Board. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. National Academy Press, Washington, DC 1997.
15. Department of Health. Report on health and social subjects; 41. Dietary Reference Values for Food

Energy and Nutrients for the United Kingdom. London: HMSO, 1991.
16. Kanis JA. Calcium nutrition and its implication for osteoporosis. Part I. Children and healthy adults. Eur J Clin Nutr 1994; 48: 757–67.
17. Nicholls L, Nimalasuriya A. Adaptation to a low calcium intake in reference to the calcium requirements of a tropical population. J Nutr 1939; 18: 563–77.
18. Begum A, Pereira SM. Calcium balance studies on children accustomed to low calcium intakes. Br J Nutr 1969; 23: 905–11.
19. Lee WTK, Leung SSF, Fairweather-Tait SJ, Leung DMY, Tsang HSY, Eagles J, *et al.* True fractional calcium absorption in Chinese children measured with stable isotopes (42Ca and 44Ca). Br J Nutr 1994; 72: 883–97.
20. Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, *et al.* Calcium supplementation and increases in bone mineral density in children. N Engl J Med 1992; 327: 82–7.
21. Slemenda CW, Reister TK, Peacock M, Johnston CC. Bone growth in children following the cessation of calcium supplements. J Bone Min Res 1993; 8 (Suppl 1): S154.
22. Dibba B, Prentice A, Ceesay M, Mendy M, Darboe S, Stirling DM, Cole TJ, Poskitt EME. Bone mineral content and plasma osteocalcin concentrations of Gambian children 12 and 24 mo after the withdrawal of a calcium supplement. Am J Clin Nutr 2002; 76: 681–6.
23. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, *et al.* Longitudinal monitoring of bone mass accumulation in healthy adolescents: Evidence for a marked reduction after 16 years of age and the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992; 75: 1060–5.
24. Magarey AM, Boulton TJ, Chatterton BE, Schultz C, Nordin BE, Cockington RA. Bone growth from 11 to 17 years: relationship to growth, gender and changes with pubertal status including timing of menarche. Acta Paediatr 1999; 88: 139–46.
25. Weaver CM, Martin BR, Plawecki KL, Peacock M, Wood OB, Smith DL, *et al.* Differences in calcium metabolism between adolescent and adult females. Am J Clin Nutr 1995; 61: 577–81.
26. O'Brian KO, Abrams SA, Liang LK, Ellis KJ, Gagel RF. Increased efficiency of calcium intake in girls. Am J Clin Nutr 1996; 63: 579–83.
27. Hallberg L, Brune M, Erlandsson M, Sandberg A-S, Rossander-Hulthén L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am J Clin Nutr 1991; 53: 112–9.
28. Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. Am J Clin Nutr 1991; 53: 106–11.
28a. Lönnerdal B. Does a high dietary intake of calcium adversely affect iron status in humans? Scand J Nutr 1999; 83: 82–84.
29. Prentice A, Shaw J, Laskey MA, Cole TJ, Frase DR. Bone and mineral content of British and rural Gambian women aged 18–80+ years. Bone Miner 1991; 12: 201–14.
30. Chapuy MC, Arlot MF, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ. Vitamin D_3 and Calcium Prevent Hip Fractures in Elderly Women. New Engl J Med 1992; 327: 1637–42.
31. Chapuy MC, Arlot ME, Delmas PD, Meunier PJ. Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. Brit Med J 1994; 308: 1081–1082.
32. Dawson-Hughes B, Harris SS, Krall EA, Dallal G. Effect of Calcium and Vitamin D supplementation on Bone Density in Men and Women 65 years of Age or Older. New Engl J Med 1997; 337: 670–6.
32a. Larsen ER. Falls and fractures among elderly community residents: Risk factors and prevention. Ph.D. Thesis, University of Aarhus 2002.

33. Dawson-Hughes B, Harris SS, Krall EA, Dallal G. Effect of withdrawal of calcium and vitamin D supplements on bone mass in elderly men and women. Am J Clin Nutr 2000; 72: 745–750.
34. Hegsted JM, Moscoso I, Collazos CH. A study of the minimum calcium requirements of adult men. J Nutr 1952; 46: 181–201.
35. FAO. Calcium requirements. Report of an FAO/WHO Expert Group. FAP Nutrition Meetings Report Series No. 30. Rome: FAO, 1962.
36. Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BE. Bone status and fracture rates in two regions of Yugoslavia. Am J Clin Nutr 1979; 32: 540–9.
37. Cooper C, Barker DJP, Wickham C. Physical activity, muscle strength, and calcium inake in fracture of the proximal femur in Britain. Br Med J 1988; 297: 1443–6.
38. Holbrook TL, Barrett-Connor E, Wingard DL. Dietary calcium and risk of hip fracture: 14-year prospective population study. Lancet 1988; 1046–9.
39. Kelly PJ, Pocock NA, Sambrook PN, Eisman JA. Dietary calcium, sex hormones, and bone mineral density in men. Br med J 1990; 300: 1361–4.
40. Horowitz M, Wishart JM, Goh D, Morris HM, Need AG, Nordin BEC. Oral calcium suppresses biochemical markers of bone resorption in normal men. Am J Clin Nutr 1994; 60: 965–8.
41. Heaney RP, Recker RR, Sville PD. Calcium balance and calcium requirements in middle-aged women. Am J Clin Nutr 1977; 30: 1603–11.
42. Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Effects of calcium supplementation on bone loss in postmenopausal women. N Engl J Med 1993; 328: 460–4.
43. Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Long-term effect of calcium supplementation on bone loss and fractures in postmenopausal women: A randomized controlled trial. Am J Med 1995; 98: 331–5.
44. Nilas L. Calcium intake and osteoporosis. World Rev Nutr Diet 1993; 73: 1–26.
45. Shenolikar IS. Absorption of dietary calcium in pregnancy. Am J Clin Nutr 1970; 23: 63–7.
46. Heaney RP, Skillman TG. Calcium metabolism in normal human pregnancy. J Clin Endocrin Metab 1971; 33: 661–70.
47. Ashe JR, Schofield FA, Gram MR. The retention of calcium, iron, phosphorus, and magnesium during pregnancy: the adequacy of prenatal diets with and without supplementation. Am J Clin Nutr 1979; 32: 286–91.
48. Kalkwarf HJ, Specker BL, Henbi JE, Vieira NE, Yergey AL. Intestinal calcium absorption of women during lactation. Am J Clin Nutr 1996; 63: 526–31.
49. Fairweather-Tait S, Prentice A, Heumann KG, Jarjou LMA, Sterling DM, Wharf SG, Turnlund JR. Effect of calcium supplements and stage of lactation on the calcium absorption efficiency of lactating women accustomed to low calcium intakes. Am J clin Nutr 1995; 62: 1188–92.
50. Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M. The effect of calcium supplenetation on bone density during lactation and after weaning. N Engl J Med 1997; 337: 523–8.
51. Affinito P, Tommaselli GA, DiCarlo C, Guida F, Nappi C. Changes in bone mineral density and calcium metabolism in breastfeeding women: A one year follow-up study. J Clin endocrinol Metab 1996; 81: 2314–8.
52. Specker BL, Vieira NE, O'Brian KO, Ho ML, Heubi JE, Abrams SA, Yergey AL. Calcium kinetics in lactating women with low and high calcium intakes. Am J Clin Nutr 1994; 59: 593–9.
53. Prentice A. Calcium requirements of breastfeeding mothers. Nutr Rev 1998; 56: 124–7.
54. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Calcium. SCF/CS/NUT/UPPLEV/64 (April 2003). http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.

CHAPTER 30

Phosphorus

Phosphorus , mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	600	600	470	540	700
Average requirement	AR	450	450			
Lower level of intake	LI	300	300			

Phosphorus is abundant in the body with the largest amounts found as hydroxyapatite in bone. In soft tissue phosphorus occurs mostly as phosphate groups bound to organic compounds.

Physiology and metabolism

Absorption of phosphorus probably takes place by several mechanisms and is partly connected to calcium. Dietary organic phosphorus compounds are hydrolysed, thus most phosphorus absorption occurs as inorganic phosphate. Net absorption is reported to be in the range 55 to 70% in adults and 65 to 90% in infants and children. The absorption of sparingly soluble phosphorus compounds, *e.g.* phytates, is estimated to be low. There does not seem to be an adaptive mechanism that improves phosphorus absorption at low intakes. Plasma concentrations of phosphorus are rather constant (0.8–1.4 mmol/L) and are regulated through kidney excretion. Vitamin D status influences absorption (calcitriol increases absorption, and a decrease in calcitriol decreases phosphorous absorption).

Hydroxyapatite, which contains phosphorus and calcium in the ratio 1:2, constitutes the most important inorganic part of the skeleton. In addition, phosphorus has an important regulatory function intracellularly, being a constituent of all major classes of biochemical compounds. Phosphorylation – dephosphorylation reactions are central in many metabolic processes and the energy required for these comes from energy-rich phosphorus bindings, *e.g.* ATP and creatinine phosphate. Phosphorus compounds also contribute to intra- and intercellular acid-base regulation. Structurally, phosphorus occurs as phospholipids, which are major constituents of most biological membranes, and as nucleotides

and nucleic acids. A tiny, but important, fraction of body phosphorus (< 0.1%) is inorganic phosphate, mainly in blood and extracellular fluid, which serves as a pool for all phosphorus functions in the body.

The total body content of phosphorus is approximately 800–1,200 g, approximately 85% of which is in bone and the rest evenly distributed in all tissues.

Phosphorus deficiency has only been observed in premature infants in situations of poorly managed parenteral nutrition, in anorexia patients (1), and when intake of aluminium hydroxide (taken as an antacid), which binds phosphorus and antagonises absorption, has been high. In such cases muscle weakness, lack of appetite (anorexia), nausea and decalcification of the skeleton have been observed.

Requirement and recommended intake

The exact requirement for phosphorus is not known. To maintain a plasma level of 0.8 mmol/L, 400 mg daily is considered adequate for adults.

The EU Scientific Committee on Food suggested that phosphorous intakes should correspond on a molar basis with those of calcium and accordingly proposed the average requirement to be 400 mg/d and the population reference intake to be 550 mg/d (2).

The US Food and Nutrition Board has set an Estimated Average Requirement (EAR) for phosphorous at 580 mg/d for both men and women aged 19–70 years, using serum inorganic phosphorus level as the criterion (3). The RDA (Recommended Dietary Allowance) is 700 mg/d for both genders, applying a coefficient of variation of 10%. For adolescents (9–18 years) RDA is 1,250 mg phosphorous per day using both dietary data and estimated additional needs during growth as criteria.

In NNR 1996, the RI for phosphorous was set to 600 mg per day for both men and women. There are no substantial new data since then to indicate that these values should be changed. This intake level adheres to the view that an equimolar relationship between calcium and phosphorus is used as a basic principle for recommendations (1 mmol calcium = 40 mg, 1 mmol phosphorus = 30.9 mg). The RI values for children are also maintained and are based on the same considerations.

Upper intake level and toxicity

The efficient regulation of the body's phosphorus content through kidney excretion prevents accumulation in healthy adults. Calcification of soft tissue has been observed in disease states where there are chronically elevated plasma levels. Acute hyperphosphataemia may lead to

hypocalcaemia and cramps. The ratio between calcium and phosphorus is more critical for infants and should not exceed 0.9:1 to 1.7:1 (mmol/mmol). Maximum tolerable daily intake for adults is approximately 70 mg/kg body weight.

The US Food and Nutrition Board has set a Tolerable Upper Intake level of 4,000 mg/day. The EU Scientific Committee on Food has so far not derived an upper tolerable level of phosphorous intake.

The NNR committee is aware of the international debate connected to the question of whether the dietary ratio of calcium to phosphorus is clinically significant. The recent recommendations from the US (3) refute any relevance of such a ratio, while others question that conclusion (4). The specific question is whether a high-phosphorus, low-calcium diet can cause hypocalcaemia and or/secondary hyperparathyroidism in humans, and thus contribute to the increasing prevalence of osteoporosis in many Western countries. The use of phosphorus-containing food additives in the processing of foods may contribute significantly to the daily phosphorus intake in some individuals, but there are limited data on the physiological and clinical relevance (3, 5).

Dietary sources and intake

Phosphorus is widely found in almost all food groups, largely as phosphate(s), and often in association with the protein content. In cereals and legumes a considerable amount of phosphorus is found as inositol-hexaphosphate (phytic acid) and is probably less available. Phosphorus compounds are used as food additives, *e.g.* for their water-binding properties. Diets in the Nordic countries contain 1,500–2,000 mg/10 MJ (see *Chapter 43*).

REFERENCES

1. Ornstein RM, Golden NH, Jacobson MS, Shenker IR. Hypophosphatemia during nutritional rehabilitation in anorexia nervosa: implications for refeeding and monitoring. J Adolesc Health 2003; 32: 83–8.
2. Reports of the Scientific Committee on Food. Nutrient and energy intakes for the European Community. Luxembourg: Commission of the European Communities, 1993.
3. Food and Nutrition Board, Institute of Medicine. Dietary reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington DC: National Academy Press, 1997.
4. Sax L. The institute of medicine's 'dietary reference intake' for phosphorus: a critical perspective. J Am Coll Nutr 2001; 20: 271–8.
5. Expert group on Vitamins and Minerals. Report on Safe Upper Levels for Vitamins and Minerals. Food Standards Agency, 2003. www.foodstandards.gov.uk/multimedia/pdfs/vitmin2003.pdf

CHAPTER 31

Magnesium

Magnesium, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	280	350	120	200	280
Average requirement	AR	–	–			
Lower level of intake	LI	–	–			

Magnesium is a divalent ion and is involved in more than 300 essential metabolic reactions. The metabolism and requirement of magnesium are still rather poorly understood.

Physiology and metabolism

The body content of magnesium is regulated via absorption and excretion. At normal dietary intakes 20–60% is absorbed, being inversely proportional to the amount of magnesium ingested (1, 2). It is uncertain to what degree the composition of the diet influences absorption. Vitamin D and its metabolites enhance magnesium absorption, but much less than their effect on calcium absorption. High levels of dietary fibre from fruits, vegetables and grains decrease fractional magnesium absorption, but the high magnesium content of these sources offsets the decreased fractional absorption. Phosphates bind divalent magnesium and thus high phosphate diets decrease magnesium absorption. Calcium probably has no effect. Less than 30 g/d of protein decreases magnesium absorption.

Plasma concentrations are probably regulated via the kidneys and are kept within a narrow range (0.75–0.95 mmol/L). At low magnesium intakes, kidney excretion is reduced.

A large number of biochemical and physiological processes are regulated by magnesium. Magnesium is necessary for energy dependent membrane transport, gene regulation, sustained electrical potential in nerves and cell membranes and for transmission of neuro-muscular impulses.

The total body content of magnesium in an adult is estimated to be 20–28 g, 40–45% being intracellular in muscles and soft tissue, 1% extracellular and the rest in the skeleton. Although we do not have a true

storage of magnesium, approximately one-third of skeletal magnesium is in equilibrium with plasma levels and functions as a buffer to maintain extracellular magnesium concentrations.

Magnesium depletion is very unusual in the absence of dietary restriction or some disorder causing magnesium loss from the body. Magnesium depletion is usually secondary to another disease process or to a therapeutic agent. The physiological manifestations of severe magnesium depletion are the followings: hypokalaemia and hypercalcaemia, neuromuscular hyperexcitability, electrocardiographic abnormalities and cardiac arrhythmias.

Therapeutic use of magnesium in heart arrhythmia conditions (3–5) and to reduce the risk of eclampsia in women with pre-eclampsia (6–9) has received wide scientific attention in recent years, and may indicate that dietary intakes are too low in parts of the population. However, no studies have so far been conducted to show a preventive potential of high *versus* low-magnesium diets in relation to reducing the risk of these conditions.

Epidemiological studies have suggested that individuals or groups with diets high in magnesium (including supplements and magnesium from water) have reduced morbidity from cardiovascular disease or less hypertension. Low magnesium intake has also been linked to osteoporosis, tension headaches and diabetes (10–12). However, considering all the confounders, it is as yet difficult to conclusively establish that too low dietary magnesium is the primary causative factor in these chronic diseases and to use these studies to help estimating dietary requirements.

Lack of reliable indicators of magnesium status has hampered magnesium research in humans for years. As plasma/serum magnesium levels are kept within such a narrow range, even extensive supplementation is not reflected in increased plasma levels. Thus, hypomagnesaemia (serum < 0.70 mmol/L) will always reflect low body stores, while serum levels within the normal range may well mask suboptimal status or deficiency (13), as serum magnesium concentrations may not reflect intracellular magnesium availability. Although serum magnesium concentration is the most available and commonly employed test to assess magnesium status, this parameter has not been validated as a reliable indicator of body magnesium status.

Studies using plasma-ionised magnesium or intracellular magnesium content (*e.g.* red blood cells or skeletal muscle) to evaluate magnesium status are few and need further evaluation before they could be used as indicators of magnesium status.

The magnesium tolerance test is based on the renal excretion of parenterally administered magnesium and has been used for many years. The method is by many currently considered the most accurate means of assessing magnesium status in adults, but its invasive nature raises concern and limits its use in clinical practice.

Requirement and recommended intake

Adults

In the absence of biochemical or functional indicators of magnesium status, the only basis we have for evaluating requirements are a few balance studies. As absorption of magnesium varies with the dietary intake and it seems possible to adapt to a low intake through more effective absorption, the value of balance data is limited. A study by Jones (14) concluded than an intake of 3.4 mg/kg body weight results in balance in almost all adult individuals. This is also considered to cover the needs during pregnancy and lactation.

The EU Scientific Committee on Food considered 150–500 mg/d to be an acceptable range of magnesium intake, based on observed intakes (15).

The US Food and Nutrition Board has set an Estimated Average Requirement (EAR) for magnesium of 255 mg/d for women and 330 mg/d for men aged 19–30 years (16). RDA (Recommended Dietary Allowance) is accordingly 310 and 400 mg/d for women and men respectively. The values are slightly higher for the age group 31–70 years: RDA for women is set at 320 mg/d and for men 420 mg/day.

The Nordic Recommendations of 1996 recommended 350 and 280 mg magnesium/d for men and women respectively. There are no substantial new data since then indicating that these values should be changed.

Children

The magnesium content in human milk is 28–40 mg/L, which reflects an intake of approximately 30 mg/d in the first months of life. The concentration of magnesium in human milk is relatively constant in the first 12 months of lactation (17). For children the RI values from 1996 are maintained (15).

Upper intake levels and toxicity

An excessive magnesium intake (0.5–5 g) gives diarrhoea but otherwise no negative symptoms when kidney function is normal.

The US Food and Nutrition Board has set a Tolerable Upper Intake level of 350 mg/d from supplements. This level is based on lowest ob-

served adverse effect levels. The EU Scientific Committee on Food has derived a level of 250 mg magnesium per day based on similar data.

Dietary sources and intake

Magnesium is found in abundance in green, leafy vegetables, legumes and wholegrain cereals. Concentrations are especially high in dark chocolate, nuts and coffee. 'Hard' water contains more magnesium than 'soft' water and may contribute to total magnesium intake. Intake of magnesium in the Nordic countries is estimated to be 340–470 mg/10 MJ (see *Chapter 43*). Cereal products contribute approximately 33% of the total magnesium intake, potatoes and vegetables 20%, milk and milk products 15%, meat and fish 10% and coffee 10%.

REFERENCES

1. Lakshmanan FL, Rao RB, Kim WW *et al.* Magnesium intakes, balances, and blood levels of adults consuming self-selected diets. Am J Clin Nutr 1984; 40: 1380–9.
2. Schwartz R, Spencer H, Welsh JJ. Magnesium absorption in human subjects from leafy vegetables, intrinsically labeled with stable ^{26}Mg. Am J Clin Nutr 1984; 39: 571–6.
3. Kalus JS, Spencer AP, Tsikouris JP *et al.* Impact of prophylactic i.v. magnesium on the efficacy of ibutilide for conversion of atrial fibrillation or flutter. Am J Health Syst Pharm 2003; 60: 2308–12.
4. Dittrich S, Germanakis J, Dahnert I *et al.* Randomised trial on the influence of continuous magnesium infusion on arrhythmias following cardiopulmonary bypass surgery for congenital heart disease. Intensive Care Med 2003; 29: 1141–4.
5. Kiziltepe U, Eyileten ZB, Sirlak M *et al.* Antiarrhythmic effect of magnesium sulfate after open heart surgery: effect of blood levels. Int J Cardiol 2003; 89: 153–8.
6. Duley L, Gulmezoglu AM, Henderson-Smart DJ. Magnesium sulphate and other anticonvulsants for women with pre-eclampsia. Cochrane Database Syst Rev 2003; CD000025.
7. Livingston JC, Livingston LW, Ramsey R *et al.* Magnesium sulfate in women with mild preeclampsia: a randomized controlled trial. Obstet Gynecol 2003; 101: 217–20.
8. Belfort MA, Anthony J, Saade GR *et al.* A comparison of magnesium sulfate and nimodipine for the prevention of eclampsia. N Engl J Med 2003; 348: 304–11.
9. Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial. Lancet 2002; 359: 1877–90.
10. Sojka JE, Weaver CM. Magnesium supplementation and osteoporosis. Nutr Rev 1995; 53: 71–4.
11. Zhao C, Stillman MJ. New developments in the pharmacotherapy of tension-type headaches. Expert Opin Pharmacother 2003; 4: 2229–37.
12. Barbagallo M, Dominguez LJ, Galioto A *et al.* Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med 2003; 24: 39–52.
13. Toto KH, Yucha CB. Magnesium: homeostasis, imbalances, and therapeutic uses. Crit Care Nurs Clin North Am 1994; 6: 767–83.

14. Jones JE, Manalo R, Flink EB. Magnesium requirements in adults. Am J Clin Nutr 1967; 20: 632–5.
15. Reports of the Scientific Committee on Food. Nutrient and energy intakes for the European Community. Luxembourg: Commission of the European Communities, 1993.
16. Food and Nutrition Board, Institute of Medicine. Dietary reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington DC: National Academy Press, 1997.
17. Dorea JG. Magnesium in human milk. J Am Coll Nutr 2000; 19: 210–9.

CHAPTER 32

Sodium as salt

Salt is nutritionally equivalent to sodium chloride (NaCl) and is used as a food ingredient or condiment. Sodium is also found in unprocessed foods but usually in very low concentrations. 1 g salt corresponds to about 0.4 g sodium and 1 g sodium is equivalent to 2.5 g salt. 1 mmol sodium corresponds to 23 mg and is equivalent to about 58 mg sodium chloride.

Physiology and metabolism

The sodium ion is essential for a number of metabolic processes in the cell and is involved in the regulation of the acid-base balance, the osmotic pressure in ECV, blood volume, nerve function and the transport mechanisms for glucose and certain amino acids (1).

The body pool of sodium is approximately 100 g. About half is found in the extracellular fluid (ECV) and 10% in the cells. The rest is mainly bound in the skeleton, of which half is exchangeable and thereby functions as a store for the body fluids.

The absorption of sodium is effective and generally amounts to more than 90% of the dietary intake. The excretion of sodium mainly occurs through the kidneys, where it is effectively regulated depending on sodium and fluid intake. Losses through the skin in our climate are generally not more than 1 mmol/d (2). Small amounts of sodium (0.1–8 mmol) are also lost daily in the faeces (3). During profound sweating, in massive diarrhoea or vomiting, the extrarenal loss may be clinically significant. Healthy kidneys can retain almost all sodium in the body, since the tubuli cells reabsorb sodium up to 99.5%. Healthy kidneys can also excrete large amounts of sodium. This requires a satisfactory water supply, since the urine cannot be concentrated more than to a limited degree. The daily excretion through kidneys and skin is normally 100–200 mmol.

Requirement

Dietary sodium deficiency does normally not occur in the Nordic countries. Acute deficiency can develop in connection with heavy sweating

in combination with large fluid intakes devoid of sodium, or in connection with prolonged vomiting and diarrhoea without salt supply. Clinical symptoms include muscle seizures, loss of appetite and circulation disturbances. Severe deficiency can result in coma and death.

Among adults, sodium balance can be maintained at intakes as low as 10 mmol (230 mg) per day corresponding to about 0.6 g of salt. An intake of 25 mmol (575 mg) per day, corresponding to about 1.5 g salt, is set as the estimated lower level of intake, accounting for variation in physical activity and climate (1).

Salt and blood pressure

From a public health perspective the role of sodium as dietary salt in the regulation of blood pressure has received most interest. The relationship between salt and blood pressure has been studied for a long time. Kempner made classical observations during the 1930s and 40s (4). He treated *e.g.* diabetics and hypertensive subjects with a salt-restricted rice and fruit diet containing less than 2 grams of salt per day and found that blood pressure was drastically reduced among most of the patients.

Cross-sectional population studies

Population studies have shown that hypertension is rare in populations with a very low salt intake (< 2 g/d) and that blood pressure does not rise with age (5). In areas with very high salt intakes (30–35 g/d) such as Northern Japan, severe hypertension is reported among 30–35% of the population (5). In the large, multi-centre Intersalt study (6) the relationship between 24-hour sodium and potassium excretion and blood pressure was investigated. The study included 10,000 men and women aged 20–59 years from 52 centres around the world. The median sodium excretion varied from 0.2 mmol/d to 242 mmol/d between centres. In four centres with very low sodium excretion, blood pressure was low and no age-related increase was observed. In the other 48 centres, sodium excretion was related to the increase in blood pressure with age but not to median blood pressure or prevalence of high blood pressure. Potassium excretion was negatively related to blood pressure on an individual basis, while the sodium : potassium ratio showed a pattern similar to that of sodium. Body mass index and heavy alcohol intake were strongly related to blood pressure.

Law *et al.* (7) analysed published data on blood pressure and sodium intake for 24 different communities (47,000 subjects) throughout the world, including the Intersalt study. Allowance was made for differences in blood pressure between economically developed and underdeveloped

communities to minimise overestimation of the association through confounding with other determinants of blood pressure. The authors found that blood pressure was higher on average in the developed communities, but the association with sodium intake was similar in both types of community. A difference in sodium intake of 100 mmol/24 h was associated with an average difference in systolic blood pressure that ranged from 5 mm Hg at age 15–19 years to 10 mm Hg at age 60–69. The differences in diastolic blood pressure were about half as great. The authors concluded that the association of blood pressure with sodium intake is substantially larger than is generally appreciated and increases with age and initial blood pressure. Data from within population studies also generally support an association (8, 9).

Clinical trials

Several meta-analyses of clinical trials of dietary salt reduction have been published (10–14). These differ in scope and inclusion criteria. Law *et al.* (10) analysed 68 crossover and 10 randomised controlled trials of salt reduction among normotensives and hypertensives, which included studies published up to 1989. They found that the blood pressure lowering effect of salt restriction was related to the duration of the study, with less effect in trials lasting less than 4 weeks. They concluded that in people aged 50–59 years, a reduction in daily sodium intake of 50 mmol (approximately 3 g of salt) would, after a few weeks, lower systolic blood pressure by an average of 5 mm Hg, and by 7 mm Hg in those with high blood pressure (170 mm Hg). The diastolic blood pressure would be lowered by about half as much.

