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Nordic Nutrition Recommendations 2012

Integrating nutrition and physical activity

Part 3

Nord 2014:005

Nordic Nutrition Recommendations 2012

Part 3
Vitamins A, D, E, K, Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Biotin, Panthothenic acid and vitamin C

5th edition

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Nordic co-operation

Nordic co-operation is one of the world's most extensive forms of regional collaboration, involving Denmark, Finland, Iceland, Norway, Sweden, and the Faroe Islands, Greenland, and Åland.

Nordic co-operation 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 co-operation 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.

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Secretary General’s Preface

There has been an increasing interest in food and nutritional science in recent years. Food programmes are a staple of most television channels and cookbooks top the bestseller lists. At the same time, it can be a bit of a challenge to find your way through the jungle of advice on what we should eat facing the average consumer.

That is why we need a work like the Nordic Nutrition Recommendations, one of the most well-researched and thoroughly documented works within nutritional science worldwide. They give a scientific basis for formulating dietary guidelines and are an excellent example of what the Nordic countries can achieve when they work together.

The Nordic Council of Ministers funds the extensive scientific effort behind the Nordic Nutrition Recommendations. We do this as a means to inform the public debate on food-related matters. But maybe more importantly, the NNR also serve as the main reference point for the various national nutrition recommendations in the Nordic countries.

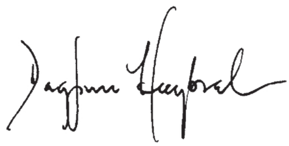
The Nordic Nutrition Recommendations are also the foundation for the criteria developed for the Nordic nutritional label the Keyhole, informing the shopping decisions of millions of consumers in the Nordic region on a daily basis.

Finally, the NNR form part of the overall Nordic action plan *A better Life through Diet and Physical Activity.* In its aim to ensure the best-possible health for the population at large, this can be seen as an expression of the Nordic model, with its focus on an inclusive and holistic approach to society and the welfare of its citizens.

This is the fifth edition of the Nordic Nutrition Recommendations. As such, this publication is one of many examples of a long and fruitful Nordic co-operation over the last decades.

As a new step, we have decided to publish a free PDF version of the NNR along with a series of e-publications of individual chapters. The NNR will also for the first time ever be published as an e-book and they have thus entered the digital era.

I would like to thank the hundreds of scientists, experts and officials involved in compiling the Nordic Nutrition Recommendations and hope that the quality of the work itself, as well as the many new forms of publication, will help ensure the widespread use that the NNR deserve.



Dagfinn Høybråten
Secretary General, Nordic Council of Ministers

Preface

The 5th edition of the Nordic Nutrition Recommendations, NNR 2012, has been produced by a working group nominated by the Working Group on Food, Diet and Toxicology (NKMT) under the auspices of the Nordic Committee of Senior Officials for Food Issues (ÄK-FJLS Livsmedel). The NNR 2012 working group was established in 2009 and consisted of Inge Tetens and Agnes N. Pedersen of Denmark; Ursula Schwab and Mikael Fogelholm of Finland; Inga Thorsdottir and Ingibjorg Gunnarsdottir of Iceland; Sigmund A. Anderssen and Helle Margrete Möltzer of Norway; and Wulf Becker (Chair), Ulla-Kaisa Koivisto Hursti (Scientific secretary), and Elisabet Wirfält of Sweden.

More than 100 scientific experts have been involved in this revision. Existing scientific evidence has been reviewed for setting dietary reference values (DRVs) that will ensure optimal nutrition and help prevent lifestyle-related diseases such as cardiovascular diseases, osteoporosis, certain types of cancer, type-2 diabetes, and obesity as well as the related risk factors for these diseases. The experts have assessed the associations between dietary patterns, foods, and nutrients and specific health outcomes. The work has mainly focused on revising areas in which new scientific knowledge has emerged.

Systematic reviews (SR) were conducted by the experts, with assistance from librarians, for the nutrients and topics for which new data of specific importance for setting the recommendations has been made available since the 4th edition. Less stringent updates of the reference values were conducted for the other nutrients and topics.