Midgley *et al.* (11) analysed 56 trials, published between 1966 and 1994, that had randomised allocation of subjects to control and dietary sodium intervention groups, monitored by sodium excretion, with outcome measures of both systolic and diastolic blood pressure, selected by blinded review of the methods section. Several of these studies, including some published before 1990, were not included in the analysis by Law *et al.* (10). The mean reduction in daily urinary sodium excretion was 95 mmol/d (71–119 mmol/d) in 28 trials with 1,131 hypertensive subjects and 125 mmol/d (95–156 mmol/d) in 28 trials with 2,374 normotensive subjects. In hypertensive subjects, a reduced urinary sodium excretion of 95 mmol/d significantly reduced systolic blood pressure by 5.9 mm Hg and diastolic blood pressure by 3.8 mm Hg . In normotensive subjects, the corresponding changes for a reduced urinary sodium excretion of 125 mmol/d were a significant reduction of 1.6 mm Hg for systolic and of 0.5 mm Hg (non-significant) for diastolic blood pressure. A weakness of the analysis of trials on normotensives was the

short duration of the trials (on average 14 days), although the authors state that there was a tendency for a greater blood pressure reduction in trials with a shorter duration (< 2 weeks). This is in contrast with the findings of Law *et al.* (10) and could be due to problems of compliance in some of the more long term-studies. Trials on normotensive subjects involved mainly young subjects, while the trials on hypertensives mainly involved middle-aged or older subjects. The decreases in blood pressure were larger in trials on older hypertensive individuals than on younger, whereas no data are given for the normotensives.

Graudal *et al.* (12) published another meta-analysis including 58 randomised trials on dietary sodium restriction among hypertensives and 56 trials among normotensives published between 1966 and 1997. In 58 trials of hypertensive persons (exact criteria not stated), a reduced urinary mean sodium excretion of 118 mmol/24 h gave a significant reduction in systolic blood pressure of 3.9 mm Hg and diastolic blood pressure of 1.2 mm Hg. In 56 trials of normotensive persons, a reduced mean sodium excretion of 160 mmol/24 h was associated with a significant average reduction in the systolic blood pressure of 1.2 mm Hg, while a non-significant reduction in the diastolic blood pressure of 0.26 mm Hg was observed. In this study too, trials on normotensives had a short duration, mean of only 8 days, and included younger subjects (mean age 27 years) with a mean systolic blood pressure of 120 mm Hg. This limits the relevance of the results for public health action. The mean duration of trials of hypertensives was 28 days and the mean age of the subjects was 49 years, which is comparable to the analysis by Midgley *et al.* (11).

The meta-analysis by Cutler *et al.* (13) included 23 trials published up to mid-1994. The lower number of trials included was due to stricter inclusion criteria. The combined weighted data showed that a decrease in sodium excretion of 100 mmol Na/24 h (5.9 g salt) was associated with a reduction in systolic blood pressure of 4.8 mm Hg in hypertensives and 2.3 mm Hg in normotensives. The corresponding figures for diastolic blood pressure were 2.5 and 1.4 mm Hg, respectively.

In the meta-analysis by Geleijnse *et al.* (14), only randomised controlled trials with duration greater than 2 weeks were included. Forty trials published between 1966 and 1991 were included. A median reduction in sodium excretion of 77 mmol/24 h (4.5 g salt) was associated with a 2.5 mm Hg reduction in systolic blood pressure and 2.0 mm Hg in diastolic blood pressure. Reductions were more pronounced in hypertensives and the same tendency was seen in older subjects. A subsequent meta-analysis including trials with a duration of 4 weeks or more with a similar reduction in sodium excretion (74–78 mmol/24h) found a 5.0

mm Hg reduction in systolic blood pressure and 2.7 mm Hg in diastolic blood pressure among hypertensives. Corresponding figures for subjects with normal blood pressure were 2.0 mm Hg and 1.0 mm Hg, respectively (14a).

Only a few studies have examined the long-term effects on blood pressure of sodium restriction. Jula *et al.* (15) studied the effects on blood pressure and serum lipids of a non-pharmacological treatment based mainly on sodium restriction in a 12-month controlled randomized study with 91 middle-aged untreated mildly hypertensive men and women. The estimated daily sodium intakes, calculated from 24-hour urines, decreased in men from 227 mmol to a mean level of 105 mmol, and in women from 129 mmol to 63 mmol. After 12 months of non-pharmacological treatment, the mean weight in men was 1.9 kg lower and in women 0.3 kg higher compared to the baseline. In the treatment group, energy derived from saturated and monounsaturated fats decreased. The net blood pressure decrease (difference in changes between treatment and control group) during the 12 months in men was 8.2 mm Hg for systolic and 5.8 mm Hg for diastolic blood pressure, and in women 9.5 mm Hg for systolic and 5.6 mm Hg for diastolic blood pressure. All changes were significant. In the treatment group LDL-cholesterol also decreased, by 6.8% in men and by 12.1% in women.

In the DASH trials (Dietary Intervention to Stop Hypertension) the effects of various controlled diets on the blood pressure of adult Americans with normal or moderately elevated blood pressure were studied (16, 17). In the study by Sacks *et al.* (17) the influence of sodium intake on blood pressure was assessed in 412 subjects who were randomly assigned to eat either a control diet typical of intake in the United States or the DASH diet, each providing three levels of sodium. The subjects were selected among adults 22 years or older, who were not taking antihypertensive medication, and with a systolic blood pressure exceeding 120 but below 160 mm Hg and a diastolic ranging from 80 to 95 mm Hg. The control diet had a fat composition corresponding to the usual American diet (36 E% total fat, 14 E% saturated fat), but a low content of fruits, vegetables and milk products. The DASH diet was rich in fruit, vegetables and low-fat dairy products, but low in edible fats, snacks and sweets, with a low content of total fat (25 E%) and saturated fat (7 E%) and cholesterol. The content of calcium, potassium and magnesium in the control diet was lower than in the average American diet, whereas the level in the DASH-diet was higher. The intake of dietary fibre was similar in both groups. Within the assigned diets, sodium levels were adjusted to provide a daily intake of 150 mmol (high, about 9 g salt), 100 mmol (intermediate, about 6 g salt), and 50 mmol (low, about 3 g

salt) for 30 consecutive days each, in random order. The estimated sodium intakes, calculated from 24-hour urines, indicated a higher intake in the low (71–74 mmol, about 4 g salt) and intermediate (120 mmol, about 7 g salt) sodium groups.

Reducing the sodium intake from the high to the intermediate level significantly reduced the systolic blood pressure by 2.1 mm Hg during the control diet and by 1.3 mm Hg during the DASH diet. A further reduction from the intermediate to the low level caused additional reductions of 4.6 mm Hg on the control diet and 1.7 mm Hg on the DASH diet. A regression analysis of these data shows that a reduction in the sodium intake of 100 mmol per day would lead to a reduction in the systolic blood pressure of about 3 mm Hg in the DASH group and of about 7 mm Hg in the control group. Corresponding values for diastolic blood pressure are 1.5–2 and about 3 mm Hg, respectively. The effects of sodium were observed in normotensive and hypertensive subjects, whites, blacks and other races, women and men, and were not dependent on weight (17, 18).

An aspect only partly addressed in the meta-analyses is the relationship between the sodium intake and the age-related change in blood pressure. Data from the Intersalt study strongly indicate a relationship between the median daily urinary sodium excretion and the difference in blood pressure with age (19). In within population analyses, individual 24-hour urinary sodium excretion higher by 100 mmol was associated with a 3–6 mm Hg higher systolic and 0–3 mm Hg diastolic blood pressure. Associations were larger at ages 40–59 than at younger ages. In cross-population analyses, median 24-hour sodium excretion higher by 100 mmol was associated with 5–7 mm Hg higher median systolic and 2–4 mm Hg higher median diastolic pressure. At age 55 the estimated mean difference in systolic and diastolic blood pressure was 10–11 and 6 mm Hg greater, respectively, compared to at age 25, indicating a strong age-related effect of high sodium intakes on blood pressure. In the DASH-trial, the blood pressure reduction was higher in older (> 45 years) than in younger subjects, *e.g.* a 100 mmol reduction in sodium excretion was associated with a 6 mm Hg lower systolic blood pressure among non-black older subjects (18).

The DASH-trials clearly showed an effect of sodium restriction, ranging from 2–5 g/d, on blood pressure, which is independent of other dietary and lifestyle factors. An important finding is that the blood pressure reduction was larger in the control group than in the DASH group. This implies that the benefits of sodium restriction are more pronounced among persons consuming a diet which is less optimal, *e.g.* with respect to fat, fruit and vegetables etc. (and similar to the current

dietary patterns in the Nordic countries), than among those already consuming a diet in line with the general nutrition recommendations. A limitation of the study is the relatively short duration (30 days) and the fact that the study excluded subjects with low (SBP < 120 mm Hg) and high (SBP > 160 mm Hg) blood pressure. However, the blood pressure lowering effect of dietary salt reduction on hypertensives is well documented, while the proportion of the adult population with systolic blood pressure below 120 mm Hg is small, especially among the middle-aged and older.

Other studies

In a Portuguese population-based intervention study, sodium intake was reduced by dietary advice (20). The mean dietary intake of salt decreased by approximately 40% (from approximately 20 to 11.5 g/d), estimated by food consumption data, while estimations based on urinary sodium to creatinine ratios indicated a lower reduction, approximately 25% (5 g salt) after one year and 9% (2 g salt) after 2 years. After 2 years of intervention, the systolic and diastolic blood pressure had both decreased by approximately 5 mm Hg.

Salt intake and blood pressure among children

There are few studies regarding the relationship between sodium intake and blood pressure among children and adolescents. In a review, Falkner and Michel (21) conclude that there are insufficient data with respect to the importance of sodium intake for blood pressure in children and adolescents. A few studies have found a positive relationship between the sodium concentration of drinking water and blood pressure (see *e.g.* 22), but there are no reliable data on the total sodium intake from the total diet in the different groups, which makes it difficult to interpret the results.

The importance of early diet on blood pressure was investigated by Geleijnse *et al.* (23). In a randomised trial among 476 Dutch newborn infants, the effect of a low (on average 120 mg/d) or normal (on average 330 mg/d) sodium diet on blood pressure during the first 6 months of life was studied. The sodium intake in the low sodium group is approximately similar to the intake of breast fed infants, whereas the intake in the normal group is similar to the sodium intake of infants fed commercial infant formula. At the end of the trial, systolic blood pressure in the low sodium group was 2.1 mm Hg lower than in the control group. The authors also measured blood pressure in 167 children from the original cohort (35%) after 15 years of follow-up. The adjusted systolic blood

pressure at follow-up was 3.6 mm Hg lower and the diastolic pressure was 2.2 mm Hg lower in adolescents who as infants had been assigned to the low sodium group compared with those assigned to the control group. Both groups had relatively low urinary sodium excretions as estimated from overnight urinary samples (around 2 g/d). These findings suggest that sodium intake in infancy may be important in relation to blood pressure in adolescence and later life.

Other dietary factors and blood pressure

A number of dietary factors have been associated with blood pressure. These include *e.g.* alcohol, potassium, calcium, magnesium, and fatty acid composition.

The influence of potassium was shown in the Intersalt study and clinical studies have indicated that potassium supplementation or an increased potassium intake can lead to blood pressure reduction in both normotensives and hypertensives (14, 16).

High alcohol consumption (> 210 g/wk) has been associated with an elevated blood pressure (24). Supplements of large doses of long-chain n-3 fatty acids (several grams per day) can lead to a blood pressure reduction in the order of 1–2 mm Hg, especially among hypertensives and older subjects (25). The effect of n-6 and monounsaturated fatty acids is more controversial. Results from intervention studies indicate that a shift from a diet with a high total fat and low linoleic acid content to a diet with a low fat and moderate linoleic acid content is associated with a reduction in blood pressure. In controlled studies an independent effect of linoleic acid has not been confirmed (26–28).

In intervention studies in which subjects have received fat-modified diets rich in fruit and vegetables, complex carbohydrates conforming to *e.g.* NNR recommendations, but without sodium restriction, a decrease in blood pressure has been observed compared to subjects receiving an average diet (16, 29). As also shown by Sacks *et al.* (17) this implies that the effect of moderate sodium restriction on blood pressure in normotensive persons is less pronounced if the overall composition of the diet is favourable. However, the effects are additive.

In a meta-analysis of controlled intervention studies, frequent coffee consumption (about 5 cups/d) was associated with 2.4 and 1.4 mm Hg higher systolic blood pressure and diastolic blood pressure, respectively, compared to no consumption (30).

Physical activity and blood pressure

Regular, moderate physical activity has been shown to reduce blood pressure, both in normotensive and hypertensive groups, with average

decreases of about 3 and 2 mm Hg for systolic and diastolic blood pressure, respectively, in normotensive groups and 8 and 6 mm Hg, respectively, in hypertensive groups (see *Chapter 10*).

Salt and morbidity and mortality

There are only few studies that have investigated the relationship between sodium intake and morbidity and mortality. The multi-centre CARDIAC study (WHO Cardiovascular Diseases and Alimentary Comparison Study) (31) investigated the relationship between biological markers of dietary factors with blood pressure and age-adjusted mortality rates of stroke and ischaemic heart disease from 55 centres in 24 countries. From each population, 100 men and 100 women aged 48 to 56 years were randomly selected for BP measurement, 24-hour urine collection and other biological parameters. Cross-centre analyses showed that stroke mortality was significantly positively related to the 24-hour sodium excretion rate in men and to the sodium/potassium ratio in both sexes.

Alderman *et al.* (32) reported an increased risk of myocardial infarction among male hypertonics who had been treated with blood pressure reducing drugs. The trend for women was the opposite, although not significant. The sodium intake was measured using single 24-hour urine, which was collected 5 days after the subjects had been asked to avoid consumption of foods with a high salt content. One can therefore question whether the assessment provided a representative measure of the subjects' usual sodium intake. The results could also have been biased due to that confounders, *e.g.* alcohol, not being accounted for. In another study Alderman *et al.* (33) reported a significant negative correlation between sodium intake estimated by 24-hour recalls and all-cause and CVD mortality in a follow-up of the first US NHANES I study. Based on these results, the authors concluded that sodium restriction might lead to negative health effects and that advice to reduce sodium intake in the general population is not justified. A critical examination of the data (34), however, favoured the opposite interpretation since the authors also found a positive correlation between the sodium content of the diet expressed as mg/kcal and mortality. A major weakness of the NHANES I data was the low energy intake, which was on average below levels associated with bed-bound or wheelchair activity. When the study population is classified into sodium density (mg Na/kcal), the energy intakes are more comparable among the quartiles, indicating that underreporting is more evenly distributed. The energy adjusted sodium intakes are thus more reliable, and only these data can, in the absence of 24-hour urine data, be used with some confidence in the analysis of a

possible relationship between sodium intake and mortality. The result of this analysis, which the authors briefly mention, is that there is a weak, but significant, positive association between the sodium content of the diet and both total and CVD mortality.

He *et al.* (35) examined the risk of cardiovascular disease associated with dietary sodium intake in 2688 overweight (BMI) and 6797 non-overweight persons in the first National Health and Nutrition Examination Survey Epidemiological Follow-up Study (NHANES I). Subjects were aged 25 to 74 years when the survey was conducted in 1971–1975. Dietary sodium and energy intakes were estimated at baseline using a single 24-hour dietary recall method. The average follow-up was 19 years. Among overweight persons, a 100 mmol higher sodium intake was associated with a 32% increase in stroke incidence, 89% increase in stroke mortality, 44% increase in coronary heart disease mortality, 61% increase in cardiovascular disease mortality, and 39% increase in mortality from all causes. Dietary sodium intake was not significantly associated with cardiovascular disease risk in non-overweight persons. The limitations of the study are the same as for the earlier mentioned study by Alderman *et al.* (34) on the same population.

In a prospective study by Tuomilehto *et al.* (36) on Finnish men and women aged 25–64 years, 24-hour urinary sodium excretion, divided into quartiles, was directly related to the incidence of coronary and stroke events, and death from coronary heart disease, cardiovascular disease, and any cause. There was a significant elevated risk for coronary heart disease, cardiovascular disease, and all-cause mortality, associated with a 100 mmol increase in 24-hour urinary sodium excretion in both men and women. The frequency of acute coronary events, but not acute stroke events, rose significantly with increasing sodium excretion. In separate analyses for each sex, the risk elevations were significant in men only. There was also a significant interaction between sodium excretion and body mass index for cardiovascular and total mortality; sodium excretion predicted mortality in men who were overweight, but not in normal weight subjects. The increase in risk was independent of blood pressure and potassium excretion (37). The sodium intake in the lowest sodium excretion groups was below 159 mmol/L in men and 119 mmol/L in women, corresponding to approximately 4 g sodium (10 g salt) and 3 g sodium (7.5 g salt) per day, respectively.

In a review Perry (38) concludes that available studies suggest that sodium intake is independently related to left ventricular hypertrophy, a condition that is associated with increased risk of coronary mortality. Long-term sodium restriction decreases left ventricular hypertrophy of hypertensive subjects (39, 40).

Several studies indicate a positive relationship between sodium and calcium excretion and that the sodium intake may play a role in the etiology of osteoporosis and kidney stones (41).

Risks associated with a reduced sodium intake

A review of controlled studies in which the sodium intake was restricted did not reveal any evidence of adverse effects of moderate sodium restriction (42). The analysis included 20 randomised intervention studies with at least 6 months follow-up and urinary excretion data. However, the authors of NNR 2004 recognise that the issue of adverse effects has not been systematically studied. Concerns that have been raised refer to subjects with impaired sodium homeostasis, high sodium losses or low food intake, and the capability of the cardiovascular system to handle extreme situations, *e.g.* during intense heat and large fluid losses. The two studies by Alderman *et al.* (32, 33) indicate an increased risk of myocardial infarction and mortality, but their interpretations can be questioned on a methodological basis (34).

Recommended intake

Data from recent individual trials and meta-analyses of previous trials show that reduction of sodium intake can independently decrease blood pressure. The effect is greater among hypertensives and overweight subjects and available data also suggest that moderate sodium restriction will attenuate the usual blood pressure increase with age (14, 19, 18). The magnitude of blood pressure decrease of sodium restriction also depends on the dietary composition, and seems to be more pronounced when the diet is less optimal, *e.g.* with respect to the balance between the energy providing nutrients, fibre, potassium and calcium and possibly other constituents, provided by *e.g.* fruit and vegetables.

The data from the DASH studies and other studies indicate that blood pressure decreases with decreasing sodium intake within the range 1.2–8 gram (3–20 g salt) per day. Blood pressure is a strong independent risk factor for CVD and moderate sodium intakes have in some studies been associated with decreased risk of CVD morbidity and mortality, primarily among overweight subjects. On the other hand, available data do not suggest any benefits of high sodium intakes.

Adults

Any recommendations on the sodium intake thus have to be based on practical and public health considerations, rather than on a precise estimate of an optimal physiological intake. Based on a pragmatic evalua-

tion of the available data, a sodium intake in the range 100–150 mmol (2.3–3.5 g) per day would be feasible at the population level. Although most of the analyses have not differentiated between men and women, it seems reasonable to set separate targets for men and women. Epidemiological data suggest that sodium intakes above 3–4 g/d are associated with increased CVD morbidity and mortality. In a British population study, urinary sodium excretion below 140 mmol/d (3.2 g sodium) was associated with a lower prevalence of hypertension than higher excretion levels. The proposed population target is to reduce the sodium intake to 2.3 g (100 mmol, about 6 g salt) per day for adult women and 2.7 g (120 mmol, about 7 g salt) per day for adult men. The differentiated recommendation for men and women is based on body size. It should be pointed out that a further reduction of the sodium intake is estimated to result in additional benefits in terms of *e.g.* blood pressure lowering and may be warranted, especially among hypertensives. A long-term goal is to reduce the average sodium intake to about 2–2.3 g/d, corresponding to 5–6 g salt.

The current average sodium intake in the Nordic countries can be estimated at 4–5 gram per day (10–12 g salt). The proposed population targets would therefore require a reduction in the average population intake of approximately 1–2 g sodium (3–5 g salt) per day. The public health implications of these targets are more important with increasing blood pressure, body weight and age.

Children

Available data, although limited, suggest that sodium intake in young age is associated with elevated blood pressure in later life. In relation to the recommendation of a moderate sodium intake in adults, it also seems prudent to limit sodium intake in childhood in order to avoid preference for a diet with a high salt level. The recommended sodium density, expressed as salt, is set to 0.5 g per 1,000 kJ, which is based on the reference values for adults.

International expert reports

As early as 1982, a WHO report on prevention of cardiovascular disease (43) recommended that the salt intake should not exceed 5 g/day. This recommendation was based on various clinical and epidemiological data. Since then, several international and national expert bodies including WHO (44, 45), US Food and Nutrition Board (46), American Heart Association (47), and a British Expert Panel (48) have published recommendations to limit salt intake to 5–6 g/day. The British Expert Panel (48) notes that the evidence linking salt intake to blood pressure

has strengthened during the last decade and also gives reference values for children and adolescents.

Public health effects of sodium restriction

Based on the Intersalt study, Thelle (49) estimated that a change in salt intake from 10 to 5 g/d would result in a reduction in the expected blood pressure increase over a 30 year period from 10 to about 5 mm Hg. According to Thelle, data from Norwegian studies show that an increase in the average blood pressure level of 5 mm Hg is associated with an increase in coronary disease and stroke of about 20% and 40%, respectively (49).

In a more recent report (50, 51) a reduction in the systolic blood pressure of 2–4 mm Hg, accomplished by a reduction of salt intake by approximately 6 grams per day, was estimated to result in a reduction of stroke and myocardial infarction mortality among Norwegian men and women below 70 years by 8–16% and 6–14%, respectively.

Advice to reduce salt intake in various population groups can result in decreases in salt intake and blood pressure, although the effects are moderate (52). Salt reduction may also allow hypertensive subjects to stop their medication (52, 53). The moderate effects of dietary advice on salt intake reflect the relatively high salt content in many processed foods and the lack of salt-reduced alternatives. To achieve a more pronounced reduction in the salt intake in the population, a general decease in the salt content of most processed foods is needed.

Dietary sources and intake

The main sources of sodium in the diet are processed foods, *e.g.* bread, cheese, spreads, meat and fish products. The contribution of sodium from added salt and salt-containing spice mixtures and condiments varies. Data on the total dietary intake of sodium in Nordic populations are scarce. According to national food balance sheets the availability of salt in the Nordic countries is estimated to be 10–12 g *per capita* and day. Estimations of the sodium intake from national dietary surveys among adults generally show somewhat lower values. Average dietary sodium contents (g/10 MJ) calculated from recent national dietary surveys among adults were in: Denmark 4.1 g Na (10 g salt), Finland 4.4 g (11 g salt), Iceland 3.7 g (9 g salt), Norway 4.1 g (10 g salt) and in Sweden 3.4 g (8.5 g salt) (54–57). The contribution from discretionary salt intake, *e.g.* from extra salt added to meals etc., is generally not included. Data from recent studies on urinary sodium excretion in small Swedish sub-populations show values corresponding to 8–11 g/d salt (58, 59).

REFERENCES

1. Commission of the European Communities. Reports of the Scientific Committee on Food (Thirty-first series). Nutrient and energy intakes for the European Community. Luxembourg, 1993.
2. Dahl LK. Salt intake and salt need. N Engl J Med 1958; 258: 1152–7.
3. Baldwin D, Alexander RW, Warner EG. Chronic sodium chloride challenge studies in man. J Lab Clin Med 1960; 55: 362–75.
4. Kempner W. Treatment of hypertensive vascular disease with rice diet. Am J Med 1948; 4: 545–77.
5. Berglund G. Kan lägre saltintag för alla minska blodtrycksproblemet? Läkartidningen 1980; 77: 1091–92.
6. Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24-hour urinary sodium and potassium excreation. Br Med J 1988; 297: 319–28.
7. Law MR, Frost CD, Wald NJ. By how much does dietary salt reduction lower blood pressure? I. Analysis of observational data among populations. Br Med J 1991; 302: 811–5.
8. Law MR, Frost CD, Wald NJ. By how much does dietary salt reduction lower blood pressure? II. Analysis of observational data within populations. Br Med J 1991; 302: 815–8.
9. Beard TC, Blizzard L, O'Brian DJ, Dwyer T. Association between blood pressure and dietary factors in the Dietary and Nutritional Survey of British Adults. Arch Intern Med 1997; 157: 234–38.
10. Law MR, Frost CD, Wald NJ. By how much does dietary salt reduction lower blood pressure? III. Analysis of data from trials of salt reduction. Br Med J 1991; 302: 819–24.
11. Midgley JP, Matthew AG, Greenwood CM, Logan AG. Effect of reduced dietary sodium on blood pressure: a meta-analysis of randomized controlled trials. JAMA 1996; 275: 1590–7.
12. Graudal NA, Galloe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols, and triglyceride: a meta-analysis. JAMA 1998; 279: 1383–91.
13. Cutler JA, Follman D, Allender PS. Randomized trials of sodium reduction: an overview. Am J Clin Nutr 1997; 65 (Suppl): 643S–51S.
14. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens. 2003; 17: 471–80.

14a. He FJ, MacGregor GA. Effect of longer-term salt reduction on blood pressure (Cochrane Review). In: The Cochrane Library, Issue 3, 2004. Chichester, UK: John Wiley & Sons, Ltd.

15. Jula A, Rönnemaa T, Rastas M, Karvetti RL, Mäki J. Long-term nopharmacological treatment for mild to moderate hypertension. J Int Med 1990; 227: 413–21.
16. Appel LJ, Moore TJ, Obarzanek E, *et al.* A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 1997; 336: 1117–24.
17. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N, Lin PH, Aickin M, Most-Windhauser MM, Moore TJ, Proschan MA, Cutler JA. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. N Engl J Med 2001; 344: 3–10.
18. Vollmer WM *et al.* Effects of a diet and sodium intake on blood pressure: subgroup analysis of the DASH-sodium trial. Ann Intern Med 2001; 135: 1019–28.
19. Elliott P, Stamler J, Nichols R, Dyer AR, Stamler R, Kesteloot H, Marmot M. Intersalt revisited: further analyses of 24 hour sodium excretion and

blood pressure within and across populations. Intersalt Cooperative Research Group. BMJ 1996; 312: 1249–53. Erratum in: BMJ 1997; 315: 458.
20. Forte JG, Pereira Miguel JM, Pereira Miguel MJ, de Padua F, Rose G. Salt and blood pressure: a community trial. J Hum Hypertens 1989; 3: 179–84.
21. Falkner B, Michel S. Blood pressure response to sodium in children and adolescents. Am J Clin Nutr 1997; 65 (Suppl): 618S–21S.
22. Pomeranz A, Korzets Z, Vanunu D, Krystal H, Wolach B. Elevated salt and nitrate levels in drinking water cause an increase in blood pressure of schoolchildren. Kidney Blood Press Res 2000; 23: 400–3.
23. Geleijnse JM, Hofman A, Witteman JC, Hazebroek AA, Valkenburg HA, Grobbee DE. Long-term effects of neonatal sodium restriction on blood pressure. Hypertension 1997 29: 913–7.
24. Fuchs FD, Chambless LE, Whelton PK, Nieto FJ, Heiss G. Alcohol consumption and the incidence of hypertension: The Atherosclerosis Risk in Communities Study. Hypertension 2001; 37: 1242–50.
25. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. J Hypertens. 2002; 20: 1493–9.
26. Knapp H. Dietary fatty acids in human thrombosis and hemostasis. Am J Clin Nutr 1997; 65 (5 Suppl): 1687S–98S.
27. Preuss, HG, Gondal JA, Liebera S. Association of macronutrients and energy intake with hypertension. Am J Coll Nutr 1996; 15: 2135.
28. Pietinen P. Dietary fat and blood pressure. Ann Med 1994; 26: 465–68.
29. Sandström B, Marckmann P, Bindslev N. An eight-month controlled study of a low-fat high-fibre diet: effects on blood lipids and blood pressure in healthy young subjects. Eur J Clin Nutr 1992; 46: 95–109.
30. Jee SH, He J, Whelton PK, Suh I, Klag MJ. The effect of chronic coffee drinking on blood pressure: a meta-analysis of controlled clinical trials. Hypertension 1999; 33: 647–52.
31. Yamori Y, Nara Y, Mizushima S, Sawamura M, Horie R. Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention. Health Rep 1994; 6: 22–7.
32. Alderman MK, Madhavan S, Cohen H, Sealey JE, Laragh JH. Low urinary sodium is associated with greater risk of myocarial infarction among treated hypertensive men. Hypertension 1995; 25: 1144–55.
33. Alderman MK, Cohen H, Madhavan S. Dietary sodium intake and mortality: the National Health and Nutrition Examination Survey (NHANES I). Lancet 1998; 351: 781–5.
34. Becker W. Dietary sodium and mortality. Scand J Nutr/Näringsforskning 1998; 42: 94.
35. He J, Ogden LG, Vupputuri S, Bazzano LA, Loria C, Whelton PK. Dietary sodium intake and subsequent risk of cardiovascular disease in overweight adults. JAMA 1999; 282: 2027–34.
36. Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P, Nissinen A. Urinary sodium excretion and cardiovascular mortality in Finland: a prospective study. Lancet. 2001a; 357: 848–51.
37. Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P, Nissinen A. Sodium excretion and cardiovascular mortality. Comment. Lancet. 2001b; 358: 666.
38. Perry IJ. Dietary salt intake and cerebrovascular damage. Nutr Metab Cardiovasc Dis 2000; 10: 229–35.
39. Jula AM, Karanko HM. Effects on left ventricular hypertrophy of long-term nonpharmacological treatment with sodium restriction in mild-to-moderate essential hypertension. Circulation 1994; 89: 1023–31.
40. Neaton JD, Grimm RH Jr, Prineas RJ, Stamler J, Grandits GA, Elmer PJ, Cutler JA, Flack JM, Schoenberger JA, McDonald R, *et al.* Treatment of Mild Hypertension Study. Final results. Treatment of Mild Hypertension Study Research Group. JAMA 1993; 270: 713–24.

41. Cappuccio FP, Kalaitzidis R, Duneclift S, Eastwood JB. Unravelling the links between calcium excretion, salt intake, hypertension, kidney stones and bone metabolism. J Nephrol 2000; 13: 169–77.
42. Kumanyika SK, Cutler J. Dietary sodium reduction: is there a cause for concern? J Am Coll Nutr 1997; 16: 192–203.
43. WHO Expert Committee. Prevention of coronary heart disease. WHO, Techn Rep Ser 678, WHO, Geneva: 1982.
44. James WPT, Ferro-Luzzi A, Isaksson B, Szostak WB. Healthy nutrition. WHO Regional Publications, European Series, No. 24, Copenhagen: 1988.
45. WHO. Diet, nutrition and the prevention of chronic diseases. Report of the WHO/FAO Joint expert consultation. WHO Techn Rep Ser 916, Geneva: 2003.
46. Food and Nutrition Board. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulphate. Institute of Medicine, National Academic Press, Washington, 2004.
47. Kotchen TA, MD, McCarron DA, for the Nutrition Committee. Dietary Electrolytes and Blood Pressure. A statement for healthcare professionals from the American Heart Association Nutrition Committee. AHA Medical/Scientific Statement. August 1998. www.americanheart.org.
48. Scientific Advisory Committee on Nutrition. Salt and health. Food Standards Agency, Department of Health. The Stationery Office: 2003.
49. Thelle D. Salt og blodtrykk – nok en gang. Tidskr Nor Lægeforen 1989; 109: 79–80.
50. Statens Ernæringsråd. Nedgang i saltforbruket i den norske befolkning; hvilke konsekvenser kan det få for sykelighet, dødelighet og samfunnsøkonomien? Rapport nr. 5/98.
51. Selmer RM, Kristiansen IS, Haglerod A, Graff-Iversen S, Larsen HK, Meyer HE, Bonaa KH, Thelle DS. Cost and health consequences of reducing the population intake of salt. J Epidemiol Community Health 2000; 54: 697–702.
52. Hooper L, Bartlett C, Davey Smith G, Ebrahim S. Systematic review of long term effects of advice to reduce dietary salt in adults. BMJ 2002; 325: 628–32.
53. Whelton PK, Appel LJ, Espeland MA, Applegate WB, Ettinger WH Jr, Kostis JB, Kumanyika S, Lacy CR, Johnson KC, Folmar S, Cutler JA. Sodium reduction and weight loss in the treatment of hypertension in older persons: a randomized controlled trial of nonpharmacologic interventions in the elderly (TONE). TONE Collaborative Research Group. JAMA 1998; 279: 839–46.
54. Becker W, Pearson M. Riksmaten 1997–98. Metod- och resultatrapport (Dietary habits and nutrient intake in Sweden 1997–98). Livsmedelsverket 2002.
55. Danskernes kostvaner 2000–2002. Hovedresultater (Dietary habits of Danes 2000–2002. Main results). Danmarks Fødevareforskning, 2005. In press.
56. Männistö S, Ovaskainen M-L, Valsta L (eds). The national Findiet 2002 study. Publications of the National Public Health Institute B3/2003. Helsinki 2003.
57. Steingrímsdóttir L, Þorgeirsdóttir H, Ólafsdóttir AE. The Diet of Icelanders. Dietary Survey of the Icelandic Nutrition Council 2002. Main findings. Public Health Institute, Reykjavík, Iceland 2003.
58. Larsson CL, Johansson GK. Dietary intake and nutritional status of young vegans and omnivores in Sweden. Am J Clin Nutr 2002; 76: 100–6.
59. Rosell M, Hellénius M-L, de Faire U, Berglund L, Gustafsson I-B, Johansson G. Contribution of a manually coded part in an optically readable, precoded seven-day food record for the intake of energy, nutrients and foods. Scandinavian Journal of Nutrition 2003; 47: 123–31.

CHAPTER 33

Potassium

Potassium, g/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	3.1	3.5	1.8	2.0	2.9/3.3
Lower level of intake	LI	1.6	1.6			

The major proportion of the potassium in the body (98%) is found in the cells and potassium is the quantitatively most important intracellular cation. Extracellular potassium, which constitutes the remaining 2%, is important for regulating the membrane potential of the cells, and thereby for nerve and muscle function, blood pressure regulation etc. Potassium also participates in the acid-base balance. 1 mmol potassium is equivalent to 39 mg.

Physiology and metabolism

The absorption of potassium is effective and about 90% of the dietary potassium is normally absorbed from the gut. The potassium balance is primarily regulated by renal excretion in urine. A small proportion can be lost in sweat.

Requirement and recommended intake

Potassium deficiency can develop as a consequence of increasing losses from the gastrointestinal tract and kidneys, *e.g.* during prolonged diarrhoea or vomiting, and in connection with the use of laxatives or diuretics. Potassium deficiency due to low dietary intake alone is very uncommon, due to the widespread occurrence of potassium in foods. Treatment with diuretics without potassium compensation can, however, lead to deficiency. Symptoms of potassium deficiency are associated with disturbed cell membrane function and include muscle weakness and disturbances in heart function, which can lead to arrhythmia and heart seizure. Mental disturbances, *e.g.* depression and confusion, can also develop.

The losses of potassium via the gastrointestinal tract, urinary excretion and sweat comprise about 800 mg/d (20 mmol), but 1.6 g/d (40 mmol) is needed to avoid low plasma levels and loss of total body potassium in adults (1). The potassium intake may affect sodium balance and potassium intakes of 10–30 mmol/d may induce sodium retention and an increase in blood pressure, both in normotensive and hypertensive subjects (2–4). In the Intersalt study a 30–45 mmol increase in urinary potassium excretion was associated with a 2–3 mm Hg lower systolic blood pressure (5). An inverse relationship between blood pressure and potassium excretion and K/Na ratio in urine was also observed (6). A number of studies of both normotensive and hypertensive subjects indicate that an increased potassium intake as supplements can lower blood pressure and increase urinary sodium excretion (7–11). However, a clear dose-response effect was not observed, and not all showed a beneficial effect. The lack of clear dose-response observed in the studies could be due to factors such as differences in duration of studies, initial blood pressure, sodium intake, habitual diet, race and age.

Two meta-analyses of randomised trials with potassium supplementation showed a significant reduction of blood pressure (7, 8). In the study by Whelton *et al.* (7) a mean increased potassium excretion of about 60 mmol/d was associated with a mean 4.4 mm Hg decrease in systolic blood pressure and a 2.5 mm Hg decrease in diastolic blood pressure among hypertensives. Corresponding figures for normotensives were 1.8 and 1.0 mm Hg, respectively, although the effects were not significantly different between hypertensives and normotensives. The median duration of the trials was 5 weeks. The blood pressure lowering effect of potassium supplementation was greater in trials with a higher urinary sodium excretion, indicating the close interrelationship between sodium and potassium in this aspect. Using urinary excretion data for potassium, the average intake of potassium in the supplemented groups is estimated at 4.5–5 g/day. In a subsequent meta-analysis including randomised controlled trials with potassium supplementation with a duration of more than two weeks (8), an increased median potassium excretion of 44 mmol/d was associated with a 2.4 mm Hg decrease in systolic and 1.6 mm Hg in diastolic blood pressure. In a randomised controlled 6-week trial, a moderate potassium supplement of 24 mmol/d (900 mg/d) resulted in a decease in systolic blood pressure of 7.6 mm Hg and in diastolic blood pressure of 6.5 mm Hg in healthy volunteers (11). This study indicates that a moderate increase in potassium intake may be sufficient to influence blood pressure. Most of the studies have used KCl supplements. A few studies with a limited number of subjects have investigated the effect of other potassium salts, *e.g.* citrate,

but the results are conflicting with respect to any differential effects on blood pressure (12, 13).

In controlled intervention studies using diets designed to meet recommended levels of *e.g.* fat, fat quality and dietary fibre similar to those in NNR, the dietary potassium intakes (estimated from urinary potassium excretion) have been of the same magnitude, *i.e.* 3–4 g/d (14–16). In these studies blood pressure reductions were observed both with and without changes in sodium intake. However, sodium restriction still decreases blood pressure at the level of 3–4 g/d of potassium intake.

The recommended daily intake in NNR 1996 was based on data on the effect of potassium on blood pressure. Several clinical trials and population surveys published thereafter support the finding that a diet rich in potassium alone, or in combination with calcium and magnesium, may have a favourable effect on blood pressure (5–11 ; 14–17). An inverse association between potassium intake and the risk of stroke has been shown in some cohort studies (18–20). The reference values are kept unchanged compared to NNR 1996, since there are no new scientific data to justify any major changes. The recommended intakes are set at 3.5 g/d (90 mmol) for men and 3.1 g/d (80 mmol) for women. The figure for women also includes pregnant and lactating women. It should be pointed out that potassium intakes somewhat over and above these values might have further beneficial effects. The reference values for children and adolescents are extrapolated from adult values based on needs for growth and adjusted for body weight.

The lower level is estimated to 1.6 g/d (40 mmol) for adults.

Upper intake levels and toxicity

Potassium chloride has been associated with acute poisoning in humans. Case reports have described heart failure, cyanosis and cardiac arrest after ingestion of high doses of potassium chloride tablets. Gastrointestinal effects have also been described after chronic ingestion of potassium chloride in case studies and supplementation studies. This is characterised by abdominal pain, nausea and vomiting, diarrhoea, and ulceration of the oesophagus, stomach and duodenum and ileum. The occurrence and severity of the effects depend on a number of factors of which formulation of the preparation, dose and gut transit time seem to be the most important. Slow release, wax-coated KCl tablets appear to induce more lesions than microencapsulated tablets (21).

Dietary potassium has not been associated with any negative effects in healthy subjects. Prolonged high potassium intakes from diet and potassium-containing salt substitutes may, however, cause hyperkalaemia

and affect heart function in subjects with renal insufficiency or impaired kidney function (21, 22).

The available data are insufficient to set an upper level for dietary potassium. A British expert group proposed an intake of 3.7 g/d from supplements as an upper guidance level for adults. Supplemental intakes up to this level are generally not associated with overt adverse effects, but certain preparations may induce mild lesions of the gastrointestinal mucosa (17). It seems prudent to include potassium from potassium-containing mineral salt in this figure.

Dietary sources and intake

Important potassium sources in the Nordic diets are potatoes, fruit and berries, vegetables, and milk products. The average dietary intake ranges from 3.6 to 4.8 g/10 MJ (see *Chapter 43*).

REFERENCES

1. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community. (Thirty-first series of Food – Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1993.
2. Gallen IW, Rosa RM, Esparaz DY, Young JB, Robertson GL, Batlle D, Epstein FH, Landsberg L. On the mechanism of the effects of potassium restriction on blood pressure and renal sodium retention. Am J Kidney Dis 1998; 31: 19–27.
3. Morris RC Jr, Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 1999; 33: 18–23.
4. Coruzzi P, Brambilla L, Brambilla V, Gualerzi M, Rossi M, Parati G, Di Rienzo M, Tadonio J, Novarini A. Potassium depletion and salt sensitivity in essential hypertension. J Clin Endocrinol Metab 2001; 86: 2857–62.
5. Dyer AR, Elliott P, Shipley M, Stamler R, Stamler J. Body mass index and associations of sodium and potassium with blood pressure in INTERSALT. Hypertension. 1994; 23: 729–36.
6. Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Br Med J 1988; 297: 319–28.
7. Whelton PK, He J, Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. J Am Med Assoc 1997; 277: 1624–1632.
8. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens. 2003; 17: 471–80.
9. Sacks FM, Willett WC, Smith A, Brown LE, Rosner B, Moore TJ. Effect on blood pressure of potassium, calcium, and magnesium in women with low habitual intake. Hypertension. 1998; 31: 131–8.
10. Gu D, He J, Wu X, Duan X, Whelton PK. Effect of potassium supplementation on blood pressure in Chinese:

a randomized, placebo-controlled trial. J Hypertens 2001; 19: 1325–31.
11. Naismith DJ, Braschi A. The effect of low-dose potassium supplementation on blood pressure in apparently healthy volunteers. Br J Nutr. 2003; 90: 53–60.
12. Overlack A, Conrad H, Stumpe KO. The influence of oral potassium citrate/bicarbonate on blood pressure in essential hypertension during unrerstricted salt intake. Klin Wochenschr 1991; 69 (Suppl XXV): 79–83.
13. Overlack A, Maus B, Ruppert M, Lennaz M, Kolloch R, Stumpe KO. Kaliumcitrat *versus* Kaliumchlorid bei essentieller Hypertonie. Dtsch med Wschr 1995; 120: 631–35.
14. Appel LJ, Moore TJ, Obarzanek E, *et al.* A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 1997; 336: 1117–24.
15. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N, Lin PH, Aickin M, Most-Windhauser MM, Moore TJ, Proschan MA, Cutler JA. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. N Engl J Med 2001; 344: 3–10.
16. Jula A, Rönnemaa T, Rastas M, Karvetti RL, Mäki J. Long-term non-pharmacological treatment for mild to moderate hypertension. J Int Med 1990; 227: 413–21.
17. Geleijnse JM, Witteman JC, Hofman A, Grobbee DE. Electrolytes are associated with blood pressure in old age: the Rotterdam Study. J Hum Hypertens 1997; 11: 421–3.
18. He FJ, MacGregor GA. Beneficial effects of potassium. BMJ 2001; 323: 497–501.
19. Fang J, Madhavan S, Alderman MH. Dietary potassium intake and stroke mortality. Stroke 2000; 31: 1532–7.
20. Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L, Whelton PK. Dietary potassium intake and risk of stroke in US men and women: National Health and Nutrition Examination Survey I epidemiologic follow-up study. Stroke 2001; 32: 1473–80.
21. Expert Group on Vitamins and Minerals. Safe Upper Levels for Vitamins and Minerals. Food Standards Agency, May 2003.
22. Doorenbos CJ, Vermeij CG. Danger of salt substitutes that contain potassium in patients with renal failure BMJ 2003; 326: 35–36.

CHAPTER 34

Iron

Iron, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	15/9*	9	8	9	11
Average requirement	AR	10/6*	7			
Lower level of intake	LI	5[1]	7			

*Post menopause.

Worldwide, iron deficiency (ID) is the most common micronutrient deficiency. That is because certain population groups have high iron requirements, but insufficient iron intake or absorption to meet their needs. The relative iron requirement is greatest in infants and young children (aged 6–24 months) and adolescents (aged 12–16 years), which is explained by the rapid growth rate in these age groups. During childbearing years women also have increased needs of iron because of iron losses due to menstrual bleeding and transfer of iron to the foetus during pregnancy. Iron overload can also occur, with people with hereditary haemochromatosis the most likely to be affected. The prevalence of this condition is much higher than previously assumed, with up to 5 per 1,000 individuals of Caucasian origin affected (1).

Physiology and metabolism

Iron is essential to virtually all living organisms. The most important biological characteristic of iron is its ability to alternate between two oxidation states – ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) – thereby accepting or donating one electron. Due to the poor solubility of ferric iron at physiological pH and the ability of ferrous iron to reduce oxygen intermediates to harmful free radicals, all organisms have developed binding molecules (chelators) in order to transport and store iron, and to control its reactivity (2, 3).

Iron has many vital functions in the body. The quantitatively dominating function of iron is to form the oxygen-binding part of haemoglobin, which transports oxygen from lungs to the tissues. Iron is also found in myoglobin, a muscle fibre protein binding oxygen. Iron is an impor-

tant part of many enzymes that transfer oxygen and electrons and many metabolic pathways in for example liver, brain and endocrine organs. It is for example a necessary part of cytochromes, one of a series of enzymes that couples energy to ATP formation during oxidative phosphorylation.

The body can store iron in ferritin and haemosiderin, which are storage proteins in liver, spleen and bone marrow. Minute amounts of ferritin are also found in plasma in iron-free form, and the serum ferritin level (s-ferritin) is considered to reflect the size of the body iron stores.

Absorption and bioavailability

Iron homeostasis is maintained through absorption. Compared to many nutrients, iron is poorly absorbed, and another feature of human iron metabolism is the absence of an excretory pathway. The absorption in the intestine depends on the iron status of the body, the amount and type of iron in the diet and the composition of the meal (4).

In foods, there are two types of iron, haeme iron and nonhaeme iron. Haeme iron constitutes about 10% of total iron in the Nordic diet. It is mainly found in meat, where it accounts for about half the total iron. Iron in grains and other plant-derived foods is nonhaeme iron. Haeme iron is generally more efficiently absorbed than nonhaeme iron and is not subjected to the same regulation mechanisms. Absorption is increased in subjects with iron deficiency compared with normal subjects, *i.e.* it depends on body iron stores (5). Usually about 25% of the total amount of haeme iron is absorbed from food and in general not affected by food components, although reduced bioavailability of haeme iron due to interaction with calcium has been reported (6). The absorption of nonhaeme iron depends on the composition of meals. The absorption is enhanced by ascorbic acid, with the most pronounced effects at moderate intake, or up to 100 mg/d (7), and an unknown factor (MFP factor) found in meat, fish and poultry, which is thought to be cysteine-containing peptides, possibly with more factors involved (8). It is still unclear to what extent organic acids other than ascorbic acid promote absorption. The absorption of nonhaeme iron is inhibited by phytates and its metabolites, iron-binding polyphenols like the tannins, and calcium (6, 9, 10, 11). Manganese in larger amounts than can be obtained from food can compete with iron for absorption in the intestines (12). It appears that zinc does not compete with iron for absorption, at least not in the amounts usually obtained from food (10), but interactions between iron and copper have been documented (13, 14). Divalent cationic metals probably share a common absorption mechanism using

DMT1 (divalent metalionic transporter 1) for transportation across the luminal side of the enterocyte (15).

The effects of enhancing and inhibiting factors on absorption can be seen from studies on different diets. Fruits and vegetables rich in vitamin C and meat can counteract the effect of inhibiting factors (16). Tea and coffee drunk with a meal diminish the absorption of nonhaeme iron because of the iron-binding polyphenols which they contain. Even cocoa diminishes absorption for the same reason, but in addition it contains considerable amount of phytates. Phytic acid is mainly found in unprocessed fibre-rich products. Part of the phytic acid is degraded during the leavening of bread. Low pH, which can be obtained for example with a lasting sourdough leavening or if acetic acid is added to the dough, increases the probability of phytic acid breakdown and iron absorption increases (4, 9, 17). Calcium, however, reduces the breakdown of phytates in dough fermentation and baking (6). Calcium also has a direct inhibiting effect on both haeme and nonhaeme iron absorption, indicating a mucosal rather than luminal effect (6). Measurements from single meals showed that 40 mg calcium did not reduce iron absorption, while one glass of milk (165 mg calcium) caused a 50% reduction in iron absorption. There was a dose-dependent effect up to a consumption of 300 mg calcium in the meal, while higher quantities of calcium did not cause any further reduction in the absorption of iron. Other experiments which evaluated iron absorption from the diet showed that iron absorption was reduced by about 40% when milk was drunk with the main meal, which contained most iron, compared to when water was drunk with this meal (18, 19). Supplemental calcium has also been shown to reduce iron absorption substantially when taken with meals (20).

The influence of enhancing and inhibiting factors on iron absorption appears to be most marked in single meal studies, while studies of whole diets show varying results. Two-week studies comparing iron absorption from a whole diet containing either enhancing or inhibiting factors on absorption found about two times higher iron absorption from the diet with the enhancing factors (21, 22). Algorithms for calculating the absorption of iron have also been developed for *adults* (23, 24). The algorithm of Hallberg and Hulthén (24) predicts the effects of dietary factors known to influence iron status based on their content in consumed foods with consideration taken of interactions between individual factors, *i.e.* phytate, polyphenols, ascorbic acid, meat, fish and seafood, calcium, egg, soy protein and alcohol (24).

Hunt and Roughead found no effect of enhancing and inhibiting components in iron-replete men over time and concluded that their

subjects biologically adapted to a diet of high or low iron bioavailability to homeostatically maintain body iron stores (25). Another study on subjects with normal iron stores showed long-term calcium supplementation with meals having no effect on iron status, while short-term supplementation decreased iron absorption (26), and a study on complete diets for 5 day periods reported no effect from calcium intake or the intake of animal foods and vitamin C on the absorption on non-haeme iron in 14 subjects of normal iron status (27). Cook and Reddy found that the facilitating effect of vitamin C on iron absorption from a complete diet was far less pronounced than that from single meals (28).

Despite varying results from studies on whole diets, subjects with poor iron status seem to benefit from a diet rich in factors enhancing iron absorption.

Iron deficiency and iron deficiency anaemia

Development

The development of iron deficiency (ID) proceeds continuously from normal iron status up to serious iron deficiency anaemia (IDA). Initially body iron stores diminish, which is reflected in decreasing concentration of s-ferritin. When iron can no longer be obtained from stores, iron deficiency in tissues develops. This leads to increasing levels of transferrin and transferrin receptors (TfR), which in turn leads to reduced transferrin saturation and higher concentration of erythrocyte protoporphyrin, often assayed as an increasing serum level of zinc protophyrin (ZPP) because zinc is incorporated into the protoporphyrin molecule in the absence of iron (29). Finally the haemoglobin (Hb) level starts to decrease. If the negative iron balance is not corrected, anaemia develops, defined as a haemoglobin value two standard deviations below the mean of the population. Iron status variables in iron-replete individuals and those with IDA overlap (30,31). Indications of the effect of iron deficiency on the formation of red blood cells, *i.e.* reduced mean cell volume (MCV) can be seen before stores are completely emptied (31–33).

Symptoms

ID can lead to various symptoms. Serious consequences of ID are anaemia (IDA), reduced work capacity and impaired cell-mediated immunological defence. Altered temperature regulation has also been noted in connection with ID (34, 35). Of particular concern is the suggestion that severe ID seems to affect children's mental development and cognitive functions, probably only when severe enough to result in anaemia

(IDA). These effects may even be irreversible, depending on the age of the child, the severity and duration of the deficiency and the socioeconomic environment (29, 36–40).

Assessment and indicators of iron status

Several indicators are used for detection of iron deficiency. S-ferritin is considered to be the best single indicator of iron status and is also the most widely used (41). It gives a good reflection of the size of the iron stores in the absence of infection and inflammation. WHO recommends 12 µg/L as the cut-off for children below the age of 5 years and 15 µg/L for males and females above 5 years (42). These cut-offs are built on global criteria that also include other races and countries, including developing countries (see *Table 34.1*). Lower s-ferritin values have been used for infants and young children, *e.g.* 10 µg/L in the Euro-Growth study (43). This reference value was also used for US children up to 5 years in a nation-wide study (44). Scientists from different countries have given < 12 µg/L as the cut-off for s-ferritin in adolescence (44–50). Other indicators of iron status are s-transferrin (total iron binding capacity, TIBC), transferrin saturation (s-iron/TIBC) and s-TfR (serum transferrin receptor). While the transferrin saturation is less useful for detecting ID due to diurnal variations in s-iron, s-TfR is considered the single most sensitive indicator of functional iron depletion (51). The transferrin saturation is, however, very useful as a screening variable for hereditary haemochromatosis (42). In recent years s-Tfr/s-ferritin has also been used as an indicator of iron status in scientific studies (45, 46). Free erythrocyte protoporphyrin (or ZPP) and MCV (mean cell volume) become abnormal relatively late in the development of ID (51), so alone they would be relatively insensitive indicators of iron deficiency and are not often used in studies on iron status.

Anaemia is defined as a reduced concentration of Hb. According to WHO, Hb < 110g/L should be used to diagnose anaemia in infants and children from 6 months up to 5 years of age, and 115 g/L for children up to 11 years (42). Higher values are recommended for children aged 12–14 years and women (120 g/L) and men (130 g/L), while during pregnancy the cut-off is lowered to 110 g/L. However, reference values for Hb (and also for other iron status variables) are poorly validated in infants and young children. In clinical practice as well as in research, the commonly used cut-off levels to identify ID and IDA in infants (Hb < 110 g/L and s-ferritin < 10–12 µg/L) are in fact extrapolated from older age groups and there are indications that they may not be appropriate (52). Emond *et al.* suggested for 8-month-old infants a cut-off of 97 g/L for Hb (53). Others have used 105 g/L (54, 55) and 100 g/L (52, 56).

IDA is defined as anaemia by Hb below given cut-off, along with abnormal iron status indicators. The number of iron status indicators used for diagnosis varies, as well as their cut-offs used for iron deficiency. Sometimes only s-ferritin is used, but some studies have used the approach that when two out of three iron status indicators are below (or above) a given cut-off together with a Hb below cut-off, the individual is diagnosed with IDA.

Table 34.1 Cut-off values for different iron status indicators

Indicator	Lower or upper limit	Adults		Children and adolescents	Infants aged 4/6/9 months[c]
		Males	Females		
Haemoglobin (Hb)	g/L	< 130[a]	< 120[a]	< 105[b] < 110[a]	< 105/105/100 < 105[d]
Serum ferritin	µg/L	< 15[a]	< 15[a]	< 12[a, i, j]	< 20/9/5
MCV	fL	< 80[c, e, f]	< 80[e, f]	< 74[g]	< 73/71/71
Serum transferrin receptors (TfR)	mg/L	> 8.5[f, h]	> 8.5[f, h]	> 8.5[f, h]	> 11/11/11
Free erythrocyte protoporphyrin	µg/dL erythrocyte	> 70[e]	> 70[e]	> 80[e]	
Transferrin saturation	%	< 16[e]	< 16[e]	< 12[e]	
TIBC	µg/dL	> 400[e, f]	> 400[e, f]		
ZPP	µmol/mol				>75/75/90

a WHO (42)
b Thorsdottir *et al.* (57)
c Domellof *et al.* (52)
d Michaelsen *et al.* for 9-month-olds (54)
e Expert Scientific Working Group (58)
f The US recommendations (7)
g Gill *et al.* (59)
h Baynes (51)
i Samuelson *et al.* (46)
j Samuelson *et al.* (45)

Prevalence of iron deficiency and iron deficiency anaemia

In a Norwegian study on one-year-old children (60), 7% had anaemia, defined as haemoglobin < 110 g/L, but iron status was not evaluated. In a Danish study, 5% of nine-month-old infants had anaemia (defined as Hb < 105 g/L), while 20% had Hb < 110 g/L, but no cases of IDA were found (54). Of Swedish 1-year-old infants, 8% had haemoglobin < 105 g/L and 13% haemoglobin < 110 g/L, and 26% were iron depleted (s-ferritin < 12 mg/L) (61). In a more recent study from the same area of Sweden, the figures for that age group using the same cut-offs (Hb < 110 g/L and s-ferritin < 12 mg/L) were anaemia 15% and ID 18%, respectively (62). In Icelandic 1-year-olds the proportion below the same cut-off for s-ferritin was higher, 45%. In that study 9% were diagnosed as anaemic (Hb

< 105 g/L) and 3% as having IDA (57). The iron status is improved at the age of two years, but at one and two years of age poorer iron status was related to faster growth from birth (57, 63). In Finland the prevalence of children aged 3–4 years with Hb < 110 g/L was 4% (64). The prevalence of ID among adolescents and adults varies between the Nordic countries. Studies on 15–16 year-old Swedish teenagers revealed that 40% of girls and 15% of boys were iron deficient (33). However, in two other studies in Sweden, the prevalence of ID proved to be lower among 15-year-olds, 5% and 4% among boys, and 15% and 14% among girls (65, 66). In a Swedish longitudinal study from 15 to 21 years of age, serum ferritin concentrations < 12 mg/L were found in 3%, 2% and 2% of males aged 15, 17 and 21 years respectively and in 18%, 26% and 21% of females aged 15, 17 and 21 years respectively (45). Bergström *et al.* have pointed out the important association between prevalence figures and diagnosis criteria used (65). In Denmark the prevalence of exhausted iron stores was 13% in adolescent boys and 18% in girls (67), but in Norwegian studies, ID was found in 5–18% and 6–30% of 13–15-year-old Norwegian boys and girls, respectively (68). Danish, Norwegian, Icelandic and Finnish studies have shown ID in 10–22% of women of childbearing age (69–74). A better founded diagnostic criterion for ID is needed for older children and adults, as well as infants. The sTfR/s-ferritin ratio might be useful for the purpose of increasing the diagnostic accuracy (45).

Not many studies have been conducted on iron status in elderly populations in the Nordic countries. In Finland the prevalence of low Hb (< 122 g/L) was 0–5% among elderly (> 65 years) men and 1–2% among women depending on differences in living conditions (75). A Danish study revealed that ID was relatively uncommon in 70-year-olds (2.4% among men and 3.3% among women). Men had higher s-ferritin values than women and high iron stores (s-ferritin > 300 mg/L) were more common in men than women, 8.7% *vs.* 3.7% (76). ID was even more uncommon in 'healthy' 80 and 85-year-old Danes (77, 78). These results are similar to those of the Framingham Heart Study, which found that an elderly white US cohort had a low prevalence of ID and IDA, but high prevalence of elevated iron stores (12.9%) using the criterion s-ferritin > 300 mg/L for men and > 200 mg/L for women (79).

Requirement and recommended intakes

Recommended daily intakes of iron for adult men, post-menopausal women and children are based on the amounts needed to cover basic losses and growth for approximately 95% of individuals in each age group. Women's need for iron during childbearing years is not normally

distributed, which makes decision-making on recommended intakes more difficult.

Vegetarians and athletes, especially female athletes, are two groups that may deserve special attention with respect to their iron needs. In the US recommendations, special considerations include recommendations for these and some other groups (7).

Children and adolescents

Children and adolescents need iron to cover basic losses and for growth.

Full-term newborn infants have iron stores sufficient to cover their needs during the first 4–6 months of life. The concentration of iron in human milk is low and similar to that in cow's milk, or 0.3–0.4 mg/L. This is to some extent counteracted by a high bioavailability from human milk, although the previous estimate of 50% absorption from human milk may be an overestimation (80). In the recent US text on dietary reference intakes, iron absorption from human milk is still considered to be quite high, *i.e.* 45–100% (7). The absorption from cow's milk is much lower. Healthy term, exclusively breast fed infants in a developed country like Sweden do not develop ID during the first 6 months of life (80) and are therefore in no need of extra iron during that period, even though their body weight doubles. It has been shown that the same is true for healthy term infants fed exclusively an infant formula with only 1.6 mg iron/L (81). Hence, no recommendation is given for the first 6 months[1]. There is evidence to suggest that during the first six months, infants may not down-regulate their absorption of iron as efficiently as children and adults (80). Therefore, excessive iron intake should be avoided particularly in that age group. After the first 6 months of life the requirement for exogenous iron is high and the infant now becomes dependent on iron-rich complementary feeds. Cow's milk consumption of infants and toddlers can influence iron status, especially when consumed in high quantities. In Iceland, where cow's milk has been a common substitute for breast milk after six months of age, consumption of cow's milk above half a litre per day was associated with worse iron status (57). In the recently published US recommendations the RDA for infants aged 7–12 months is 11 mg/d, but 7 mg/d in children aged 1–3 years (7). The basic iron requirements are similar for these age groups but the absorption is considered to be

1. Infants who are not breast fed during the first 6 months should be fed an iron-fortified infant formula. Infant formulas that are not fortified with iron should not be used.

different because the infants consume more cereals with low iron bioavailability, while the older children consume more haeme iron. The recommendations are higher (10 mg/d) for children aged 4–8 years. The NNR 1996 for the ages 6 months to 5 years was 8 mg/d, which is not changed in NNR 2004. This is supported by the recent observation that infants consuming on average 9 mg iron per day during the end of infancy, with most of the iron provided by iron-fortified phytate-rich cereals, do not develop ID (62). Moreover, a higher recommendation would require a diet unrealistically dense in iron for that age group, much denser than for older children and adults. For older children, 6–9 years, the recommendation 2004 is set to 9 mg/day.

The need for iron is relatively high in adolescence (see *Table 34.2*) since it is a period of rapid growth. For 10–13 year-old boys, an iron intake of 9 mg meets the requirements of 95%, given 15% absorption, and for 14–17-year-old boys about 12 mg are needed to meet those requirements. For boys aged 14–17 years, the recommended intake is set to 11 mg/day. Changes in recommended values for adolescent boys from earlier recommendations are based on more correct body weight values for the Nordic population and therefore new values for basal losses.

In addition to their requirement for growth and basal losses, adolescent girls need iron to cover losses during menstruation. The total iron need for menstruating girls and women is estimated by the sum of basal losses, need for growth (when appropriate) and menstrual losses (see *Table 34.2*). The variation in menstrual losses is used to estimate the 90th and 95th percentiles of total need, with basal losses and need for growth constant assuming high menstruation loss to be accompanied by homeostatic regulation of iron, basal losses and absorption.

Using Nordic body weight values and menstrual blood losses for 15-year-old girls (84), 10 mg iron per day is required to satisfy the iron need of half of all adolescent girls (see *Table 34.2*). To satisfy the need of 95% of adolescent girls, 19 mg/d is required when assuming that the absorption is 15%. The iron need of 95% of 10–13 year-old pre-menarchal girls is met at a lower level or 9 mg/day. The iron need of about 90% of post-menarchal girls aged 10–17 years is satisfied by 15 mg/day. Recommended intake is set to 15 mg/d for 14–17 year-old girls and 11 mg/d for 10–13 year-old girls. The 90th percentile of need represents the recommended intake, and is justified by the fact that those in the top 5th centile of iron need probably have a higher absorption rate than 15%, and also by the fact that it is difficult to obtain much more iron in a typical Nordic diet. However, there will always be some small proportion of girls and women with higher iron needs, which must be satisfied with iron supplements.

Women of childbearing age

Daily iron requirement is high among women during childbearing years, because of blood loss during menstruation. This loss is very variable between women, but the variation is less among adolescent girls than among adult women (83). The amount of menstrual blood is relatively constant for every woman, but is diminished by contraceptive pills while contraceptive sponges enhance menstrual blood loss (83). The daily iron amount required to meet the need of 50% and 95% of adult women is 9 mg/d and 19 mg/d, respectively, given 15% absorption (see *Table 34.2*). The iron need for about 90% of women is satisfied by 15 mg/day. Recommended daily iron intake is set to 15 mg/d for women of childbearing age.

It should be emphasised that the calculations assume that blood losses in menstruation are the same as in the 1960s, although it is known that today oral contraceptive use is higher with less blood loss. By the same criteria discussed for menstruating girls, the 90th percentile is chosen to represent the recommended intake. As discussed earlier in this chapter under 'Absorption and bioavailability', the composition of meals can affect the absorption of iron, especially in iron-deficient individuals. Including foods with factors enhancing iron absorption is therefore important for many women of childbearing age. The composition of a typical Swedish diet, according to Hallberg *et al.* 1991 (83), gives about 14% iron absorption, while a regular French diet in the 1980s, which includes more meat and less phytates, gives 18% iron absorption (86). It is difficult to put together a diet that contains enough iron to meet the needs of almost all women if the recommendations about the proportions of the energy-providing nutrients and fibre are to be maintained and iron-fortified products are not used. It is therefore important to improve the bioavailability of the iron consumed by evaluating the meal composition.

Pregnancy and lactation

Maternal iron need during pregnancy increases slowly as the pregnancy progresses, because of growth and maintenance of the foetus, uterus, increase in red blood cell count and expected iron losses when giving birth. According to Hallberg the total iron requirement is 1,040 mg during pregnancy, of which 840 mg goes to the foetus or is lost while giving birth (87). During the first trimester of pregnancy iron intake must cover basal losses. Iron demand increases during the second trimester and is greatest during the third trimester and can be 10 mg/d in the last six weeks. Iron absorption increases during this period (88). However,

Table 34.2 Iron requirements of adolescents and adults

Age years	Weight kg	Need for growth mg/d	Basal losses mg/d[1]	Menstrual losses mg/d[2]			Total iron need mg/d			Necessary intake of iron from foods to cover 50%, 90% and 95% of iron need of groups on a diet for which iron absorption is assumed to be 15%		
	mean		median	median	90%	95%	median	90%	95%	50%	90%	95%
Girls												
10–13[h]	38.5[e]	0.55	0.54				1.09		1.36	7.3		9.1
10–13	38.5[e]	0.55	0.54	0.46[c]	1.05	1.69[d]	1.55	2.14[g]	2.78[g]	10.3	14.3	18.5
14–17	53.5[e]	0.30	0.75	0.46[c]	1.05	1.69[d]	1.51	2.1[g]	2.74[g]	10.1	14	18.3
Women												
18+	62[f]		0.87	0.48[c]	1.35	1.90	1.35[b]	2.22[g]	2.77[g]	9	14.8	18.5
Women after menopause	62[f]		0.87				0.87		1.13	5.8		7.5
Boys												
10–13	37.5[e]	0.55	0.53				1.08		1.35	7.2		9.0
14–17	57[e]	0.60	0.80				1.40		1.75	9.3		11.7
Men												
18+	76[f]		1.05				1.05		1.37	7		9.1

a Basal losses are estimated to be 0.014 mg/kg/d (82).

b Evaluated from the amount of menstrual blood, ml/28 days (83,84). For girls median and 90% are based on reference 68 and 95% on reference 6. Menstrual losses for girls are assumed to be the same in both age groups. Haemoglobin concentration is calculated as 135g/L and it is assumed that 1g of haemoglobin contains 3.34 mg iron. Menstrual iron loss (mg) = [blood loss (ml)/28 days* 135 g Hb/L* 3.34 mg iron/g Hb / (1,000 ml/L)]

c Calculated with the median blood loss of 28.4 ml for adolescent girls and 30 ml for adult women, per 28 days (83).

d Calculated from equation in US recommendations derived from a fitted log normal distribution with a Monte Carlo simulation [ln (blood loss) = 3.3183+ 0.6662(SD)] (7).

e Children weights 1973–77 (85).

f Reference weight based on KOST95. Mean weight of men and women aged 15–80 years.

g Sum of basal losses, need for growth and 90th and 95th percentiles of menstrual losses, respectively. It is assumed that there is no distribution in values for basal losses and need for growth.

h Not menstruating

even though iron absorption increases during the last two thirds of pregnancy, for some women the amount of iron in foods is not enough to satisfy the greatly increased iron demand that takes place during pregnancy. Appropriate iron balance during pregnancy presumes that

iron stores possesses about 500 mg of iron in the beginning of pregnancy. Recommendations about the intake of iron as a supplement during pregnancy are based on measurements made in Norway on ferritin level in serum (89). When planning diets for pregnant women, the same recommendation is used as for women who are not pregnant. However, the physiological need of some women for iron cannot be satisfied during the last two thirds of pregnancy with foods only and supplemental iron is needed. NNR 2004, in accordance with the recommendations of SCF (90), do not give recommendations for dietary iron during pregnancy. The US recommendation for pregnancy is 27 mg/d (7).

During the first months of lactation, menstruation has often not yet resumed and therefore the need for iron during lactation is less than usual (91), which is the basis for the lower US recommendations for lactating women compared to others of the same age (7). Their median iron needs are assumed to be the sum of basal losses and iron secretion in the human milk, with no menstrual losses. Many women in the northern countries breastfeed their infants for quite a long time, and menstrual losses start within the breastfeeding period. Recommendations for lactating women are the same as for women of childbearing age who are not pregnant, or 15 mg/day.

Post-menopausal women and adult men

Older women and adult men have to cover basal losses of iron. Minimum iron intake has not changed since the NNR 1989 and varies from 5 or 7 mg/d, depending on body size. Median need is set to 6 mg/d for post-menopausal women and 7 mg/d for adult men. Recommended intake of iron is 9 set to mg/d for both post-menopausal women and adult men. The US recommendation is 8 mg/d for both groups (7).

Upper intake levels and toxicity

Under physiological conditions iron status is almost exclusively regulated by adaptation of intestinal iron absorption to demand, which is well described for deficiency and supply via food. Several studies seem to indicate that this regulation also operates up to the level of an additional 10–15 mg Fe/d (7, 22, 92–96). However, Fleming and co-workers found that an additional iron intake of > 30 mg Fe/d was associated with an increased risk of high iron stores, defined as plasma-ferritin > 300 and 200 µg/L in men and women, respectively (97). Thus, the homeostatic regulation of iron absorption seems able to prevent iron overload at a total iron intake of 17.5–25 mg Fe/d (10 mg/d habitual dietary intake +7.5–15 mg/d), but not at a total intake of 40 mg Fe/d (10 mg/habitual

dietary intake +30 mg/d), particularly not in the elderly. Theoretical calculations of prolonged intake of pharmaceutical iron and serum ferritin levels showed that ingestion of an extra 60 mg Fe/d during 5 years or more would risk building up excessive iron stores in a fertile woman of 63 kg (98).

Acute effects of iron overload in humans

Ingestion of an acute overdose of pharmaceutical iron preparations causes mucosal erosion in the stomach and intestine. Young children are especially at risk (99). Due to damage to the intestinal mucosa noncontrolled iron absorption can be high and cause acute systemic symptoms such as shock due to vascular dilatation, capillary leakage and heart failure. A high iron concentration in the liver causes hepatocellular necrosis including hepatic failure and bleeding disorders (100). Other organs such as the pancreas, kidney, CNS and red blood cells may also be damaged (99). A dose of 180–300 mg Fe/kg bw may be lethal, whereas oral doses below 10–20 mg Fe/kg bw do not seem to induce systemic toxicity (101).

Nausea, vomiting, heartburn and epigastric discomfort, along with constipation and occasionally diarrhoea, are common side effects of oral therapeutic doses of iron (102–106). The mechanisms are mucosal irritation, alteration of gastrointestinal motility and rapid transfer of iron to the circulation. The occurrence of side effects of therapeutic iron is dependent on the dose and luminal iron concentration. The lower dose level associated with such acute effects seems to be 50–60 mg Fe/d (102, 104).

Chronic effects of iron in humans

In chronic systemic iron overload – haemochromatosis – iron is mainly accumulated and stored in the liver. Haemochromatosis is either primary or secondary. Primary haemochromatosis is a hereditary disease causing an increased iron absorption (2–3 times in homozygotes) due to a genetic defect in the HFE gene. Three mutations have been described and the two most common, C282Y and H63D, have prevalences in homozygotes of about 0.4 and 2% in Norway and Sweden respectively (107, 108). Heterozygotes for the mutations are about 15 and 24% and compound heterozygotes about 7%. They have slightly increased iron absorption (109). Homozygosity for the C282Y mutation is most often associated with clinical signs of haemochromatosis and risk of developing serious symptoms even at the iron levels normally present in the diet. These are hepatomegaly, hepatic fibrosis and hepatoma in addition to joint inflammation, diabetes mellitus, cardiomyopathy and car-

diac failure. The treatment is phlebotomy. If untreated the risk of symptoms in males and females is 5:1, which is due to a constantly higher loss of iron among females via menstrual bleeding. The penetrance of C282Y mutation, which is the most frequent one leading to haemochromatosis, has been estimated to be between 1 and 25%; it depends on the study design and endpoints used (110, 111).

A threshold of 22.3 mg Fe/g dry weight (corresponding to 2,100 µmol/g protein) for hepatic fibrosis has been proposed (112, 113). However, in two recent studies moderate and slight liver fibrosis was seen in several cases with hepatic iron concentrations from 51 to 240 µmol/g dry weight (113, 114). Hepatic fibrosis and iron concentration have also been correlated to s-ferritin. In the study by Bell and co-workers a dose response curve for severity of fibrosis and s-ferritin was found (114). S-ferritin was mostly above 1,000 µg/l for those with liver fibrosis. The median s-ferritin for the mildest form of fibrosis was 858 µg/L with values down to 520 µg/L. Åsberg and co-workers found a clear correlation between hepatic iron and s-ferritin with quite a wide distribution (113). To keep hepatic iron below 400 µmol/g dry weight, the limit for s-ferritin would be about 250 µg/L. In this study, 4 of 12 patients with moderate liver fibrosis had s-ferritin below 1,000 µg/L, ranging from 311 to 629 µg/L.

In Bantu subjects, siderosis due to excess oral iron intake of 50–100 mg Fe/d from home-brewed beer fermented in iron drums in sub-Saharan Africa was found. Regular consumption leads to hepatic cirrhosis (with a threshold in liver of 5 mg Fe/g wet weight, close to that for primary haemochromatosis) and diabetes (112, 115–117). Due to the possibility of confounding, these studies are not suitable for deriving an UL for iron (118–120).

Secondary haemochromatosis has been reported in patients who have ingested pharmaceutical iron over more than a decade at doses of 160–1,200 mg/d (114, 121–124). Homo- and heterozygotes for haemochromatosis were not excluded in the early studies, but there was no mutation in the HFE gene or close relatives affected in the more recent reports.

Iron and risk of cardiovascular disease

In a considerable number of studies the relationship between iron overload and cardiovascular disease has been examined. However, meta-analysis of studies performed between 1994 and 1997 argued against a relationship between iron stores and cardiovascular disease (125). In these studies the control of confounders such as liver disease, inflammation and cancer was considered to be insufficient (126, 127). However, some more recent prospective studies did support a relationship (128,

129), whereas others gave more unclear results (130, 131). Increased risk was found in heterozygotes for haemochromatosis (132). Although some of the later studies seem more reliable and indicate a relationship (128), an absolute causal relationship between iron and risk of cardiovascular disease cannot be established nor can any quantitative relationship between iron intake and risk of cardiovascular disease be established.

Iron and risk of cancer and other diseases

Homozygotes of haemochromatosis have an increased risk of hepatocellular carcinoma (133). This is in agreement with results from dietary iron overload in black Africans (134). Regarding extra-hepatic malignancies in the general population there have been some studies indicating an increased risk of colon cancer and also other cancers related to high iron stores (135–138). An increased risk of colorectal cancer was also reported for heterozygotes of haemochromatosis (139). However, a causal relationship between iron and extra-hepatic cancer cannot be established.

Jiang and co-workers showed that high iron stores as expressed by elevated s-ferritin and a lower ratio of transferrin receptors to ferritin were associated with an increased risk of diabetes type 2 in healthy women independent of known diabetes risk factors (140). Adjustment for inflammation and other potential confounders did not change the association. In another study by Jiang and co-workers iron intake and blood donations in relation to risk of type 2 diabetes in men was studied (141). Haeme iron from meat was positively associated with diabetes type 2 whereas total iron intake, haeme iron intake from other sources and blood donations were not related to diabetes type 2.

Upper level of iron intake

Epidemiological data on the risk of cancer or cardiovascular disease and iron do not permit any dose-response relationships with dietary iron. Consequently, a quantitative UL for iron intake cannot be set on this basis, nor is it possible to directly derive a limit based on liver fibrosis and increased hepatic iron and s-ferritin. However, it seems clear that an s-ferritin level above 300 µg/L, which is often referred to as 'biochemical iron overload', when caused by increased iron stores, is associated with an increased risk of slight liver fibrosis.

Based on homeostatic control of iron absorption and risk of 'biochemical iron overload', this seems to occur at intake levels between 10 and 30 mg per day of additional Fe over and above typical dietary intakes. A regular intake of 60 mg Fe/d in a fertile woman has been calculated to lead to 'biochemical iron overload'. A quantitative UL for iron

intake additional to habitual dietary iron is set to 10 mg (non-haeme) Fe/d in order to protect against 'biochemical iron overload'.

Although it is not possible to establish a cause-effect relationship between iron and cardiovascular disease, it seems prudent at least in subpopulations such as adult males, post-menopausal women and heterozygotes of haemochromatosis to avoid intake of iron above the current recommendation, which provides for the higher need.

The UL would also protect against local intestinal toxicity manifested by side effects of therapeutic iron, the lower dose level associated with such acute effects seems to be 50–60 mg Fe/day.

The UL and advice do not apply to individuals receiving iron prophylaxis and pharmaceutical iron preparations under medical supervision.

Dietary sources and intake

Recent dietary surveys in the Nordic countries show that iron intake among adult men and women is 10–15 mg/d on average, using information from Norway and Denmark and with iron fortified products where found included in those calculations. The intake is considerably lower among women than men. Average intake in Iceland is 10.3 mg/d among women and 13 mg/d among men. The intake is slightly higher among adolescents aged 15–19 years, 11.7 mg/d among girls and 13.7 mg/d among boys (142). In Finland the figures for adults are 13.2 mg/d for men and 10.0 mg/d for women, after the fortification of wheat flour was ceased (143). The figures for Swedish adolescents are 14 mg/d for boys and 9 mg/d for girls compared to 12.3 and 10.4 mg/d for adult men and women respectively (144).

The majority of iron in the Nordic diet comes from cereal products, some of which, such as breakfast cereals, are iron-fortified. However, the bioavailability of that iron seems to be low and today fortification of flour in Finland, Sweden and Denmark has ceased. Lower proportions of iron have come from iron-rich foods such as variety meats and lean meat.

To ensure a higher absorption of iron in the diet, people with high iron needs, such as adolescent girls and women of childbearing age, can increase the bioavailability of iron by several means. For these vulnerable groups, consumption of foods inhibiting absorption should be limited or avoided with main meals containing iron, and if possible foods with enhancing factors, such as vitamin C, included. This could for example be fresh vegetables or vegetable salad, fresh fruits and berries or juice. Meat, fish and poultry contain the MFP-factor, which also enhances iron absorption in the meal. In main meals, foods containing substances that inhibit iron absorption, such as cow's milk and other

dairy products containing calcium, should be consumed in moderation. However, cow's milk is an important staple food and should not be eliminated from the general diet. Finally, tea, coffee and chocolate products contain iron-binding polyphenols that inhibit absorption and should be consumed in moderation and avoided with main meals.

REFERENCES

1. Schumann K. Safety aspects of iron in foods. Ann Nutr Metab 2001; 45: 91–101.
2. Bothwell TH. Contributions of the Brisbane Liver Group to knowledge of iron metabolism. J Gastroenterol Hepatol. 1996; 11: 1025–7.
3. Domellöf M. Iron requirements of term, breast fed infants: a study in Sweden and Honduras. Umeå University medical dissertation New series No759 ISSN 0346–6612, 2001.
4. Bothwell TH, Baynes RD, MacFarlane BJ, MacPhail AP. Nutritional requirements and food iron absorption. J Intern Med 1989; 226: 357–65.
5. Lynch SR, Skikne BS, Cook JD. Food iron absorption in idiopathic hemochromatosis. Blood. 1989; 74: 2187–93.
6. Hallberg L, Brune M, Erlandsson M, Sandberg A-S, Rossander-Hultén L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am J Clin Nutr 1991; 53: 112–9.
7. Institute of Medicine. Dietary Reference Intakes for vitamin A,vitamin K, arsenic, boron, chromium, copper, iodine, iron, molybdenum, nickel, silicon, vanadium and zinc. Food and Nutrition Board. Washington, DC: National Academy Press; 2001.
8. Engelmann MD, Davidsson L, Sandstrom B, Walczyk T, Hurrell RF Michaelsen KF. The influence of meat on nonheme iron absorption in infants. Pediatr Res 1998; 43: 768–73.
9. Brune M, Rossander-Hultén L, Hallberg L, Gleerup A, Sandberg A-S. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J Nutr 1992; 122: 442–9.
10. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: importance of different phenolic structures. Eur J Clin Nutr 1989; 43: 547–8.
11. Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 1989; 49: 140–4.
12. Rossander-Hultén L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr 1991; 54: 152–6.
13. Lonnerdal B, Kelleher SL, Lien EL. Extent of thermal processing of infant formula affects copper status in infant rhesus monkeys. Am J Clin Nutr 2001; 73: 914–9.
14. Lonnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast fed infants and infants fed trace element fortified milk-based infant formula. Acta Pædiatr 1994; 83: 367–73.
15. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF *et al.* Cloning and characterization of a mammalian proton-coupled metalion transporter. Nature. 1997; 388: 482–8.
16. Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG *et al.* Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 1991; 53: 537–41.

17. Sandberg A-S. Antinutrient effects of phytate. Nutrition 1994; 18: 429–32.
18. Gleerup A, Rossander-Hulthén L, Gramatkowski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr 1995; 61: 97–104.
19. Gleerup A, Rossander-Hulthén L, Hallberg L. Duration of the inhibitory effect of calcium on non-haem iron absorption in man. Eur J Clin Nutr 1993; 47: 875–9.
20. Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. Am J Clin Nutr 1991; 53: 106–11.
21. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991; 54: 717–22.
22. Hultén L, Gramatkovski E, Gleerup A, Hallberg L. Iron absorption from whole diet. Relation to meal composition, iron requirements and iron status. Eur J Clin Nutr 1995; 49: 794–808.
23. Reddy M, Hurrell RF, Cook JD. Estimation of nonheme-iron bioavailability from meal composition. Am J Clin Nutr 2000; 71: 937–43.
24. Hallberg L, Hulthén L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am J Clin Nutr 2000; 71: 1147–60.
25. Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low bioavailability, Am J Clin Nutr 2000; 71: 94–102.
26. Minihane AM, Fairweather-Tait SJ. Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. Am J Clin Nutr 1998; 68: 96–102.
27. Reddy M, Cook JD. Effect of calcium intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 1997; 65: 1820–5.
28. Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr. 2001; 73: 93–8.
29. Aggett PJ, Agostoni C, Axelsson I, Bresson JL, Goulet O *et al*. Iron metabolism and requirements in early childhood: do we know enough? A commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 2002; 34: 337–45.
30. Hallberg L, Hulthén L, Bengtsson C, Lapidus L, Lindstedt G. Iron balance in menstruating women. Eur J Clin Nutr 1995; 49: 200–7.
31. Domellöf M, Hernell O. Iron-deficiency anaemia during the first two years of life. Scand J Nutr 2002; 46: 20–30.
32. Hallberg L, Bengtsson C, Lapidus L, Lundberg P-A, Hulthén L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. Brit J Haematol 1993; 85: 787–98.
33. Hallberg L, Hulthén L, Lindsted G, Lundberg PA, Mark A, Purens J, *et al.* Prevalence of iron deficiency in Swedish adolescents. Pediatric Research 1993; 34: 680–7.
34. Hercberg S, Galan P. Nutritional anaemias. Baillieres Clin Haematol 1992; 5: 143–68.
35. Hallberg L, Sandstrom B, Aggett PJ. Iron, zinc and other trace elements. In: Garrow JS, James WPT, Ralph A, eds. Human Nutrition and Dietetics. 10th ed. London: Churchill Livingstone, 2000: 174–207.
36. Lozoff B. Behavioural alterations in iron deficiency. Adv Pediatr 1988; 35: 331–59.
37. Walter T, De Andraca I, Chadud P, Perales CG. Iron deficiency anaemia: Adverse effects on infant psychomotor development. Pediatrics 1989; 84: 7–17.
38. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics 2000; 105: E51.
39. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993; 341: 1–4.
40. Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases

weight gain and psychomotor development. Arch Dis Child 1986; 61: 849–57.
41. Hulthén L, Lindstedt G, Lundberg PA, Hallberg G. Effect of a mild infection on serum ferritin concentration – clinical and epidemiological implications. Eur J Clin Nutr 1998; 52: 376–9.
42. WHO. Iron deficiency anaemia. Assessment, prevention and control. A guide for programme managers. WHO/NHD/01.3, Geneva: WHO, 2001.
43. Male C, Persson LA, Freeman V, Guerra A, van't Hof MA, Haschke F. Prevalence of iron deficiency in 12-mo-old infants from 11 European areas and influence of dietary factors on iron status (Euro-Growth study). Acta Paediatr 2001; 90: 492–8.
44. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. J Am Med Assoc 1997; 277: 973–6.
45. Samuelson G, Lonnerdal B, Kempe B, Elverby JE, Bratteby LE. Serum ferritin and transferrin receptor concentrations during the transition from adolescence to adulthood in a healthy Swedish population. Acta Paediatr. 2003; 92: 5–11.
46. Samuelson G, Lonnerdal B, Kempe B, Elverby JE, Bratteby LE. A follow-up study of serum ferritin and transferrin receptor concentrations in Swedish adolescents at age 17 age 15. Acta Paediatr. 2000; 89: 1162–8.
47. Anttila R, Cook JD, Siimes MA. Body iron stores decrease in boys during pubertal development: the transferrin receptor-ferritin ratio as an indicator of iron status. Pediatr Res. 1997; 41: 224–8.
48. Samuelson G, Bratteby LE, Berggren K, Elverby JE, Kempe B. Dietary iron intake and iron status in adolescents. Acta Paediatr. 1996; 85: 1033–8.
49. Bergstrom E, Hernell O, Lonnerdal B, Persson LA. Sex differences in iron stores of adolescents: what is normal? J Pediatr Gastroenterol Nutr. 1995; 20: 215–24.
50. Olsson KS, Marsell R, Ritter B, Olander B, Akerblom A, Ostergard H, Larsson O. Iron deficiency and iron overload in Swedish male adolescents. J Intern Med. 1995; 237: 187–94.
51. Baynes RD. Assessment of iron status. Clin Biochem 1996; 29: 209–15.
52. Domellof M, Dewey KG, Lonnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. J Nutr. 2002; 132: 3680–6
53. Emond AM, Hawkins N, Pennock C *et al.* Haemoglobin and ferritin concentrations in infants at 8 months of age. Arch Dis Child 1996; 74: 36–9.
54. Michaelsen KF, Milman N, Samuelson G. Iron status and dietary iron intake in a longitudinal study of Danish infants. Acta Paediatr 1995; 84: 1035–44.
55. Siimes MA, Salmenperä L, Perheentupa J. Exclusive breastfeeding for 9 months: risk of iron deficiency. J Pediatr 1984; 104: 196–9.
56. Sherriff A, Emond A, Hawkins N, Golding J. Haemoglobin and ferritin concentrations in children aged 12 and 18 months. ALSPAC Children in Focus Study Team. Arch Dis Child 1999; 80: 153–7.
57. Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G. Iron status at 12 months of age – effects of body size, growth and diet in a population with high birth weight. Eur J Clin Nutr 2003; 57: 505–13.
58. Expert Scientific Working Group. Summary of a report on assessment of the iron nutritional status of the United States population. Am J Clin Nutr 1985; 42: 1318–30.
59. Gill DG, Vincent S, Segal DS. Follow-on formula in the prevention of iron deficiency: a multicentre study. Acta Paediatr 1997; 86: 683–9.
60. Bærug A, Kjærnes U. Får norske spedbarn for lite jern i kosten? Tidsskr Nor Lægeforen 1991; 111: 1238–9.
61. Persson LA, Lundstrom M, Lonnerdal B, Hernell O. Are weaning foods causing impaired iron and zinc status in 1-year-old Swedish infants? A cohort study. Acta Paediatr 1998; 87: 618–22.
62. Lind T, Lönnerdal B, Persson L-Å, Stenlund H, Tennefors C, Hernell O. Effect of weaning cereals with different

phytate content on hemoglobin, iron stores, and serum zinc – a randomized intervention from 6 to 12 months of age. Am J Clin Nutr 2003; 78: 168–75.
63. Gunnarsson BS, Thorsdottir I, Palsson G. Iron status in 2-year-old Icelandic children and associations with dietary intake and growth. Eur J Clin Nutr 2004; 58: 901–6.
64. Niinikoski H, Koskinen P, Punnonen K, Seppanen R, Viikari J, Ronnemaa T *et al*. Intake and indicators of iron and zinc status in children consuming diets low in saturated fat and cholesterol: the STRIP baby study. Special Turku Coronary Risk Factor Intervention Project for Babies. Am J Clin Nutr. 1997; 66: 569–74.
65. Bergström E, Hernell O, Lönnerdal B, Persson L. Sex difference in iron stores of adolescents: what is normal? J Pediatr Gastroenterol Nutr 1995; 20: 215–24.
66. Samuelson G, Bratteby L-E, Berggren K, Elverby J-E, Kempe B. Dietary iron intake and iron status in adolescents. Acta Paediatr 1996; 85: 1033–8.
67. Milman N. Serum ferritin in Danes: studies of iron status from infancy to old age, during blood donation and pregnancy. Int J Hematol 1996; 63: 103–35.
68. Borch-Iohnsen B. Järnmangel i tenårene. Tidskr Nor Lægeforen 1993; 113: 2940–1.
69. Milman N, Heitman BL, Lyhne N, Rosdahl N, Jensen KH Graudal N. Iron status in 1113 Danish men and women aged 35–65 years. Relation to dietary and supplemental iron intake. Scand J Nutr 1993; 37: 98–103.
70. Milman N, Kirchoff M. Iron stores in 1359 30- to 60-year old Danish women: evaluation by serum ferritin and hemoglobin. Ann Hematol 1992; 64: 22–7.
71. Borch-Iohnsen B, Meltzer HM, Stenberg V, Reinskou T. Iron status in a group of Norwegian menstruating women. Eur J Clin Nutr 1990; 44: 23–8.
72. Fogelholm M, Alopaeus K, Silvennoinen T, Teirilä J. Factors affecting iron status in non-pregnant women from urban South Finland. Eur J Clin Nutr 1993; 47: 567–74.
73. Jonsson JJ, Johannesson GM, Sigfusson N, Magnusson B, Thjodleifsson B, Magnusson S. Prevalence of iron deficiency and iron overload in the adult Icelandic population. J Clin Epidemiol 1991; 44: 1289–97.
74. Lahti-Koski M, Valsta LM, Alfthan G, Tapanainen H, Aro A. Iron status of adults in the capital area of Finland. Eur J Clin Nutr 2003; 42: 287–92.
75. Rajala T. Nutritional status of the elderly living in southwestern Finland. Publications of the Social Insurance Institution, Turku, Finland 1991; 106: 258.
76. Milman N, Schultz-Larsen K. Iron stores in 70-year-old men and women. Evaluation in 469 individuals by serum-ferritin and hemoglobin. Aging (Milano) 1994; 2: 97–103.
77. Milman N, Pedersen AN, Ovesen L, Schroll M. Iron status in 358 apparently healthy 80-year-old Danish men and women: relation to food composition, dietary and supplemental iron intake. Ann Hematol 2004; 83: 423–9.
78. Milman N, Ingerslev J, Graudal N. Serum ferritin and iron status in a population of 'healthy' 85-year-old individuals. Scand J Clin Lab Invest 1990; 50: 77–83.
79. Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB, Wilson PWF, Wood RJ. Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. Am J Clin Nutr 2001; 73: 638–46.
80. Domellof M, Lonnerdal B, Abrams SA, Hernell O. Iron absorption in breast fed infants: effects of age, iron status, iron supplements, and complementary foods. Am J Clin Nutr 2002; 76: 198–204.
81. Hernell O, Lönnerdal B. Iron status of infants fed low-iron formula: no effect of added bovine lactoferrin or nucleotides. Am J Clin Nutr 2002; 76: 858–64.
82. Green R, Charlton R. Seftel H, Bothwell T, Mayet F, Adams B *et al*. Body

iron excretion in man. Am J Med 1968; 45: 336–53.
83. Hallberg L, Rossander-Hulthén L. Iron requirements in menstruating women. Am J Clin Nutr 1991; 54: 1047–58.
84. Hallberg L, Högdahl A-M, Nilsson L, Rybo G. Menstrual blood loss – a population study. Variation at different ages and attempts to define normality. Acta Obstet Gynecol Scand 1966; 45: 320–51.
85. Andersen E, Hutchings B, Jansen J, Nyholm M. Højde og vægt hos danske born. Ugeskr Lægere 1982; 144: 1760–5.
86. Galan P, Hercberg S, Soustre Y, Dop MC, Duprin H. Factors affecting iron stores in French female students. Hum Nutr Clin Nutr 1985; 39C: 279–87.
87. Hallberg L. Iron balance in pregnancy. In: Berger H (ed). Vitamins and minerals in pregnancy and lactation. Nestl, Nutrition Workshop Series 1988; 16: 115–27.
88. Barrett JFR, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. Br Med J 1994; 309: 79–82.
89. Borch-Iohnsen B, Halvorsen R, Andrew M, Forde R, Matheson I, Rytter E, *et al.* Trenger vi nye retningslinjer for bruk av jerntilskudd i svangerskapet? Tidsskr Nor Lægeforen 1993; 113: 2414–5.
90. Commission of The European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community. (Thirty-first series of Food – Science and Techniques), Luxembourg, Office for Official Publications of the European Communities, 1993.
91. Habicht JP, DaVanzo J, Butz WP, Meyers L. The contraceptive role of breastfeeding. Popul. Stud 1985; 39: 213–32.
92. Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe(III) EDTA in an iron-deficient population: Report of a controlled iron-fortification trial. Am J Clin Nutr 1989; 49: 156–61.
93. Beard J. Dietary iron intakes and elevated iron stores in elderly: is it time to abondon the set-poit hypothesis of regulation of the iron absorption? Am J Clin Nutr 2002; 76: 1189–90.
94. Hallberg L, Hulthen L, Gramatkowsky E. Mechanisms of iron accumulation from the whole diet in men: how effective is the regulation of iron absorption. Am J Clin Nutr 1997; 66: 347–56.
95. Hallberg L, Hulthen L, Garby L. Iron stores in man in relation to diet and iron requirements. Eur J Clin Nutr 1997; 52: 623–31.
96. Sayers MH, English G, Finch CA. The capacity of the store regulator in maintaining iron balance. Am J Hematol 1994; 47: 194–7.
97. Fleming DJ, Tucker KL, Jaques PF, Dallal GE, Wilson PWF, Wood RJ. Dietary factors associated with risk of high iron stores in the elderly Framingham Heart Study Cohort. Am J Clin Nutr 2002; 76: 1375–84.
98. Borch-Iohnsen B, Pettersson Grawe K. Iron. In Oskarsson A. Risk evaluation of essential trace elements. Copenhagen, Nordic Council of Ministers, 1995: 67–110.
99. Anderson AC. Iron poisoning in children. Curr Opin Pediatr 1994; 6: 289–94.
100. Engle JP, Polin KS, Stile IL. Acute iron intoxication: treatment controversies. Drug Intell Clin Pharm 1987; 21: 153–9.
101. Ellenham MJ, Barceloux DG. Iron. In: Medical Toxicology. New York, Amsterdam, London: Elsevier; 1988. pp 1023–30.
102. Brock C, Curry H, Hama C, Knipfer M, Taylor L. Adverse effects of iron supplementation: A comparative trial of wax-matrix iron preparation and conventional ferrous sulfate tablets. Clin Ther 1985; 7: 568–73.
103. Choplin M, Schuette S, Leichtmann G, Lasher B. Tolerability of iron: A comparison of bis-glycino iron (II) and ferrous sulfate. Clin Ther 1991; 13: 606–12.

104. Frykman E, Bystrom M, Jansson U, Edberg A, Hansen T. Side effects of iron supplements in blood donors: Superior tolerance of heme iron. J Lab Clin Med 1994; 123: 561–4.
105. Hallberg L, Ryttinger L, Söllvell L. Side-effects of oral iron therapy. Acta med Scand 1966; 459 (Suppl): 3–10.
106. Liguori L. Iron protein succinylate in the treatment of iron deficiency: Controlled, double blind, multicenter clinical trial on over 1,000 patients. Int J Clin Pharmacol Ther Toxicol 1993; 31: 103–23.
107. Distante S, Berg JP, Lande K, Haug E, Bell H. High prevalence of the hemochromatosis-associated Cys282Tyr HFE gene mutation in a healthy Norwegian population in the city of Oslo, and its phenotypic expression. Scand J Gastroenterol. 1999; 34: 529–34.
108. Holmstrom P, Marmur J, Eggertsen G, Gafvels M, Stal P. Mild iron overload in patients carrying the HFE S65C gene mutation: a retrospective study in patients with suspected iron overload and healthy controls. Gut. 2002; 51: 723–30.
109. Beutler E, Waalen J. Response to Moirand *et al.* – 'HFE based re-evaluation of heterozygous hemochromatosis'. Am J Med Genet. 2004; 124A: 218–9.
110. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G – A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002; 359: 211–8
111. European Haemochromatosis Consortium. Letter to the Editor. Lancet 2002; 360: 412.
112. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early haemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology 1986; 6: 24–9.
113. Åsberg A, Hveem K, Thorstensen K, Ellekjter E, Kannelonning K, Fjosne U *et al.* Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol. 2001; 36: 1108–15.
114. Bell H, Berg JP, Undlien DE, Distante S, Raknerud N, Heier HE *et al.* The clinical expression of hemochromatosis in Oslo, Norway. Excessive oral iron intake may lead to secondary hemochromatosis even in HFE C282Y mutation negative subjects. Scand J Gastroenterol. 2000; 35: 1301–7.
115. Bothwell TH, Bradlow BA. Siderosis in Bantu. Arch Pathol 1960; 70: 279–92.
116. Isaacson C, Seftel HC, Keeley KJ, Bothwell TH. Siderosis in the Bantu: the relationship between iron overload and cirrhosis. J Lab Clin Med 1961; 58: 845–53.
117. Seftel HC, Keeley KJ, Isaacson C, Bothwell TH. Siderosis in the Bantu: the clinical incidence of hemochromatosis in diabetic subjects. J Lab Clin Med 1961; 58: 837–44.
118. Bothwell TH, Seftel H, Jacob P, Torrance JD, Baumslag N. Iron overload in Bantu subjects. Am J Clin Nutr 1964; 14: 47–51.
119. Gordeuk U, Mukiibi J, Hasstedt SJ. Iron overload in Africa. Interaction between a gene and dietary iron control. N Engl J Med 1992; 326: 95–100.
120. McNamara L, MacPhail AP, Gordeuk VR, Hasstedt SJ, Rouault T. Is there a link between African iron overload and the described mutations of the hereditary haemochromatosis gene. Br J Haematol 1998; 102: 1176–8.
121. Turnberg LA. Excessive oral iron therapy causing haemochromatosis. Br Med J 1965; 1: 1360.
122. Johnson BF. Haemochromatosis resulting from prolonged oral iron therapy. N Engl J Med 1968; 278: 1100–1101.
123. Green P, Evitan JM, Sivota P, Avidor I. Secondary hemochromatosis due to prolonged iron ingestion. Isr J Med Sci 1989; 25: 199–201.
124. Jalil SS, Barlow AM. Haemochromatosis following prolonged iron ingestion. J R Soc Med 1984; 77: 690–2.
125. Danesh J, Appleby P. Coronary heart disease and iron status: Meta-analy-

sis of prospective studies. Circulation 1999; 99: 852–4.
126. Sullivan JL. Iron and the genetics of cardiovascular disease. Circulation 1999; 100: 1260–3.
127. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis. Circulation 1997; 96: 3300–07.
128. Tuomainen T-P, Punnonen K, Nyyssönen K, Salonen JT. Association between body iron stores and the risk of acute myocardial infarction in men. Circulation 1998; 97: 1461–6.
129. Klipstein-Grobusch K, Koster JF, Grobbee DE, Lindemans J, Boeing H, Hofman A, Witteman JCM. Serum ferritin and risk of myocardial infraction in the elderly: the Rotterdam study. Am J Clin Nutr 1999; 69: 1231–6.
130. Claeys D, Walting M, Julmy F, Waillermin WA, Mayer BJ. Haemochromatosis mutations and ferritin in myocardial infarction: a case-control study. Eur J Clin Invest 2002; 32 (Suppl 1): 2–8.
131. Sempos CT, Looker AC, Gillum RF, McGee DL, Vuong CV, Johnson CL. Serum ferritin and death from all causes ans cardiovascular diseases: The NHANES II mortality study. Ann Epidemiol 2000; 10: 441–8.
132. Tuomainen T-P, Kontula K, Nyyssönen K, Lakka TA, Heliö T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation. Circulation 1999; 100: 1274–9.
133. Powell LW, Jazwinska E, Halliday JW. Primary iron overload. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron Metabolism in Health and Disease. London: WB Sanders Co Ltd, 1994: 227–70.
134. Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA; Paterson AC, Rouault TA, Kew MC. Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. Hepatology 1998; 27: 1563–6.
135. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliövaara M, Hakulinen T. Body iron stores and risk of cancer. Int. J. Cancer 1994; 56: 379–82.
136. Nelson RL, Davis FG, Sutter E, Sobin LH, Kikendall JW, Bowen P. Body iron stores and risk of colonic neoplasia. J. Natl. Cancer Inst 1994; 86: 455–60.
137. Stevens RG Jones Y, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. N Engl J Med 1988; 319: 1047–52.
138. Stevens RG, Graubard BI, Micozzi MS, Neriishi K, Blumberg BS. Moderate elevation of body iron level and increased risk of cancer occurrence and death. Int J Cancer 1994; 56: 364–9.
139. Nelson RL, Davis FGg, Persky V, Becker E. Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis. Cancer 1995; 6: 875–9.138.
140. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA. 2004; 291: 711–7.
141. Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr. 2004; 79: 70–5.
142. Steingrimsdottir L, Thorgeirsdottir H, Olafsdottir AS. The Diet of Icelanders. Dietary Survey of The Icelandic Nutrition Council 2002. Main findings.
143. Männistö S, Ovaskainen ML, Valsta L, eds. the national Findiet 2002 Study. Helsinki, Publications of the National Public Health Institute B3/2003, 2003.
144. Becker W, Pearson M. Riksmaten 1997–98. Kostvanor och näringsintag i Sverige. Metod och resultatrapport. Uppsala; Livsmedelsverket, 2002.

CHAPTER 35

Zinc

Zinc, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	7	9	6	7	8/11
Average requirement	AR	5	6			
Lower level of intake	LI	4	5			

Zinc has been known to be an essential nutrient for humans for more than 40 years, but only in the last 20–25 years have clinical zinc deficiency in humans and negative effects of a low zinc intake been recognized. Due to the lack of specific and sensitive indicators of zinc status, the prevalence of sub-optimal zinc intakes is not known and thus the functional consequences and public health importance of sub-optimal zinc intake are not well understood. Well-defined, clinical zinc deficiency has only been reported in a limited number of cases, related to incomplete TPN, malabsorption etc. Information about negative effects of low zinc intake under more 'normal' conditions is very rare but includes reduced growth velocity. Recent estimates based on evaluation of zinc intakes and diet composition in different parts of the world suggest that the populations of many countries in Asia and Africa have a high risk for developing zinc deficiency, while the risk is low in European countries and North America.

Physiology and metabolism

Absorption of zinc is dependent on dose and mainly takes place in the upper part of the small intestine. Absorbed zinc is transported in the blood, mostly bound to albumin. The main proportion of body zinc is located within the cells. The total body content of zinc in an adult is estimated at 2 g, of which approximately ⅔ is located in muscle tissue and 30% in bone tissue. Plasma zinc only represents 0.1% of total body zinc. High concentrations of zinc are found in parts of the eye and in prostate liquid. Zinc is excreted through the kidneys, skin and gastrointestinal tract. Strong homeostatic mechanisms keep the zinc content of

tissues and fluids constant over a wide range of intakes through changes in excretion and absorption. The molecular mechanisms involved in this regulation are not fully understood.

The biochemical role of zinc is as an essential part of more than 300 enzymes involved in synthesis, metabolism and turnover of proteins, carbohydrates, lipids, nucleic acids and some of the vitamins, for example vitamin A. Zinc is a part of the cell nucleus and participates in the regulation of gene expression. The third important role of zinc is structural, in stabilizing organic cell components and cell membranes.

The clinical manifestations of severe zinc deficiency are growth retardation, delayed sexual maturation, skin lesions adjacent to the body orifices, hair loss and behavioural disturbances (1). These clinical signs have almost exclusively been observed in subjects with an inborn error in zinc transport (Acrodermatitis enteropathica) and in adolescents subsisting on diets with a presumably very low availability of zinc. The consequences of moderate and mild zinc deficiency are still unclear. Impaired growth and a reduced immune defence are regarded as early signs of zinc deficiency, but are not specific for zinc.

Requirement and recommended intake

Adults

Sensitive and specific biochemical or functional indicators of zinc status are lacking. At severe zinc depletion, reduction of plasma zinc is observed. However, plasma zinc concentration is influenced by factors unrelated to zinc status such as food intake, infection, tissue anabolism or catabolism and cannot be used for estimating zinc requirements. In addition, the activities of the zinc dependent enzymes explored so far have not proven sensitive enough to identify optimal or desired levels of zinc intake. In populations where signs of zinc deficiency have been observed, reliable food intake data are usually not available. Consequently, zinc requirements have to be estimated by the factorial method, *i.e.* estimates of the daily losses of zinc and the corresponding amount of zinc to be ingested to replace these losses. Additional zinc is needed during periods of tissue growth. The use of the factorial method to estimate zinc requirements is complicated by a strong homeostatic regulation of body zinc, primarily through changes in endogenous zinc excretion and by the pronounced impact of diet composition on zinc absorption and potentially also on excretion of zinc. At zinc intakes close to zero, total endogenous zinc losses through urine, faeces and skin are of the order of 0.5–0.6 mg/d (2, 3), while on an intake of 10–15 mg of zinc, losses will amount to > 4 mg/day. During the first days on

low zinc intakes, before adaptive mechanisms have had full effect, zinc losses are approximately 1.0 and 1.4 mg/d for women and men, respectively (2, 3). These values were chosen as the basis for the NNR 1996 recommendations as estimates of the average *physiological* requirement for zinc. The same figures have been used by WHO/FAO 1996 (4) for determinations of population dietary zinc requirements.

To reach a *dietary recommendation* from a figure for *physiological* requirement, an estimate of the efficiency of absorption has to be applied. Fractional zinc absorption is dependent on dietary zinc content; when intakes are increased, fractional absorption decreases. However, the relationship is not linear and the amount of zinc absorbed increases when zinc intake increases. Superimposed on the relationship between intake and fractional absorption is the effect of enhancing and inhibiting components in the diet (4). At low intakes of zinc in diets with no inhibitors the fractional absorption can be > 50% (5), while at more common intakes 15–40% is absorbed, depending on the composition of the diet. Phytic acid, which is present in cereals and leguminous plants, inhibits zinc absorption, while animal protein counteracts this inhibition (6, 7). From a cereal-based meal with a high content of phytic acid 10–15% of the zinc is absorbed, while from meals based on animal protein sources 20–40% can be absorbed depending on zinc content. The negative effect of phytic acid is in some foods partly counteracted by a higher zinc content.

A number of single meal studies using radioisotope techniques have been undertaken to identify the dietary factors affecting absorption and their relative impact. Relatively few studies have measured zinc uptake from total diets with realistic compositions and the techniques used in these studies are based on the use of stable zinc isotopes, which are typically added in amounts which account for 20% or more of the total zinc content. In the NNR 1996, zinc absorption was estimated at 25% based on two total diet studies using stable isotopes and with a composition that would be plausible within Nordic countries (8, 9), *i.e.* a fibre intake of ≥ 3 g/MJ with at least 50% of the fibre intake from fruits and vegetables, less than 50% from whole grain phytic acid-rich cereal products and with an intake of animal protein accounting for at least 50% of total protein intake. For strictly vegetarian diets, 25–30% higher intake of zinc was recommended. Due to the limited number of data a relatively large safety factor was applied. (The inter-individual variation in physiological zinc requirement was set to 1 SD = 30%). The resulting recommended daily intake for adults consuming a mixed animal vegetable protein diet was 9 mg for men and 7 mg for women and increased to 12 mg and 10 mg, respectively, with a vegetarian cereal-based diet.

The 1991 British recommendations give an RNI of 9.5 mg/d for men and 7.0 mg/d for women (10). This committee estimated the daily physiological losses to be 2.2 mg/d and 1.6 mg/d respectively, and assumed 30% absorption.

The Food and Nutrition Board, USA, recommends a daily intake of 11 mg/d for men and 8 mg/d for women (11). Although the absolute numbers are similar to those of the other reports and the approach used is the same factorial method, they have introduced a partly different concept in the calculations. The data used are almost exclusively derived from total diet studies using semi-synthetic basic diets or blended low zinc foods with added zinc and stable zinc isotopes for the absorption estimates. This committee uses a three-step approach to reach the average requirement of zinc. First, the losses of zinc via routes other than the intestine are estimated. These losses are regarded as constant over the range of intake that encompasses zinc requirements. For men the estimates for losses via kidneys and sweat, integumental losses and losses in semen are estimated to be 0.63, 0.54 and 0.1 mg/d, respectively. For women menstrual zinc losses are estimated to be 0.1 mg/d and losses via kidney and skin 0.44 mg/d and 0.46 mg/d, respectively. Thus, total losses via these routes are 1.27 mg/d and 1.0 mg/d for men and women, respectively. The second step and partly new concept is the use of the relationship between quantity of zinc absorbed and the excretion of endogenous zinc via the intestine. In the stable isotope/balance studies used for this calculation the data suggest a linear relationship between absorbed zinc and intestinal (endogenous) excreted zinc. The constant losses via other routes are added and the point where the absorbed zinc is equal to the sum of the endogenous intestinal excretion and the other losses is taken as the minimum requirements for absorbed zinc (*i.e.* the *physiological* requirement) (3.84 mg/d for men and 3.3 mg/d for women). The same studies are then used to calculate the amount of zinc that has to be ingested to give this amount of absorbed zinc. These calculations give an average dietary requirement of zinc of 9.4 mg/d and 6.8 mg/d, respectively. (It should be noted that these values correspond to fractional absorption of 0.41 and 0.48). A CV of 10% is used as an estimate of the inter-individual variations and the RDA is set to 11 mg/d and 8 mg/day. Thus, the major differences in the American estimates compared to other reports are a much higher estimate of the physiological requirement, especially the estimates of the endogenous intestinal losses, a higher estimate of the fractional absorption and a smaller figure for the inter-individual variations.

The major limitation of the data and approach used to derive the American recommendations is the composition of the test diets applied

in the underlying studies resulting in a high availability and consequently a high endogenous intestinal secretion. In studies performed with a zinc intake in the order of 15 mg/d, the basic diets have been either egg protein-based formula (12) or based on low zinc foods (13) providing approximately 5 mg of zinc. To these basic diets approximately 10 mg of zinc or the same amount in the form of oysters (15) has been added. The observed absorption of zinc varied from 17 to 44% (mean 28.9%), 4–5.5 mg of zinc was absorbed and the endogenous intestinal excretion varied from 1.4 to 4.2 mg. In studies with a dietary zinc content of 4–5.5 mg/d (14), fractional absorption has been 53% to 65% and the intestinal excretions 1.4–1.8 mg/day. Only one study has used a zinc content which falls close to the average requirement figure (13). In two 4-day periods a diet based on low zinc food and chicken as the main protein source and providing 7 mg zinc/d was given to *one* subject. Absorption was 46.6% and 57.8% and endogenous intestinal excretion 3.8 mg and 3.0 mg.

In the NNR 2004, the following estimates have been made. For the estimate of the endogenous losses, routes other than the intestine (the Food and Nutrition Board figures, 11) have been used. It should be noted however, that the majority of the studies quoted in that report are > 10 years old and performed at a time when *e.g.* reference urine samples were not available for quality control. Thus, the losses via kidneys, skin, semen or menses are set to 1.27 mg/d for men and 1.0 mg/d for women. Endogenous intestinal losses are estimated to 1.4 mg/d for both genders based on the observed losses at low intakes (1–5 mg/d). Thus, 2.67 mg/d and 2.4 mg/d for men and women, respectively, has to be absorbed in order to replace these losses. At these levels of intake, absorption from a mixed animal and vegetable protein diet more realistic for Nordic conditions is assumed to be 40%. The average dietary requirement of zinc is consequently 6.4 mg and 5.7 mg, respectively. The inter-individual variation in requirement is set to 15%, resulting in unchanged recommended intakes of 9 (8.3) mg/d for men and 7 (7.4) mg/d for women. This recommended intake probably has a high safety margin as the ability to adapt to lower intakes appears to be substantial.

Lower level of intake

Balance studies with a combination of a semi-synthetic formula based on egg white and low zinc foods have shown that an intake of 4.4 mg/day for 35 days does not give any indications of an impaired zinc status or the need for adaptation, based on plasma levels and zinc excretion in urine (15), nor did a recent study of seven men given a zinc intake of 4.6 mg/d for 10 weeks (16). The latter study also showed no changes in

exchangeable zinc pool mass during the low intake. These data are used as the basis for the lower level of zinc intake.

Children

Data on endogenous losses of zinc at different intakes are almost completely lacking for children. In relation to body weight, children appear to have larger losses of zinc than adults. The need of zinc for growth is approximately 175 µg/kg/d during the first month and then decreases to approximately 30 µg/kg/d at 9–12 months (17). For growing children the need for zinc is based on basal losses of 0.1 mg/kg and a zinc content in new tissue of 30 mg/kg. For adolescents, growth is assumed to result in an average zinc content in new tissue of 23 mg/kg, due to an increase in fat tissue with a lower zinc content than in children. The *physiological* needs for rapidly growing adolescents can consequently be increased by 0.3–0.4 mg/day. Applying the same principles as for adults, the recommended zinc intake varies from 2 mg in the youngest age group to 12 mg for adolescent boys.

Pregnancy and lactation

The total need for zinc during pregnancy for the foetus, placenta and other tissues is approximately 100 mg (18). This additional need for zinc in pregnancy can be met by an increase in zinc intake or by adjustment in zinc homeostasis. There is no evidence that pregnant women increase their intake of zinc, therefore homeostatic adjustments in zinc utilization must be the primary mechanism for meeting the additional zinc demands for reproduction (18). It is assumed that an increased efficiency of zinc absorption or other metabolic changes occur during pregnancy and these changes ensure that the requirement for zinc can be met at unchanged intake. However, studies in this area are inconclusive and there are some that show increased absorption during pregnancy (19), while other studies found no significant increase in fractional absorption (20). The results from the latter study might reflect inadequate power of the study design. The US recommendations 2001 for zinc intake in pregnancy are based on this reference. The Nordic Recommendations are based on an increase of the physiological requirement by 0.7 mg/day.

Ortega *et al.* (21) showed lower zinc concentration in human milk of women consuming less than 7.5 mg/d during the third trimester.

Zinc content in human milk is approximately 2.5 mg/L in the first month of lactation and thereafter falls to approximately 0.7 mg/L after 4 months (17). Theoretically this means that the zinc requirement of lactating women is double that of non-lactating women. A fractional

increase in zinc absorption of 75–80% has been shown for lactating women compared with non-lactating-postpartum or never-pregnant women (20, 22). Release of zinc from bone tissue could also possibly be an explanation why zinc concentrations in human milk are relatively independent of the mother's zinc intake and do not seem to result in zinc deficiency of the mother even after a long time of lactation. An elevated intake corresponding to the zinc content in human milk is recommended to women lactating for a long time, *i.e.* a physiological need of 1.7 mg/day.

Upper intake levels and toxicity

The risk of excessive intake of zinc from food alone is very low. Symptoms of acute toxicity from excessive intake occur at intakes of gram quantities of zinc and are related to consumption of dietary supplements. At considerably lower intakes, there is a risk for negative effects on the metabolism of other trace elements, especially copper. Reduced activity of copper-containing enzymes has been observed with intakes of 50 mg Zn/d and with a slightly higher intake ≥ 150 mg, more pronounced signs of impaired copper metabolism have been observed and also negative changes in immune defence and blood lipids (23–26). More recent studies in which strictly controlled intakes of copper and zinc were given showed that at intakes of 50 mg zinc/d, no adverse effects on a wide range of relevant indicators of copper status could be observed (27–30). Based on these data, the EU Scientific Committee on Food set an uncertainty factor of 2 and arrived at an upper level of 25 mg zinc per day. As there are no data on adverse effects of zinc intakes on children and adolescents, they extrapolated upper levels for children on a surface area basis.

With this background it is recommended that the intake of zinc does not exceed 25 mg/d for adults and 12.5 mg/d for children.

Dietary sources and intake

Good sources of zinc are meat, milk and milk products and wholegrain cereals. Foods with a high content of fat and sugar have a low content of zinc. Intake of zinc in the Nordic countries is approximately 12–13 mg/10 mg/MJ (see *Chapter 43*).

REFERENCES

1. Prasad AS. Zinc deficiency. BMJ 2003; 326: 409–10.
2. Baer MT, King JC. Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am J Clin Nutr 1984; 39: 556–70.
3. Hess FM, King JC, Margen S. Effect of low zinc intake and oral contraceptive agents on nitrogen utilization and clinical findings in young women. J Nutr 1977; 107: 2219–27.
4. Sandstrom B, Lonnerdal B. Promoters and antagonists of zinc absorption. In: Mills CF, editor. Zinc in human biology. Berlin Heidelberg: Springer-Verlag, 1989: 57–78.
5. Sandstrom B. Dose dependence of zinc and manganese absorption in man. Proc Nutr Soc 1992; 51: 211–8.
6. Rossander-Hulthén L, Sandberg A-S, Sandstrom B. The influence of dietary fibre on mineral absorption and utilization. In: Schweizer T, Edwards C, editors. Dietary fibre – a component of food – nutritional function in health and disease. London: Springer-Verlag, 1992: 195–216.
7. Sandstrom B, Arvidsson B, Cederblad A, Bjorn-Rasmussen E. Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. Am J Clin Nutr 1980; 33: 739–45.
8. Swanson CA, Turnlund JR, King JC. Effect of dietary zinc sources and pregnancy on zinc utilization in adult women fed controlled diets. J Nutr 1983; 113: 2557–67.
9. Knudsen E, Sandstrom B, Solgaard P. Zinc, copper and magnesium absorption from a fibre-rich diet. J Trace Elem Med Biol 1996; 10: 68–76.
10. Department of Health. Report on health and social subjects. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. London: HMSO, 1991.
11. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
12. Turnlund JR, Durkin N, Costa F, Margen S. Stable isotope studies of zinc absorption and retention in young and elderly men. J Nutr 1986; 116: 1239–47.
13. Jackson MJ, Jones DA, Edwards RH, Swainbank IG, Coleman ML. Zinc homeostasis in man: studies using a new stable isotope-dilution technique. Br J Nutr 1984; 51: 199–208.
14. Wada L, Turnlund JR, King JC. Zinc utilization in young men fed adequate and low zinc intakes. J Nutr 1985; 115: 1345–54.
15. Johnson PE, Hunt CD, Milne DB, Mullen LK. Homeostatic control of zinc metabolism in men: zinc excretion and balance in men fed diets low in zinc. Am J Clin Nutr 1993; 57: 557–65.
16. Pinna K, Woodhouse LR, Sutherland B, Shames DM, King JC. Exchangeable zinc pool masses and turnover are maintained in healthy men with low zinc intakes. J Nutr 2001; 131: 2288–94.
17. Krebs NF, Hambidge KM. Zinc requirements and zinc intakes of breast fed infants. Am J Clin Nutr 1986; 43: 288–92.
18. King JC. Determinants of maternal zinc status during pregnancy. Am J Clin Nutr 2000; 71: Suppl–43S.
19. Swanson CA, King JC. Zinc utilization in pregnant and nonpregnant women fed controlled diets providing the zinc RDA. J Nutr 1982; 112: 697–707.
20. Fung EB, Ritchie LD, Woodhouse LR, Roehl R, King JC. Zinc absorption in women during pregnancy and lactation: a longitudinal study. Am J Clin Nutr 1997; 66: 80–8.
21. Ortega RM, Andres P, Martinez RM, Lopez-Sobaler AM, Quintas ME. Zinc levels in maternal milk: the influence of nutritional status with

respect to zinc during the third trimester of pregnancy. Eur J Clin Nutr 1997; 51: 253–8.
22. Moser-Veillon PB, Patterson KY, Veillon C. Zinc absorption is enhanced during lactation. FASEB J 1995; 9: A729.
23. Fischer PW, Giroux A, L'Abbe MR. Effect of zinc supplementation on copper status in adult man. Am J Clin Nutr 1984; 40: 743–6.
24. Yadrick MK, Kenney MA, Winterfeldt EA. Iron, copper, and zinc status: response to supplementation with zinc or zinc and iron in adult females. Am J Clin Nutr 1989; 49: 145–50.
25. Hooper PL, Visconti L, Garry PJ, Johnson GE. Zinc lowers high-density lipoprotein-cholesterol levels. JAMA 1980; 244: 1960–1.
26. Chandra RK, McBean LD. Zinc and immunity. Nutrition 1994; 10: 79–80.
27. Davis CD, Milne DB, Nielsen FH. Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins. Am J Clin Nutr 2000; 71: 781–8.
28. Milne DB, Davis CD, Nielsen FH. Low dietary zinc alters indices of copper function and status in postmenopausal women. Nutrition 2001; 17: 701–8.
29. Bonham M, O'Connor JM, McAnena LB, Walsh PM, Downes CS, Hannigan BM *et al.* Zinc supplementation has no effect on lipoprotein metabolism, hemostasis, and putative indices of copper status in healthy men. Biol Trace Elem Res 2003; 93: 75–86.
30. Bonham M, O'Connor JM, Alexander HD, Coulter J, Walsh PM, McAnena LB *et al.* Zinc supplementation has no effect on circulating levels of peripheral blood leucocytes and lymphocyte subsets in healthy adult men. Br J Nutr 2003; 89: 695–703.

CHAPTER 36

Iodine

Iodine, µg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	150	150	90	120	150
Average requirement	AR	100	100			
Lower level of intake	LI	70	70			

Iodine deficiency is considered to be one of the most common nutritional disorders in the world and the most common cause of goitre (1). In Sweden and Finland iodine deficiency goitre was common during the first decades of the 1900s. The introduction of iodine fortification of salt resulted in a sharp decrease in the prevalence (2). In 2000, mandatory iodisation of table salt and bread salt was introduced in Denmark as a response to studies showing low iodine status and goitre in certain population groups (3, 4).

Physiology and metabolism

Iodine is essential for a number of animal and plant species. Only vertebrates have developed a thyroid gland to cater for synthesis, storage and secretion of the iodine-containing hormones, thyroxine (T4) and the biologically active form triiodothyronine T3 (5).

The thyroid hormones increase metabolism in body cells. The mechanism of action is not completely known, but protein synthesis increases, *e.g.* with respect to those enzymes necessary for an increased metabolic activity. The thyroid hormones also increase the size and number of mitochondria – a sign of increased ATP-production.

Dietary iodine is generally efficiently absorbed as iodide, although some sources of iodine, such as certain seaweed and protein-bound iodine, may be absorbed less efficiently (6, 7). A recent study showed that about 90% of the iodine in a mixed diet, providing about 200 µg/d, was excreted in the urine (6). Iodide is mainly excreted through the kidneys (iodine clearance approximately 40 mL/min). Faecal losses vary, but are in general only 10–20 µg/day. Small amounts are lost through the skin.

The iodine concentration in breast milk varies with the iodine intake (8). Reported levels in breast milk from Danish mothers, before the introduction of salt iodination, were about 30 µg/L (9). Older data from Finland reported average levels of 25 µg/L in breast milk from goitrous areas compared to 53 µg/L in non-goitrous areas (8). In Sweden, breast milk samples have been reported to contain 50–90 µg/L (8). Smoking is associated with lower iodine concentrations in breast milk, possibly due to impaired iodine uptake in the mammary gland (10).

The utilisation of iodine in the thyroid gland occurs via: 1) active uptake of iodide (iodide concentration is approx. 30 times higher in the gland than in plasma), 2) incorporation of iodine in thyroglobulin and iodine tyrosine, and 3) secretion of the iodine thyronines triiodothyronine and thyroxine. The thyroid-stimulating hormone (TSH) from the pituitary gland regulates the formation of the thyroid hormones.

Iodine absorption and utilisation may be affected by goitrogens, mainly sulphur-containing glucosides (glucosinolates). These are dietary constituents that may inhibit the uptake of iodine into the thyroid gland, *e.g.* thiocyanates, or interact with hormone production (goitrins) (7). They occur in *e.g.* Brassica species such as cabbage, Brussels sprouts, turnip, and rapeseeds. Generally the levels of glucosinolates in the current Nordic diet are too low to have an impact on iodine status.

Iodine status can also be affected by selenium or iron intake and in areas with marginal iodine status, low selenium intakes (11) or iron intakes (12) might have a negative impact on thyroid function.

Iodine status has traditionally been assessed by urinary iodide excretion or by assessment of the goitre rate, *e.g.* thyroid gland size. Recently serum thyroglobulin concentration has been shown to be a useful marker of thyroid function and iodine status (13, 14).

Iodine deficiency primarily occurs as non-toxic goitre, *i.e.* an enlarged thyroid gland with a normal production of thyroid hormones. A non-toxic goitre can gradually develop to a toxic goitre with an increased secretion of hormones and an increased metabolism (thyrotoxicosis). In hyperthyroidism the thyroid gland can be enlarged (toxic goitre) either in a diffuse form (Basedow's or Graves' disease) or with focal changes (nodular goitre). In more severe iodine deficiency cretinism can occur, which is characterised by impaired growth, mental disturbances and disturbances in speech and acuity (deaf mutism) as well as hypothyroidism (myxoedema) among adults (5).

The prevalence of goitre due to iodine deficiency is nowadays generally low in the Nordic countries. In Denmark prevalence levels up to 20–30% were found among elderly females in Jutland prior to the introduction of iodination of salt in 1998 (14). In Sweden, lower mean thyroid

volumes have been reported among adults (15). In a study of Swedish adolescents, 4 of 59 subjects (7%) had mild goitre defined as a thyroid volume above 16 ml (16).

Hypothyroidism due to iodine deficiency does not normally occur in the Nordic countries, but is a severe health problem in many countries of the world, especially in mountainous areas far from the sea (17).

Requirement and recommended intake

The iodine requirement to prevent goitre (increased thyroid gland size) is estimated to be 50–75 µg/d or approximately 1 µg/kg body weight and day (18, 19). The recommendations in NNR 1996 are kept unchanged, since no new data are available. The average requirement is estimated to be 100 µg/d for both adult women and men. At this level the iodine concentration in the thyroid gland reaches a plateau. Iodine turnover in euthyroid subjects is at a similar level (20). The recommended intake is set to 150 µg/d for adults and adolescents and includes a safety margin for any goitrogenic substances in foods.

The recommended intakes for infants and children are based on data on goitre prevalence and urinary iodine excretion in European children (21) and extrapolations from adults based on energy and growth requirements.

In pregnancy an extra daily supply is needed to cover the needs of the foetus. Balance studies indicate that iodine retention of full-term infants is approximately 7 µg/kg (22). In a group of pregnant Swedish mothers, free T4 levels tended to decrease and TSH levels to increase during pregnancy, despite normal urinary iodine levels (23). The significance of this finding is not known (the fall in free T4 is physiological during pregnancy and not due to iodine insufficiency). An extra 25 µg above the RI for non-pregnant women would normally cover the needs. During lactation an additional supply of 50 µg/d is recommended to provide sufficient iodine in the breast milk.

The lower level of intake for adults is estimated at 70 µg/d, corresponding to urinary iodine excretion of 35–40 µg/L. It is lower for children.

Upper intake levels and toxicity

An iodine intake in excess of 2 mg/d can in rare cases cause sensitivity reactions such as rhinitis, nasal congestion, swollen salivary glands, headache and acne-like skin changes (24).

High iodine intakes can also cause disturbances in the thyroid function. Symptoms include inflammation in the thyroid gland (auto-

immune thyroiditis), goitre, and hypo- or hyperthyroidism (24, 25). A high iodine intake from *e.g.* drugs, certain types of seaweed or supplements in amounts corresponding below 1 mg up to 10 mg iodine per day has resulted in increased incidence of iodine goitre, in certain cases with hyperthyroidism or myxoedema (24–29).

An increased incidence of hyperthyroidism has been observed after introduction of iodine fortification, *i.e.* in populations where iodine intake is increased from a low baseline level (30–33). Most studies indicate that this is a transient phenomenon.

The risk of auto-immune thyroiditis, in some cases followed by hypothyroidism, seems to increase following an increased iodine intake from fortification (34, 35). Studies also indicate that an increased iodine intake during pregnancy increases the prevalence of auto-immune disease in the thyroid gland in the mother after birth (post partum thyroiditis) (36, 37). There are more recent studies that do not show an increased risk of post partum thyroiditis with iodine supplementation (38). Other factors that might affect thyroid function include humic substances in drinking water (39).

In summary it can be concluded that there is a substantial inter-individual variation with respect to the dose of iodine that can cause adverse effects. This complicates the assessment of an upper safe limit of intake. Persons with a normal thyroid function can in general tolerate prolonged consumption of iodine up to 1 mg/d (24). The Scientific Committee on Food has proposed 600 µg/d of iodine as the safe upper level (UL) for adults (40). The UL is based on elevations in TSH levels after iodine intake and an enhanced response in TSH levels to TRH stimulation. The effects are of a biochemical nature and are not associated with any clinical adverse effects. The UL includes an uncertainty factor and is also considered acceptable for pregnant and lactating women. No data are available for children, but guidance levels can be calculated from the adult value corrected for body surface area.

The most important risk group for excessive iodine intakes is elderly persons with one or several independently functioning areas of the thyroid gland, *i.e.* areas where TSH does not regulate the hormone production.

Dietary sources and intake

In plants, iodine predominantly occurs in inorganic form and the content varies with the iodine content in the environment. The iodine content in sea-plants is higher than in plants grown on land. In certain seaweed species, the iodine content can be up to 4.5 g/kg dry weight. The

iodine content of milk and milk products varies considerably depending on feed and use of iodine-containing disinfectants in connection with milking. The iodine content is generally higher in winter than in summer milk (41). The iodine in drinking water varies considerably between regions and can locally be a significant iodine source (42, 43). Fish, especially marine fish and shellfish, generally have a high iodine content. Eggs can also be an important iodine source.

Iodised table salt is available in Denmark, Sweden, Finland and Norway and contributes to iodine intake. The levels vary from 5–50 µg/g salt. Denmark also fortifies salt used in bread. In Norway iodisation of cow fodder has been more important for the iodine intake than iodised table salt (41, 44).

The dietary supply of iodine is difficult to assess in dietary surveys, since data for iodised table salt and drinking water are commonly lacking. Data from national dietary surveys show average intakes of about 200 µg/10 MJ in Denmark, 320 µg/10 MJ in Finland, 200 µg/10 MJ in Iceland and 160–170 µg/10 MJ in Norway (45, see *Chapter 43*). Urinary iodine excretion per 24 hours in different population groups has been reported to be on average 70–120 µg in Denmark before salt iodisation (43), 150–200 µg in Finland and Sweden (16, 23, 46), and 150–250 µg in Norway (47). In a recent Norwegian study, mean iodine excretion per 24 hours in selected population samples from Bergen and Tromsø was close to the lower end of that range among men but at or below 100 µg among women (48). Very high iodine excretion (up to 1,700 µg per 24 hours) has been reported in subjects consuming seaweed preparations (49).

REFERENCES

1. WHO. Assessment of iodine disorders and monitoring their elimination. A guide for programme mangers. 2nd ed. WHO/NHD/01.1. 2001.
2. Sjöberg K-H. Berikat koksalt som jodkälla (Fortified table salt as iodine source). Vår Föda 1980; 32: 338–44.
3. Jod – er der behov for berigelse af kosten? Jodindtagelsen i Danmark og betydningen for forekomsten af struma og stofskiftesygdomme. Publikation nr. 230. Søborg: Levnedsmiddelstyrelsen, 1995. Rasmussen LB, Andersen G, Haraldsdóttir J, Kristiansen E, Molsted K, Laurberg P, Overvad K, Perrild, H, Ovesen L. Iodine. Do we need an enrichment program in Denmark. Int J Food Sci Nutr 1996; 47: 377–81.
4. Knudsen B, Bülow I, Jørgensen T, Laurberg P, Ovesen L, Perrild H. Goitre prevalence and thyroid abnormalities at ultrasonography: a comparative epidemiological study in two regions with slightly different iodine status. Clin Endocrin 2000; 53: 479–85.
5. Stanbury JB. Iodine deficiency and the iodine deficiency disorders. In: Present knowledge in nutrition, 7th ed. Washington, DC: ILSI Press: 1996: 378–83.
6. Jahreis G, Hausmann W, Kiessling G, Franke K, Leiterer M. Bioavailability

of iodine from normal diets rich in dairy products – results of balance studies in women. Exp Clin Endocrinol Diabetes 2001; 109: 163–7.
7. Hurrell RF. Bioavailability of iodine. Eur J Clin Nutr 1997; 51 (Suppl 1): S9–S12.
8. Dorea JG. Iodine nutrition and breastfeeding. J Trace Elem Med Biol 2002; 16: 207–20.
9. Nøhr SB, Laurberg P, Børlum K-G, Pedersen KM, Johannesen PL, Damm P, *et al.* Iodine status in neonates in Denmark: regional variations and dependency on maternal iodine supplementation. Acta Pædiatr 1994; 83: 578–82.
10. Laurberg P, Nohr SB, Pedersen KM, Fuglsang E. Iodine nutrition in breast fed infants is impaired by maternal smoking. J Clin Endocrinol Metab. 2004; 89: 181–7.
11. Zagrodzki P, Szmigiel H, Ratajczak R, Szybinski Z, Zachwieja Z. The role of selenium in iodine metabolism in children with goiter. Environ Health Perspect 2000; 108: 67–71.
12. Hess SY, Zimmermann MB, Adou P, Torresani T, Hurrell RF. Treatment of iron deficiency in goitrous children improves the efficacy of iodized salt in Cote d'Ivoire. Am J Clin Nutr 2002; 75: 743–48.
13. Eltom A, Elnagar B, Elbagir M, Gebre-Medhin M. Thyroglobulin in serum as an indicator of iodine status during pregnancy. Scand J Clin Lab Invest 2000; 60: 1–8.
14. Knudsen N, Bulow I, Jørgensen T, Perrild H, Ovesen L, Laurberg P. Serum Tg – a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiological studies. J Clin Endocrinol Metab 2001; 86: 3599–603.
15. Gutekunst R, Smolarek H, Hasenpusch U, Stubbe P, Friedrich H-J, Wood WG, Scriba PC. Goitre epidemiology: thyroid volume, iodine excretion, thyroglobulin and thyrotropin in Germany and Sweden. Acta Endocronologica 1986; 112: 494–501.
16. Milakovic M, Berg G, Eggertsen R, Lindstedt G, Nystrom E. Screening for thyroid disease of 15–17-year-old school-children in an area with normal iodine intake. J Intern Med 2001; 250: 208–12.
17. Hetzel BS, Dunn JT. The iodine deficiency disorders: Their nature and prevention. Ann Rev Nutr 1989; 9: 21–38.
18. National Research Council. Recommended dietary allowances. 10th ed. Washington DC: National Academy Press, 1989.
19. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community (Thirty-first series of Food Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1993.
20. Food and Nutrition Board. Dietary reference intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of Medicine, National Academic Press, Washington: 2000
21. Delange F, Benker G, Caron P, Eber O, Ott W, Peter F, Podoba J, Simescu M, Szybinsky Z, Vertongen F, Vitti P, Wiersinga W, Zamrazil V. Thyroid volume and urinary iodine in European schoolchildren: standardization of values for assessment of iodine deficiency. Eur J Endocrinol 1997; 136: 180–7.
22. Delange F, Bourdoux P, Vo Thi LD, Ermans AM, Senterre J. Negative iodine balance in preterm infants. Ann Endocrinol 1984; 45: 77.
23. Elnagar B, Eltom A, Wide L, Gebre-Medhin M, Karlsson FA. Iodine status, thyroid function and pregnancy: study of Swedish and Sudanese women. Eur J Clin Nutr 1998; 52: 351–55.
24. Alexander J, Borch-Iohnsen B, Frey H, Kumpulainen J, Meltzer HM, Grawé KP, *et al.* Risk Evaluation of Essential Trace Elements – essential *versus* toxic levels of intake. Oskarsson A, editor. København: Nordic Council of Ministers, Nord 1995:18.

25. Wolff J. Iodide goiter and the pharmacological effects of excess iodide. Am J Med 1969; 47: 101–24.
26. Coakley JC, Francis I, Gold H, Mathur K, Conelly JF. Transient primary hypothyroidism in the newborn: Experience of the Victorian Neonatal Thyroid Screening Programme. Aust Pediatr J 1989; 25: 25–30.
27. Lamberg BA. Jodin terveydellinen merkitys (Betydelsen av jod för hälsan). Kivennäis- ja hivenaineet. Valtion Neuvottelukunnan Julkaisuja N: o 3: 43–50, Helsinki 1975.
28. Skare S, Frey HMM. Iodine induced thyrotoxicosis in apparently normal thyroid glands. Acta Endocrinol 1980; 94: 332–6.
29. Jørgensen H, Svinland O. Hyper- og hypotyreose etter bruk av jodholdige 'naturprodukter' og jodholdige vitamin- og mineraltilskudd (Hyper- and hypothyreosis after use of iodine containing 'health products' and iodine containing vitamin and mineral supplements). Tidsskr Nor Lægeforen 1991; 111: 3153–5.
30. Stewart JC, Vidor GI, Buttfield IH, Hetzel BS. Epidemic thyrotoxicosis in Northern Tasmania: Studies of clinical features and iodine nutrition. Aust N Z J Med 1971; 3: 203–11.
31. Schröder F. Hyperthyreosemanifestation vor und nach alimentärer Jodkomplettierung. Verlaufsuntersuchungen in Brandenburg 1983–1988. Z Gesamt Med 1991; 46: 573–80.
32. Galofré JC, Fernàndez-Calvet L, Rios M, Garcia-Mayor RVG. Increased incidence of thyrotoxicosis after iodine supplementation in an iodine sufficient area. J Endocrinol Invest 1994; 17: 23–7.
33. Laurberg P, Jørgensen T, Knudsen N, Bülow Pedersen I, Perrild H, Banke Rasmussen L, Ovesen L, Implementation and monitoring of iodine supplementation in Denmark: The DanThyr Program. IDD Newsletter, 2003; 19: 55–9.
34. Harach HR, Escalante DA, Onativia A, Outes JL, Day ES, Williams ED. Thyroid carcinoma and thyroiditis in an endemic goitre region before and after iodine prophylaxis. Acta Endocrinol 1985; 108: 55–60.
35. NNT. The influence of chemicals in the food and the environment on the thyroid gland function. TemaNord 2002:520, Nordic Council of Ministers, Copenhagen.
36. Kämpe O, Jansson R, Karlsson FA. Effects of L-thyroxine and iodide on the development of autoimmune postpartum thyroiditis. J Clin Endocrinol Metab 1990; 70: 1014–8.
37. Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregersen HE, Rasmussen OS, Larsen KR *et al.* Amelioration of some pregnancy-associated variations in thyroid function by iodine supplementation. J Clin Endocrinol Metab 1993; 77: 1078–83.
38. Nøhr SB, Jørgensen A, Pedersen KM, Laurberg P. Postpartum thyroid dysfunction in pregnant thyroid peroxidase antibody-positive women living in an area with mild to moderate iodine deficiency: is iodine supplementation safe? J Clin Endocrinol Metab 2000; 85: 3191–98).
39. Laurberg P, Andersen S, Pedersen IB, Ovesen L, Knudsen N. Humic substances in drinking water and the epidemiology of thyroid disease. Biofactors, 2003; 19, 145–53.
40. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of iodine (expressed on 26 September 2002). SCF/CS/NUT/UPPLEV/ 26 Final. 7 October 2002. European Commission. Health and Consumer Protection Directorate General.
41. Dahl L, Opsahl JA, Meltzer HM, Julshamn K. Iodine concentration in Norwegian milk and dairy products. British Journal of Nutrition 2003; 90: 679–85.
42. Sjöström G. Jodhalten i svenska vatten. Nord Hyg Tidskr 1956; 27: 265–82.
43. Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P, Perrild H. Dietary intake and urinary iodine excretion in a Danish population: effect of geography, supple-

ments and food choice. Br J Nutr 2002; 87: 61–69.
44. Pedersen JI, Frølich W, Johansson L, Nordgård H, Trygg K. Behovet for tilsetning av næringsstoffer til matvarer i Norge. Scand J Nutr 1995; 39: 84–87.
45. Dahl L, Meltzer HM, Johansson L, Julshamn K. The iodine content of Norwegian foods and diets. Publ Health Nutr 2003; 7: 569–76.
46. Valsta L, Lamberg-Allardt C, Bourdoux P, Laurinen M, Kärkkäinen M, Outila T, Aro A, Pietinen P. Iodine status of middle-aged subjects in Finland. Abstract. Finnish Society for Nutrition Research. Spring meeting 20.03.03.
47. Frey H, Rosenlund D, Try K, Theodorsen L. Urinary excretion of iodine in Norway. pp 297–300. I: Delange F *et al.* editors. Iodine deficiency in Europe. New York: Plenum Press, 1993.
48. Dahl L, Meltzer HM, Opsahl JA, Julshamn K. Iodine intake and status in two groups of Norwegians. Scand J Nutrition 2003; 47: 170–78.
49. Rauma A-L, Törmöla M-L, Nenonen M, Hänninen O. Iodine status in vegans consuming a living food diet. Nutr Res 1994; 14: 1789–95.

CHAPTER 37

Selenium

Selenium, µg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	40	50	25	30	40
Average requirement	AR	30	35			
Lower level of intake	LI	20	20			

Selenium is found in all tissues, mainly as selenomethionine, an analogue of the sulphur-containing methionine, and as selenocysteine in various selenoproteins. The main biological functions of selenium are thought to be mediated by the glutathione peroxidases and other selenoproteins. Severe selenium deficiency may cause cardiomyopathy but, on the other hand, toxic symptoms are caused by excessive selenium intake. Organic and inorganic selenium compounds have different kinetics and different bioavailability for man.

Physiology and metabolism

Water-soluble selenium compounds and dietary selenium (mainly organic selenium in forms such as selenomethionine and selenocysteine) are effectively absorbed; selenates and organic selenium somewhat better than selenites. Selenium compounds are converted to selenides before they are incorporated into specific selenoproteins. Selenomethionine is incorporated as such in a number of unspecific proteins, and inorganic selenium salts are retained less effectively since a major proportion is excreted in the urine. At high intakes, detoxified excretory products such as dimethyl selenide and trimethyl selenonium ions are formed. The former is exhaled via the lungs and the latter excreted in the urine. Dietary selenium affects the selenium concentration in serum and red blood cells, which are good biomarkers for organic selenium intake.

The bioavailability of selenium from fish has been poor in some studies in experimental animals, but there have been differences between various fish species. In humans, selenium has been shown to be readily available from Baltic herring and rainbow trout (9, 16). In a study with

stable isotopes, selenium from trout showed apparent absorption equal to, and retention higher than, that of selenate (9). Even some human studies have suggested reduced bioavailability from fish compared with other selenium-containing foods (15, 20). The reasons for the observed differences in bioavailability are not clear.

Men and women have similar selenium concentrations in the serum despite different intakes.

Part of selenium in tissues is composed of functional selenoproteins. The human selenoproteome has recently been reported to consist of 25 selenoproteins. These include the glutathione peroxidases: cellular (cGSHPx), extracellular (eGSHPx), phospholipid hydroperoxide (phGSHPx) and gastrointestinal (giGSHPx) which, together with certain other metalloenzymes, protect tissues against oxidative damage. It is anticipated that the essentiality of selenium is based on the effect of GSHPx and other selenoproteins. The types I, II and III iodothyronine deiodinases that produce tri-iodothyronine and related metabolites from thyroxine are also selenoproteins. Selenium also affects the activity of the selenoproteins thioredoxin reductases, which have a number of physiological functions (3). Other selenoproteins with unknown functions are selenoprotein P, selenoprotein W and prostatic epithelial selenoprotein (14).

Requirement and recommended intake

A type of cardiomyopathy, affecting particularly children and fertile women and associated with low intake of selenium (< 20 µg/d), has been found in certain parts of China (13). Similar findings have been observed in some isolated cases during parenteral nutrition without selenium supplementation. In two Finnish studies from the 1970s, low serum selenium levels (< 45 µg/L) were associated with increased risk of cardiovascular death (18), and in Denmark men with serum selenium concentrations within the lowest third of the population were shown to have increased risk of myocardial infarction (19).

The daily losses of selenium are determined by previous dietary intake and tissue stores and give only limited information about requirements. The requirement is assumed to depend on body size. Selenium intakes of 30–40 µg/d are needed in order to achieve maximal GSHPx activity in the serum. In red blood cells and platelets, intakes of 80 µg/day and 120 µg/d, respectively, are needed for maximal GSHPx activity. It is not probable, however, that maximal GSHPx activity in all tissues is necessary for optimal health. The effect of varying selenium intakes on

the activity of newly discovered selenoproteins has not been studied in humans.

Information on selenium requirements for children and pregnant and lactating women is incomplete. During continued lactation, the selenium concentration of mother's milk is reduced over time when selenium intake is less than 45–60 µg/d, but remains unchanged at intakes of 80–100 µg/day.

The recommendations of different countries are usually based on a Chinese study showing maximal stimulation of plasma GSHPx activity by selenium supplementation (30 µg/d) in people whose basal intake was 11 µg/d (21). The NNR recommendation is based on the mean + 2 SD of this study and is adjusted for difference in mean body weight. The recommended intake is 50 µg/d for men and 40 µg/d for women, which is the same as in NNR 1996. The recommendation of the EU SCF and the US Institute of Medicine is 55 µg/d both for men and women (10). For pregnant and lactating women, the SCF recommendation is 55 and 70 µg/d, respectively, and the recent US recommendation 60 and 70 µg/day. The NNR 1996 recommendation of 55 µg/d for both pregnant and lactating women is maintained. The lower level of intake for adults is also kept unchanged from NNR 1996, *i.e.* 20 µg/day. The RI values for children and adolescents are derived from the values for adults.

According to experimental animal studies and some observational epidemiological studies, higher selenium intakes might reduce the risk of certain types of cancer (11). In a Chinese supplementation study with selenium combined with beta-carotene and vitamin E, the incidence of stomach cancer was reduced. In a US study in skin cancer patients, supplementation with 200 µg/d organic selenium failed to affect the risk of new skin cancer but reduced the risk of lung, colorectal and prostate cancer (7). There is no information from clinical trials on the effects of selenium supplementation in healthy Western populations (17). Therefore it is at present impossible to take into account the possible benefits of increased selenium intakes on cancer risk in the recommendations. Some findings suggest that selenium deficiency might be associated with the development of new, more virulent virus strains (5) and that the selenium-dependent cardiomyopathy might be one example of the interaction between selenium deficiency and viral infection.

Upper intake levels and toxicity

Selenium intoxication is rare in man but well known in animals. Acute toxicity has been observed after consumption of a large (250 mg) single dose or after multiple doses of ~30 mg. The symptoms include nausea,

vomiting and garlic-like breath odour. Other toxic symptoms are nail and hair deformities and, in severe cases, peripheral nerve damage and liver damage. Because of the risk of toxicity, high doses of selenium are not recommended and an upper level of intake is needed. A no observed adverse effect level (NOAEL) for clinical signs of selenium toxicity and a threshold of 850 µg/d for inhibited prothrombin synthesis were found in Chinese studies. An upper level of 300 µg/d was derived by the SCF using a factor of 3 to allow for uncertainties in different studies.

Dietary sources and intake

Foods contain a number of selenium compounds. In animal foods there are specific selenoproteins containing selenocysteine. Foods of both animal and plant origin contain selenomethionine and possibly also some selenocysteine incorporated into proteins. Inorganic forms of selenite and selenate are used in dietary supplements but they are not normally found in food.

The selenium concentration of foods varies in different countries depending on the origin. Fish and other seafood, eggs and offal are relatively rich in selenium everywhere. Cereal products and vegetables grown in the Nordic countries, with the exception of Finland after 1984, have a low selenium content whereas wheat imported from North America has a high selenium content. The selenium concentration of meat and milk depends on the amount of organic selenium in animal feeds. Fodder is generally enriched with selenite, which has only a limited effect on the selenium concentration of meat and milk. In Finland, agricultural fertilizers have been supplemented with selenium since 1984 (4). This has increased the intake of organic selenium of both people and animals. Meat (40%), dairy products and eggs (25%) and cereal products (20%) are the most important sources of selenium in the diet of Finns nowadays (8). In Norway and Iceland, the intake of selenium has been influenced by high-selenium wheat imported from North America.

Different chemical forms of selenium have different bioavailability and metabolism. Organic selenium and most inorganic selenium salts are easily absorbed from the gut. Selenium in serum is most effectively increased by selenium-rich wheat or yeast, which is non-specifically incorporated into proteins. Inorganic selenite and selenate are less effective in increasing serum selenium concentration but they effectively increase the activity of GSHPx by incorporation via selenide and selenocysteine.

Selenium intake (per 10 MJ) of the Nordic countries according to recent dietary surveys is around 40 µg in Sweden, 40–45 µg in Denmark,

60–80 µg in Norway and around 90 µg in Finland and Iceland (1, 2, 6, 8, 12). The average serum selenium concentration in Sweden and Denmark is 70–80 µg/L, and in Finland 100–120 µg/L (1, 8).

REFERENCES

1. Alexander J, Melzer HM. Selenium. In: Oskarsson A (editor). Risk evaluation of essential trace elements – essential *versus* toxic levels of intake. Copenhagen: Nordic Council of Ministers, Nord 1995:18: 9–54.
2. Andersen NL, Fagt S, Groth MV *et al.* Danskernes kostvaner 1995. Hovedresultater. Publikation nr. 235. Søborg: Levnedsmiddelstyrelsen, 1996.
3. Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem. 2000; 267: 6102–6109.
4. Aro A, Alfthan G, Ekholm P, Varo P. Effects of selenium supplementation of fertilizers on human nutrition and selenium status. In: Environmental chemistry of selenium. Frankenberger WT Jr, Engberg RA, eds. New York: Marcel Dekker 1998: 81–97.
5. Beck MA, Levander OA. Host nutritional status and its effect on a viral pathogen. J Infect Dis 2000; 182: S93–S96.
6. Becker W, Pearson M. Riksmaten 1997–98 Kostvanor och näringsintag i Sverige. Metod- och resultatsanalys. (Dietary habits and nutrient intake in Sweden 1997–98). National Food Administration, 2002.
7. Clark LC, Combs GF, Turnbull BW, Slate EH, Chalker DK, Chow J, *et al.* Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. JAMA 1996; 276: 1957–1963
8. Eurola M, Alfthan G, Aro A *et al.* Results of the Finnish selenium monitoring program 2000–2001. Agrifood Research Reports 36. MTT Agrifood Research Finland, Jokioinen 2003.
9. Fox TE, Van den Heuvel EGHM, Atherton CA, Dainty JR, Lewis DJ, Langford NJ, *et al.* Bioavailability of selenium from fish, yeast and selenate: a comparative study in humans using stable isotopes. Eur J Clin Nutr 2004; 58: 343–349
10. Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. Washington DC: National Academy Press 2000: 284–324
11. Ip C. Lessons from basic research in selenium and cancer prevention. J Nutr 1998; 188: 1845–1854.
12. Johnsson L, Åkesson B, Alexander J. Availability of selenium from soils in relation to human nutritional requirements in Sweden – is there a need for supplementation? Swedish Environmental Protection Agency, Report 4711, 1997: 1–104.
13. Keshan Disease Research Group. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. Chin Med J 1979; 92: 477–482.
14. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science 2003; 300: 1439–1443.
15. Meltzer HM, Bibow K, Paulsen IT, Mundal HH, Norheim G, Holm H. Different bioavailability in humans of wheat and fish selenium as measured by blood platelet response to increased dietary Se. Biol Trace Element Res 1993; 36: 229–241.
16. Mutanen M. Bioavailability of selenium. Ann Clin Res 1986; 18: 48–54.
17. Rayman MP. The importance of selenium in human health. Lancet 2000; 356: 233–241
18. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982; ii: 175–179.

19. Suadicani P, Hein HO, Gyntelberg F. Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3,000 males. Atherosclerosis 1992; 96: 33–42.
20. Thorngren M, Åkesson B. Effect of dietary fish on plasma selenium and its relation to haemostatic changes in healthy adults. Int J Vitamin Nutr Res 1987; 57: 429–435.
21. Yang G-Q, Zhu L-Z, Liu S-J *et al*. Human selenium requirements in China. In: Selenium in biology and medicine, pp 589–607. Combs GF Jr, Levander OA, Spallholz JE, Oldfield JE, eds. New York: Avi 1987.
22. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the tolerable upper intake level of selenium. SCF/CS/NUT/UPPLEV/25 Final. 28 November 2000. European Commission. Health and Consumer Protection Directorate General.

CHAPTER 38

Copper

Copper, mg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	0.9	0.9	0.4	0.5	0.7
Average requirement	AR	0.7	0.7			
Lower level of intake	LI	0.4	0.4			

Copper has two oxidation states and is involved in oxidation and reduction processes inside cells. Copper functions as a component of a number of enzymes involved in energy metabolism, formation of connective tissue and defence against free radicals.

Physiology and metabolism

Copper absorption occurs primarily in the small intestine. At normal dietary intakes (1–5 mg/d) absorption varies between 35 and 70%. Copper is bound to intestinal metallothionein, a protein that is induced by zinc. At high zinc intakes (> 50 mg/d), copper absorption is therefore inhibited. Absorption of copper probably occurs by a saturable, active transport mechanism at lower levels of dietary copper; at high levels of dietary copper, passive diffusion plays a role (1) The majority of the absorbed copper is transported to the liver where it is incorporated into newly synthesised caeruloplasmin, metallothionein or cuproproteins. Homeostasis of copper is regulated to some extent by absorption, but primarily through excretion via bile and approximately 0.5 to 1.5 mg copper per day is excreted through the intestinal tract in this way. Urinary excretion of copper is low.

The total body content of copper for an adult is approximately 50 to 120 mg: 40% is contained in muscle tissue, 15% in liver, 10% in brain, and approximately 6% in blood. Newborn infants have a higher content of copper in the liver than adults, and this might act as a store of copper during the first couple of months. Copper deficiency in humans is rare, but has been found in a number of circumstances. Copper deficiency has been observed in premature infants fed milk formula, in infants recovering from malnutrition associated with chronic diarrhoea and

fed cow's milk (2), and in patients with prolonged total parenteral nutrition without additional copper. Symptoms of copper deficiency in children are low concentrations of white blood cells, anaemia, and hair and skin depigmentation (3). Heart and skeletal abnormalities have also been observed. Most of the symptoms can be related to the copper-containing enzymes.

There is substantial evidence from animal studies to suggest that diets low in copper reduce the activity of many of the copper-dependent metalloenzymes. The activity of some of these metalloenzymes has also been shown to decrease during human copper depletion (4, 5).

There is also evidence that immune and cardiac dysfunction can occur during experimental copper deficiency and the development of such signs of deficiency has been demonstrated in infants (5, 6).

Serum copper and caeruloplasmin concentration are currently used as biochemical indices of copper status and may be used to detect severe copper deficiency.

The decline in serum copper and caeruloplasmin concentrations observed when healthy young men were fed a diet containing 0.38 mg/d of copper for 42 days was reversed by copper supplementation (7). In a number of other studies with higher levels of copper intake, (0.66 mg/d and above), serum copper and caeruloplasmin concentrations did not decline significantly (8, 9), suggesting sufficient intake.

The dietary copper intake at which caeruloplasmin concentration no longer increases in response to increased dietary copper might be considered the copper requirement for caeruloplasmin synthesis. Other suggested indices of copper status include platelet copper concentration and cytochrome C oxidase activity, both of which declined in adults who had a copper intake of 0.57 mg/d (10).

Requirement and recommended intake

Adults

The precise requirement for copper is not known. Indications of deficient copper status, using superoxide dismutase (SOD) activity as a marker of Cu status, have been reported with intakes of 0.7 to 1 mg/d (11–13). However, other studies with less extreme intervention diets have not found indications of changes in copper status; SOD, caeruloplasmin or plasma Cu at intakes of 0.79 mg/d for 42 days (9). In a subsequent study, an intake of 0.66 mg/d for 24 days followed by an intake of 0.38 mg/d for 42 days resulted in decreasing indicators of copper status with time in young men (7, 14). Although the levels did not fall into the deficient range, a steady state was not completely reached. There are

thus limited data to establish an average requirement for copper for adults, but the available data indicate that an intake of approximately. 0.7–0.8 mg/d will maintain adequate copper status, *i.e.* plasma copper, caeruloplasmin and SOD. The US Food and Nutrition Board base their recommended copper intake for adults on a number of indicators including plasma and platelet copper concentration, serum ceruloplasmin concentration and erythrocyte SOD in controlled depletion-repletion studies (15). Data on obligatory copper losses were also used. Based on these indicators an average requirement was estimated to be 0.7 mg (700 µg)/d for adults. With a coefficient of variation of 15%, the RDA was calculated to be 0.9 mg (900 µg)/day. This approach is also adopted in NNR.

Children

The copper content of human milk is highest during early lactation and then declines during the course of lactation. The mean copper content of human milk during the first 6 months of lactation is approximately 0.25 mg/L (16–18). There are no indications of inadequate copper status in breast fed infants. For infants 6–11 months the requirements are based on extrapolation from adults with allowance for growth.

The copper requirements for children more than one year old have been calculated from estimates of adult requirement with allowance for growth (15).

Pregnancy and lactation

The extra requirement for copper in pregnancy is relatively low, approximately 0.15 mg/d in the last trimester, and is probably met by adaptation.

The copper content of human milk is approximately 0.22 mg/L. With a milk production of approximately 750 ml/d and an estimated 50% absorption, an extra 0.3 mg/d is recommended.

Upper intake levels and toxicity

Intake of high doses of copper leads to acute toxicity, which produces symptoms of gastric pain, nausea, vomiting and diarrhoea. Storage of food in non-galvanised copper containers is associated with the risk of childhood sclerosis (19). In areas with soft water, copper can leach from copper tubes and result in high copper concentrations (more than 100 mg/L) in drinking water. Gastro-intestinal disturbances have been seen with intakes of copper-contaminated water containing 3.7 mg/L (20). Infants are probably the most sensitive group and case studies have indicated an association with intake of copper. Recent controlled and

population-based studies found weak evidence for a role of copper from drinking water at concentrations up to 2 mg/L (21). However, it is considered prudent to recommend letting water run before it is used for consumption by that age group, especially when used for formula.

Recently, the Scientific Committee on Food has proposed an upper limit of 5 mg/d to be safe for adults (22). This is based on the absence of negative effects during copper supplementation and includes a safety factor.

Dietary sources and intake

Copper is widely distributed in food. The highest levels of copper are found in liver and other offal, while milk and milk products have a low copper content. The intake of copper in the Nordic countries varies between 1.0 and 2.0 mg/d (23).

REFERENCES

1. Turnlund JR. Future directions for establishing mineral/trace element requirements. J Nutr. 1994; 124 (9 Suppl): 1765S–1770S.
2. Shaw JCL. Copper deficiency in term and preterm infants. In: Nutritional Anemias. Fomon SJ, Zlotkin S eds. New York, Vevey/Raven Press. 1992, 105–117.
3. Danks DM. Copper deficiency in humans. Ann Rev Nutr 1988; 8: 235–57.
4. Milne DB. Assessment of copper nutritional status. Clin Chem. 1994; 40: 1479-1484.
5. Turnlund JR. Copper. In: Modern nutrition in Health and disease (eds Shils M, Olson JA, Shike M, Ross AC), Williams and Wilkins, Baltimore. 1999, 241–252.
6. Olivares JA and Uauy R. Limits of metabolic tolerance to copper and biological basis for present recommendations and regulations. Am J Clin Nutr 1996; 63: 846S–852S.
7. Turnlund JR, Scott KM, Pfeiffer GL, Jang AM, Keyes WR, Keen CL, Sakanashi TM. Copper status of young men consuming a low copper diet. Am J Clin Nutr 1997; 65: 72–78.
8. Milne DB. Copper intake and assessment of copper status. Am J Clin Nutr 1998; 67: suppl 1041S–1045S.
9. Turnlund JR, Keen CL, Smith RG. Copper status and urinary and salivary copper in young men at three levels of dietary copper. Am J Clin Nutr 1990; 51: 658–664.
10. Milne DB, Nielsen FH. Effects of a diet low in copper on copper-status indicators in postmenopausal women. Am J Clin Nutr 1996; 63: 358–364.
11. Reiser S, Smith JC Jr, Mertz W, Holbrook JT, Schofield DJ, Powell AS, Canfield WK, Canary JJ. Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am J Clin Nutr 1985; 42: 242–251.
12. Lowy SL, Fisler JS, Drenick EJ, Hunt IF, Swenseid ME. Zinc and copper nutriture in obese men receiving very low caloric diets of soy or collagen protein. Am J Clin Nutr 1986; 42: 272–287.
13. Lukaski HC, Klevay LM, Milne DB. Effects of dietary copper on human autonomic cardiovascular function. Eur J Appl Physiol; 1988; 58: 74–80.

14. Turnlund JR, Thompson KH, Scott KC. Key features of copper *versus* molybdenum metabolism models in humans. Adv Exp Med Biol. 1998; 445: 271–81.
15. Institute of Medicine. Dietary reference intakes for vitamin A, Vitamin K, Arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC 2001.
16. Biego GH, Joyeux M, Hartemann P, Debry G. Determination of mineral contents in different kinds of milk and estimation of dietary intake in infants. Food Addit Contam 1998; 15: 775–781.
17. Raiten DJ, Talbot JM, Walters JH. Assessment of nutrient requirements for infant formulas. J Nutr 1998; 128: 2059S–2294S.
18. Rossipal E, Krachler M. Pattern of trace elements in human milk during the course of lactation. Nutr Res 1998; 18: 11–24.
19. Bhargave SK. Indian childhood cirrhosis. Indian Pediatr 1982; 19: 961–962.
20. Spitalny KC, Brindum J, Vogt RL, Sargent HE, Kappel S. Drinking water induced copper intoxication in an Vermont family. Pediatr 1984; 74: 1103–1106.
21. Pettersson R, Rasmussen F, Oskarsson A. Copper in drinking water: not a strong risk factor for diarrhoea among young children. A population-based study from Sweden. Acta Pædiatr 2003; 92: 473–80.
22. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper. SCF/CS/NUT/UPPLEV/57 Final 27 March 2003. http://europa.eu.int/comm/food/fs/sc/scf/out176_en.pdf
23. Alexander J, Borch-Johnson B, Frey H, Kumpulainen J, Meltzer HM, Grawé KP *et al.* Risk evaluation of essential trace elements – essential *versus* toxic levels of intake. Oskarsson A editor. København, Nordic Council of Ministers, Nord 1995:18.

CHAPTER 39

Chromium

In ionic form chromium exists in many valence states. Trivalent chromium (III) is the most stable form and the principal form of chromium found in foods and supplements. It is ubiquitous in nature, occurring in air, water, soil and biological materials. Hexavalent chromium (VI) makes chromates and dichromates, which are strongly oxidizing and transverse biological membranes. Hexavalent chromium compounds occur only rarely in the environment, and are almost always man-made. They are toxic, mutagenic and environmental contaminants.

Physiology and metabolism

The absorption of trivalent chromium from the diet is low, 0.4–2.5% (1). The element is mainly excreted via urine, with only small amounts being eliminated in sweat and bile. Organic chromium compounds are absorbed more efficiently but are rapidly excreted via bile. Simultaneous ascorbate administration increases chromium uptake both in humans and animals. Chromium absorption is also higher in both zinc- and iron-deficient animals.

The exact biological function of chromium has not yet been determined. Experimental chromium deficiency in animals results in reduced glucose tolerance in spite of normal insulin levels. Other deficiency signs in animals include impaired growth, elevated serum cholesterol and triglycerides, increased incidence of aortic plaques, corneal lesions and decreased fertility and sperm count.

Chromium is considered to be a cofactor for insulin, possibly through influencing membrane receptors. A low molecular weight chromium-binding substance is believed to be involved in the process (2). It has also been suggested that chromium influences carbohydrate, lipid and protein metabolism via its effect on insulin action.

Three cases have been reported of possible chromium deficiency in humans after long-term, parenteral nutrition (3–5). The symptoms observed were impaired glucose tolerance and glucose utilization, weight loss, neuropathy, elevated plasma fatty acids, depressed respiratory quo-

tient and abnormalities in nitrogen metabolism. The symptoms improved after chromium supplementation (200 µg/d). However, the reported concentrations of chromium in blood and urine were above those considered normal even before the supplementation was initiated. As with foodstuffs, analytical data on chromium concentrations in biological specimens produced before 1978 should be regarded with caution, as possible contamination in sampling and processing may have led to spuriously high values of chromium (1).

A number of chromium supplementation studies have been published investigating effects on insulin and blood glucose levels, but control groups have been missing or the experimental design has in other ways been inadequate. Only one study in which controlled amounts of dietary chromium were given has been reported (6). In addition, lack of reliable biomarkers for chromium status has had a major contributory role for the current uncertainties about the biological significance of chromium as an essential micro-mineral.

There are also quite a few studies looking at chromium supplementation in relation to body composition, exercise and weight loss. Most studies have used chromium picolinate, but have failed to show an independent effect of chromium supplements (1).

Requirements and recommended intake

As described above, the role of chromium as an essential nutrient is still unclear. If chromium is an essential metal it must have a specific role in an enzyme or cofactor, and a deficiency should produce a disease or impairment of function. Methods for evaluating chromium status are missing. Furthermore, there is still uncertainty about how chromium deficiency in humans manifests itself, and the requirement for chromium is accordingly not known.

The EU SCF stated in 1993 stated that 'Since data on the essentiality and metabolism of chromium are so sparse, the Committee is unable to specify any requirements' (7).

The UK Committee on Medical Aspects of Food Policy calculated a theoretical requirement for adults from balance studies of 23 µg/d by using regression equations and mentioned that a safe and adequate level of intake is believed to lie above 25 µg/d for adults (8).

Using well-balanced diets as a basis, the US Food and Nutrition Board calculated Adequate Intakes for chromium for different age groups: for men (19–50 years) 35 µg/d and for women 25 µg/d (9).

The NNR 1996 did not include recommendations for chromium intake. As very few relevant new human studies have been conducted

since then, requirements are also impossible to establish this time and accordingly, recommendations have not been set for any age group.

Data are also lacking on the requirements during pregnancy. The US Food and Nutrition Board suggests an increase of 5 µg/d during pregnancy (9). Human milk contains 0.06–0.7 µg/L, which indicates that the requirement in humans may be very low. Exclusively breast fed infants would receive only 130 ng/d based on a concentration of 0.18 µg/L (10). A study on lactating Finnish mothers found an average concentration of 0.4 µg/L (range 0.2–0.7) (11). Women do not appear to reduce urine chromium excretion during lactation to compensate for increased need (12).

Upper intake levels and toxicity

Chromium III has low toxicity, no adverse effects were observed at intakes of 1,000–2,000 µg/day.

Due to the lack of adequate data, the EU Scientific Committee on Food has not suggested a tolerable upper intake level (UL) for chromium (III) salts (13). The same conclusion was reached by the US Food and Nutrition Board (9) and the UK Expert Group on Vitamins and Minerals (17).

Chromium picolinate, a trivalent chromium compound popular in many food supplements, is being discussed because of possible adverse health effects. It may influence the central nervous system and thus behaviour (14). Another study has suggested kidney damage as a result of high doses of chromium supplementation (15). Potential clastogenecity is being discussed (16). It is still unclear whether these effects are due to the picolinate fraction or to the higher extent of chromium absorption. The UK Food Standards Agency advises people not to take chromium picolinate and has consulted on a proposal to ban the use of this form of chromium in the manufacture of food supplements because there is a chance that it could cause cancer (17).

Dietary sources and intake

Analysis of chromium in foods requires special sampling procedures to avoid chromium contamination from the environment (air, stainless steel, etc.). Old data on chromium contents of foods should therefore be used with care. Processed meats, fish, wholegrain products, nuts, pulses and spices are the best sources, while most other foods have low concentrations (< 100 µg/kg). Foods high in simple sugars, such as soft drinks and table sugar, are not only low in chromium content, but also promote chromium losses (18). Analysed or estimated intakes of chro-

mium in the diet of the Nordic countries are scarce, but are in the range 20–160 µg/d (19). Many food supplements contain chromium in doses of 50–100 µg per serving unit.

REFERENCES

1. Lukaski HC. Chromium as a supplement. Annu Rev Nutr 1999; 19: 279–302.
2. Sun Y, Ramirez J, Woski SA *et al.* The binding of trivalent chromium to low-molecular-weight chromium-binding substance (LMWCr) and the transfer of chromium from transferrin and chromium picolinate to LMWCr. J Biol Inorg Chem 2000; 5: 129–36.
3. Jeejeebhoy KN, Chu RC, Marliss EB *et al.* Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. Am J Clin Nutr 1977; 30: 531–8.
4. Freund H, Atamian S, Fischer JE. Chromium deficiency during total parenteral nutrition. JAMA 1979; 241: 496–8.
5. Brown RO, Forloines-Lynn S, Cross RE *et al.* Chromium deficiency after long-term total parenteral nutrition. Dig Dis Sci 1986; 31: 661–4.
6. Anderson RA, Polansky MM, Bryden NA *et al.* Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. Am J Clin Nutr 1991; 54: 909–16.
7. Reports of the Scientific Committee on Food. Nutrient and energy intakes for the European Community. Luxembourg: Commission of the European Communities, 1993.
8. COMA (Committee on Medical Aspects of Food Policy). Dietary reference values for food energy and nutrients for the United Kingdom. Department of Health Report 41. London: HMSO, 1991.
9. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
10. Anderson RA, Bryden NA, Patterson KY *et al.* Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. Am J Clin Nutr 1993; 57: 519–23.
11. Kumpulainen J, Vuori E. Longitudinal study of chromium in human milk. Am J Clin Nutr 1980; 33: 2299–302.
12. Mohamedshah FY, Moser-Veillon PB, Yamini S *et al.* Distribution of a stable isotope of chromium (53Cr) in serum, urine, and breast milk in lactating women. Am J Clin Nutr 1998; 67: 1250–5.
13. Scientific Committee on Food. Tolerable upper intake level of trivalent chromium. SCF/CS/NUT/UPPLEV/67 Final. 23 April 2003. European Commission. http://europa eu int/comm/food/fs/sc/scf/out80_en.html, 2003.
14. Reading SA. Chromium picolinate. J Fla Med Assoc 1996; 83: 29–31.
15. Cerulli J, Grabe DW, Gauthier I *et al.* Chromium picolinate toxicity. Ann Pharmacother 1998; 32: 428–31.
16. Bagchi D, Stohs SJ, Downs BW *et al.* Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology 2002; 180: 5–22.
17. Food Standards Agency. Safe upper levels for vitamins and minerals. London: Available on: www.foodstandards.gov.uk/, 2003.
18. Kozlovsky AS, Moser PB, Reiser S *et al.* Effects of diets high in simple sugars on urinary chromium losses. Metabolism 1986; 35: 515–8.
19. Jorhem L, Becker W, Slorach S. Intake of 17 elements by Swedish women, determined by a 24-hour duplicate portion study. J Food Comp Anal 1998; 11: 32–46.

CHAPTER 40

Manganese

Chemically, manganese is closely related to iron. It is a catalytic cofactor for arginase, pyruvate carboxylase and mitochondrial superoxide dismutase (SOD), but also functions as a specific or unspecific activator for a large number of other enzymes, some of which participate in the synthesis of proteins, mucopolysaccharides and cholesterol.

Physiology and metabolism

The total body content of manganese is estimated to be 10–20 mg. The concentration is relatively high in bone and in organs rich in mitochondria, such as liver, pancreas and kidney, while muscle and plasma have low concentrations. Absorption from the diet is low, approximately 5%, and excretion is primarily through the bile. Animal studies have shown that iron, calcium and phytic acid reduce the absorption of manganese (1). A negative effect of calcium has been confirmed in humans, while the effect of iron and phytic acid does not seem to be pronounced (2). High intakes of manganese inhibit iron absorption (3), and a higher absorption of manganese has been reported in iron deficiency (4).

Manganese deficiency in experimental animals results in reduced growth, skeletal abnormalities and defects in lipid and carbohydrate metabolism (1). In humans, only a limited number of possible manganese deficiency symptoms have been described in experimental studies with a manganese-deficient diet (5). Dermal changes and hypocholesterolaemia are possible signs of manganese deficiency, as well as diffuse bone demineralization and poor growth in children.

Requirement and recommended intake

Our knowledge of manganese metabolism and the consequences of low intakes are insufficient for determining requirements and recommended daily intakes for humans. Balance studies have suggested that an intake of 0.74 mg/d should be sufficient to replace daily losses of manganese (6). The EU Scientific Committee on Food considered a 'safe and adequate intake' to be 1–10 mg/person/d (7).

The US Food and Nutrition Board found data to be insufficient for setting an Estimated Average Requirement (EAR) for manganese (8), but used median intakes reported from the US Total Diet Study 1982–9 (9) as a basis for setting adequate intakes. The AI for adult men and women is set at 2.3 and 1.8 mg/d, respectively.

The NNR 1996 did not include recommendations for manganese intake. As very few relevant new human studies have been conducted since then, requirements are also difficult to determine this time, and accordingly, recommendations are not given for any age group.

Data are also too limited to determine requirements for manganese during pregnancy and lactation, and manganese deficiency of pregnant or lactating women has not been observed in humans.

Upper intake levels and toxicity

Manganese is regarded as one of the least toxic trace elements. Manganese toxicity, which manifests as psychological and neurological changes, has been observed in workers in manganese mines (4). The neurological symptoms are reminiscent of those seen in Parkinson's disease. Inhalation of manganese dust may be the explanation, while toxicity caused by a high dietary intake is unknown. The EU Scientific Committee on Food found that data for setting a tolerable upper intake level of manganese were too uncertain (10). The UK Foods Standards Agency has also found data to be insufficient to establish a safe upper level for manganese (11).

Dietary sources and intake

Unrefined cereals, nuts and leafy vegetables have high manganese content. Tea is a substantial contributor to manganese intake, containing about 2.7 mg/L. Accordingly, manganese intake varies from very low, < 2 mg/d, to high, >8 mg/d, in vegetarian diets.

The average content of manganese in the Swedish diet analysed in market baskets and duplicate portions collected in the late 1980s was 3.6–3.7 mg/d (12, 13). A Danish study where 100 men collected duplicate portions of their regular diets for 48 hours showed a manganese intake of 3.9 mg/d (14). The manganese intake of Finnish children 3–18 years of age was in the range of 3–7 mg/d calculated from food consumption data and food contents (14). These data indicate that manganese intake is adequate in these countries. Many multivitamin-mineral and mineral supplements for adults may provide 2–5 mg manganese/dose.

REFERENCES

1. Hurley LS, Keen CL. Manganese. In: Mertz W, ed. Trace elements in human and animal nutrition. San Diego: Academic Press, 1987: 185–223.
2. Davidsson L, Cederblad A, Lonnerdal B *et al.* The effect of individual dietary components on manganese absorption in humans. Am J Clin Nutr 1991; 54: 1065–70.
3. Rossander-Hulthén L, Brune M, Sandstrom B *et al.* Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr 1991; 54: 152–6.
4. Mena I, Horiuchi K, Burke K *et al.* Chronic manganese poisoning. Individual susceptibility and absorption of iron. Neurology 1969; 19: 1000–6.
5. Friedman BJ, Freeland-Graves JH, Bales CW *et al.* Manganese balance and clinical observations in young men fed a manganese-deficient diet. J Nutr 1987; 117: 133–43.
6. Freeland-Graves JH, Behmardi F, Bales CW *et al.* Metabolic balance of manganese in young men consuming diets containing five levels of dietary manganese. J Nutr 1988; 118: 764–73.
7. Reports of the Scientific Committee on Food. Nutrient and energy intakes for the European Community. Luxembourg: Commission of the European Communities, 1993.
8. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
9. Pennington JA, Young BE. Total diet study nutritional elements, 1982–1989. J Am Diet Assoc 1991; 91: 179–83.
10. Scientific Committee on Food. Tolerable upper intake level of manganese. SCF/CS/NUT/UPPLEV/22 Final. 28 November 2000. http://europa eu int/comm/food/fs/sc/scf/out80_en. 2003.
11. Expert Group on Vitamins and Minerals. Safe upper levels for vitamins and minerals. Food Standards Agency, London: 2003. Available on: www.foodstandards.gov.uk/.
12. Becker W, Kumpulainen J. Contents of essential and toxic mineral elements in Swedish market-basket diets in 1987. Br J Nutr 1991; 66: 151–60.
13. Jorhem L, Becker W, Slorach S. Intake of 17 elements by Swedish women, determined by a 24-hour duplicate portion study. J Food Comp Anal 1998; 11: 32–46.
14. Bro S, Sandström B, Heydorn K. Intake of essential and toxic trace elements in a random sample of Danish men as determined by the duplicate portion sampling technique. J Trace Elem Electrolytes Health Dis 1990; 4: 147–55.
15. Räsänen L, Ahola M, Kara R, Uhari M. Atherosclerosis precursors in Finnish children and adolescents. VIII. Food consumption and nutrient intakes. Acta Paediatr Scand Suppl. 1985; 318: 135–53.

CHAPTER 41

Molybdenum

Molybdenum has a number of valences and functions in oxidation-reduction reactions in plants and lower organisms. In humans only three molybdenum-containing enzymes are known: sulphite oxidase, xanthine oxidase and aldehyde oxidase. The enzymes are involved in catabolism of sulphur amino acids and heterocyclic compounds, including purines and pyridines.

Physiology and metabolism

Molybdenum absorption from the diet is efficient (> 80%). The body content is primarily regulated via the kidneys.

There is only one recorded case of apparent molybdenum deficiency, which occurred in a subject receiving total parenteral nutrition (50 µg Mo/d) for 18 months due to Crohn's disease (1). Unconsciousness, heart disturbances and night blindness were observed; the symptoms disappeared after supplementation with 160 µg Mo/day.

Turnlund *et al.* (2, 3) have used stable isotopes to investigate molybdenum metabolism in healthy men. Molybdenum absorption was efficient (about 90%) when subjects ingested diets containing five levels of the metal (ranging from 22 to 1,490 µg/d) for 24 days each. Excess molybdenum was rapidly excreted in urine, although whole-body retention was increased when the dietary level was low.

Requirement and recommended intake

WHO in 1996 estimated a daily requirement for molybdenum of between 0.1 and 0.3 mg/d for adults (4).

In 1993, the EU Scientific Committee on Food concluded that a requirement cannot be established reliably and, in the absence of evidence to the contrary, current intakes appear to be adequate and safe (5). At that point in time the research by Turnlund *et al.* had not been published.

Adult men fed a diet with only 22 µg/d molybdenum for 102 days did not develop any symptoms of molybdenum deficiency, leading Turn-

lund *et al.* (3) to suggest that the minimum daily requirement for this trace element is about 25 µg.

Based on the findings of Turnlund *et al.* (3), the US Food and Nutrition Board has set a Recommended Dietary Allowance (RDA) for adult men and women to 45 µg/d (6). The average dietary intake of molybdenum in US men and women is more than twice this level.

The requirement for pregnant and lactating women is not known, but the typical intake in the Nordic countries is high enough to allow for possible increased needs of this group.

The Nordic Recommendations of 1996 did not include recommendations for molybdenum intake. Although two human studies have been conducted since then, these are not considered sufficient to establish requirements. Accordingly, recommendations are not given for any age group.

Upper intake levels and toxicity

The absence of toxicity symptoms in men fed 1,490 µg molybdenum per day for 24 days (7) provides a working upper boundary for further studies.

The US Food and Nutrition Board set a Tolerable Upper Intake Level (UL) of 2 mg/d based on impaired reproduction and growth in animals (6). A British expert group concluded that there are insufficient data from animal and human studies to establish a safe upper level for molybdenum (8).

Dietary sources and intake

Molybdenum is ubiquitous in food and water as soluble molybdates, although the content of molybdenum in plants varies widely with soil concentration and pH. Good food sources are grains, legumes, nuts, offal, milk and milk products and eggs, while fruits, root vegetables and muscle meat are poor sources (5). High concentrations have been found in shellfish. Molybdenum levels in drinking water are mostly low, typically less than 0.01 mg/L. However, in areas near mining sites, molybdenum concentrations up to 0.2 mg/L have been reported (4).

There are few published data on the dietary intake of molybdenum in the Nordic countries. Typical intakes according to market baskets or dietary surveys are in the range 100–150 µg/d (9–11). Many multivitamin-mineral supplements contain molybdenum and must be taken into consideration when estimating total dietary intake.

REFERENCES

1. Abumrad NN, Schneider AJ, Steel D, Rogers LS. Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. Am J Clin Nutr 1981; 34: 2551–9.
2. Turnlund JR, Keyes WR, Peiffer GL. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. Am J Clin Nutr 1995; 62: 790–6.
3. Turnlund JR, Keyes WR, Peiffer GL, Chiang G. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men during depletion and repletion. Am J Clin Nutr 1995; 61: 1102–9.
4. WHO. Guidelines for drinking-water quality. World Health Organization, Vol. 1: 3rd ed. 1996. Geneva 2004.
5. Reports of the Scientific Committee on Food. Nutrient and energy intakes for the European Community. Luxembourg: Commission of the European Communities, 1993.
6. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
7. Turnlund JR, Keyes WR, Peiffer GL. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. Am J Clin Nutr 1995; 62: 790–6.
8. Expert Group on Vitamins and Minerals. Safe upper levels for vitamins and minerals. Food Standards Agency. London: 2003. Available on: www.foodstandards.gov.uk.
9. Räsänen L, Ahola M, Kara R, Uhari M. Atherosclerosis precursors in Finnish children and adolescents. VIII. Food consumption and nutrient intakes. Acta Paediatr Scand Suppl. 1985; 318: 135–53.
10. Bro S, Sandström B, Heydorn K. Intake of essential and toxic trace elements in a random sample of Danish men as determined by the duplicate portion sampling technique. J Trace Elem Electrolytes Health Dis. 1990; 4: 147–55.
11. Becker W, Kumpulainen J. Contents of essential and toxic mineral elements in Swedish market-basket diets in 1987. Br J Nutr 1991; 66: 151–60.

CHAPTER 42

Fluorine

Fluorine occurs as fluoride in food and drinking water either in an ionic form or bound in complexes. Fluoride has a well-documented role in the prevention and treatment of dental caries but the mechanism is attributed to local rather than systemic effects. The role of fluoride as an essential trace element is debated.

Physiology and metabolism

Fluoride in drinking water is effectively absorbed (> 90%), while complex-bound fluoride in foods is less well absorbed. Most of the ingested fluoride is excreted by the kidneys, but parts are built into the bone and, in childhood, into the teeth. Thus, the main proportion of fluoride in the body is complex-bound to calcium in the skeleton and tooth tissues, where upon replacement of hydroxyl ions in hydroxyapatite crystals it leads to less soluble crystals. This was previously considered to render fluoride its caries preventive property. Today, the presence of fluoride in the mouth and subsequent deposition of CaF_2 in the tooth biofilm acting as a fluoride reservoir ready to interact with the balance between enamel demineralisation and remineralisation is recognized as the basis for the anti-caries power of fluoride (1). Apart from local effect, biological functions of fluoride in man remain largely unclear.

Requirement and recommended intake

No recommendation for daily fluoride intake is given since it is not considered an essential trace element. This agrees with the EU Scientific Committee on Foods, which also did not set any recommended intake (2). The US Institute of Medicine was unable to establish an RDA but has set a reference value for fluoride, which is based on the observed estimated intake judged to reduce the incidence of dental caries in a group of healthy adults (3). For adults this level was set to 3 mg/d and 4 mg/d for women and men, respectively (3).

Upper intake levels and toxicity

An intake of 2.2 g/kg body weight is lethal in adults (4). In children 15 mg/kg body weight is lethal and 5 mg/kg body weight causes acute symptoms, such as nausea, stomach pain and vomiting (4, 5). Chronic high intakes may affect skeletal mineralisation and kidney function.

The most common side effect of high fluoride intake is enamel fluorosis also called 'mottled teeth'. Fluorosis develops during tooth formation, *i.e.* between 0 to 6–7 years of age. Thus, for 0–6 year olds a maximum daily dose of 0.75 mg fluoride is recommended and for 6–12 year olds 1.0 mg (1, 5) and the water content is recommended to be below 0.8–1 mg/L.

Dietary sources and intake

Fluoride levels in foods (except water) are generally low, with a few exceptions. Fish eaten with bones, such as canned sardines, some teas and mineral waters and drinking water in some areas have the highest content. The average daily intake from food is estimated to be less than 1 mg in adults and 0.2 mg in small children (6). A major proportion of fluoride intake is from water sources. Estimated intake from water sources, given a water content of 1 mg/L, is 1.7 mg/d in adults and 0.3 mg/d in small children (6), but since fluoride concentration in drinking water varies between areas, intake also varies considerably due to water source. Another source of fluoride in small children is toothpaste. A child younger than 6 years of age is estimated to swallow approximately 0.15 mg fluoride per exposure (7).

REFERENCES

1. Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand. 1999; 57: 325–329.
2. Commission of the European Communities. Reports of the Scientific Committee on Food: Nutrient and energy intakes for the European Community (Thirty-first series of Food Science and Techniques). Luxembourg: Office for Official Publications of the European Communities, 1993.
3. Food and Nutrition Board. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Institute of Medicine, National Academic Press, Washington DC: 1997.
4. FASS 2001. LINFO Läkemedelsinformation. AB Elanders. Kungsbacka 2001.
5. Waldbott GL. Mass intoxication from accidental overfluoridation. Clin Toxicol, 1981; 18: 531–541.
6. Calgary Health Region. Fluoridation. www.crha-health.ab.ca.
7. Naccache H, Simard PI, Trahan T *et al.* Factors affecting the ingestion of fluoride dentifrice by children. J Publ Health Dent 1992; 52: 222–226.

CHAPTER 43

Intake of vitamins and minerals in the Nordic countries

A diet composed according to the recommended distribution of energy from fat, carbohydrate and protein and the principle of variety in food choice will usually have a satisfactory content of vitamins and minerals. If such a diet is eaten in amounts that meet energy requirements, the intake of vitamins and minerals will generally be sufficient.

The nutrient density of average diets in the Nordic countries is presented in *Table 43.1.* Data are calculated from recent dietary surveys. Some of the observed differences may be explained by different dietary patterns (*i.e.* consumption of fish), levels of micronutrients added to foods (thiamine, riboflavin, vitamin B_6, iron and iodine) or differences in soil and composition of fertilizers (selenium). There may also be significant differences caused by the various survey methods and calculation procedures, *e.g.* recipes and correction for losses in cooking. Contributions to intakes of vitamins and minerals from supplements are not included.

For comparison the NNR 2004 recommended nutrient density for planning diets (*Table 1.3*) is included in *Table 43.1.* These values are intended for groups of individuals with a heterogeneous age and sex distribution and they form a rather strict reference based on the principle of the 'most demanding subject' (explained in *Chapter 4*). It is obvious that the average diets do not meet the reference nutrient density for all micronutrients. However, this does not mean that food supply is inadequate, but rather it should be seen as a reminder to the diet planner of where to focus.

Table 43.1 Nutrient density (per 10 MJ) of selected vitamins and minerals in the average diet in the Nordic countries

		Denmark	Finland	Iceland	Norway	Sweden	NNR 2004
Vitamin A	RE	1,150[4]	1,310	1,590	1,620	1,370	800
Vitamin D	µg	3.5	6.1	7.2	5.5	6.3	10
Vitamin E	α-TE	7.7	13.4	10.0	8.2	9.9[1]	9
Thiamin	mg	1.3	1.6	1.5	1.4	1.6	1.2
Riboflavin	mg	1.8	2.3	2.2	1.8	2.0	1.4
Niacin	NE	31	34	40	33	40	16
Vitamin B_6	mg	1.6	2.3	2.0	1.5	2.3	1.3
Folate	µg	340	330	330	240	250	450
Vitamin B_{12}	µg	5.4	7.6	8.1	7.7	7.3	2
Vitamin C	mg	115	140	103	120	98	80
Calcium	mg	1,120	1,410	1,240	1,030	1,210	1,000
Phosphorus	mg	1,490	2,000	1,890	–	1,610	800
Potassium	g	3.7	4.8	3.6	–	3.9	3.5
Magnesium	mg	375	469	338	380	360	350
Iron	mg	10.7	15.1	13.0	12.2	12.8	16
Zinc	mg	12	–	13	–	12.7	11
Iodine	µg	210	322	198	–	–	170
Selenium	µg	41	87	90	–	38	40
Corrected for cooking losses		Yes	Yes	Yes	Yes[2]	Yes[3]	
Age group	years	4–75	25–64	15–80	16–79	18–74	
Survey method		7-d food record	48-hour recall	24-hour recall	QFFQ[5]	7-d food record	
Reference		1	2	3	4	5	

1 Calculated from α-tocopherol
2 Refers to vitamin A and E, thiamine, riboflavin, vitamin C and folate
3 Refers to thiamin, riboflavin, preformed niacin, vitamin B_6 and vitamin C
4 Contribution from β-caroten is calculated as 1/12
5 Quantitative food frequency questionnaire

REFERENCES

1. Danskernes kostvaner 2000–2002. Hovedresultater (Dietary habits of Danes 2000–2002. Main results). Danmarks Fødevareforskning, 2005. In press.
2. Männistö S, Ovaskainen M-L, Valsta L. The National Findiet 2002 Study. Helsinki: National Public Health Institute, 2003.
3. Steingrímsdóttir L, Þorgeirsdóttir H, Ólafsdóttir AE. The Diet of Icelanders. Dietary Survey of the Icelandic Nutrition Council 2002. Main findings. Public Health Institute, Reykjavík, Iceland 2003.
4. Johansson L, Solvoll K. Norkost 1997. Landsomfattende kostholdsundersøkelse blant menn og kvinner i alderen 16–79 år. Rapport 2/1999. Statens råd for ernæring og fysisk aktivitet. Oslo 1999.
5. Becker W, Pearson M. Riksmaten 1997–98. Kostvanor och näringsintag i Sverige. Metod- och resultatrapport (Dietary habits and nutrient intake in Sweden 1997–98) Uppsala: Livsmedelsverket, 2002.

INDEX

C

D

G

N

Q

R

S

T