Peer reviewers for each nutrient and topic have also been engaged in the process of reading and commenting on the SRs and the updates conducted by the expert groups. A reference group consisting of senior experts representing various fields of nutrition science both within and outside the Nordic countries has also been engaged in the project. A steering group with representatives from national authorities in each country has been responsible for the overall management of the project.

All chapters were subject to public consultations from October 2012 to September 2013. The responses and actions to the comments by the NNR working group are published separately.

The SRs and the updates form the basis for deriving the DRVs. In the process of deriving the NNR 2012, emphasis has been put on the whole diet and the current dietary practices in the Nordic countries. This evaluation was performed by the NNR 2012 working group and was not part of the SRs conducted by the expert groups. The SRs were used as major and independent components – but not the only components – for the decision-making processes of the working group that was responsible for deriving the NNR 2012.

The SRs are published in the Food & Nutrition Research journal and the other background papers can be found on the Nordic Council of Ministers (NCM) website.

The 5th edition, the Nordic Nutrition Recommendations 2012, is published by the NCM and is also available in electronic form.

The following experts and peer reviewers have been engaged in performing SRs and chapter updates.

Systematic reviews

Calcium experts: Christel Lamberg-Allardt, Kirsti Uusi-Rasi and Merja Kärkkäinen, Finland.
Peer reviewers: Christian Mølgaard, Denmark and Karl Michaëlsson, Sweden.

Carbohydrates – including sugars and fibre experts: Emily Sonestedt, Sweden, Nina C Överby, Norway, Bryndis E Birgisdottir, Iceland, David Laaksonen, Finland.
Peer reviewers: Inger Björck, Sweden, Inge Tetens, Denmark.

Elderly experts: Agnes N Pedersen, Denmark, Tommy Cederholm, Sweden, Alfons Ramel, Iceland.
Peer reviewers: Gunnar Akner, Sweden, Merja Suominen, Finland, Anne Marie Beck, Denmark.

Fat and fatty acids experts: Ursula Schwab and Matti Uusitupa, Finland, Thorhallur Ingi Halldorsson, Iceland, Tine Tholstrup and Lotte Lauritzen, Denmark, Wulf Becker and Ulf Risérus, Sweden.
Peer reviewers: Jan I Pedersen, Norway, Ingibjörg Hardardottir, Iceland, Antti Aro, Finland, Jorn Dyerberg, Denmark, Göran Berglund, Sweden.

Folate experts: Cornelia Witthöft, Sweden, Georg Alfthan, Finland, Agneta Yngve, Norway.
Peer reviewers: Margaretha Jägerstad and Jörn Schneede, Sweden.

Food based dietary guidelines experts: Lene Frost Andersen, Norway, Asa Gudrun Kristjansdottir, Iceland, Ellen Trolle, Denmark, Eva Roos and, Eeva Voutilainen, Finland, Agneta Åkesson, Sweden, Elisabet Wirfält, Sweden.
Peer reviewers: Inge Tetens, Denmark, Liisa Valsta, Finland, Anna Winkvist, Sweden.

Infants and children experts: Agneta Hörnell, Sweden, Hanna Lagström, Finland, Britt Lande, Norway, Inga Thorsdottir, Iceland.
Peer reviewers: Harri Niinikoski, Finland, Kim Fleischer Michaelsen, Denmark.

Iodine experts: Ingibjörg Gunnarsdottir, Iceland, Lisbeth Dahl, Norway.
Peer reviewers: Helle Margrete Meltzer, Norway, Peter Lauerberg, Denmark.

Iron experts: Magnus Domellöf, Sweden, Ketil Thorstensen, Norway, Inga Thorsdottir, Iceland.
Peer reviewers: Olle Hernell, Sweden, Lena Hulthén, Sweden, Nils Milman Denmark.

Overweight and obesity experts: Mikael Fogelholm and Marjaana Lahti-Koski, Finland, Sigmund A Anderssen, Norway, Ingibjörg Gunnarsdottir, Iceland.
Peer reviewers: Matti Uusitupa, Finland, Mette Svendsen, Norway, Ingrid Larsson, Sweden.

Pregnancy and lactation experts: Inga Thorsdottir and Anna Sigridur Olafsdottir, Iceland, Anne Lise Brantsaeter, Norway, Elisabet Forsum, Sweden, Sjurdur F Olsen, Denmark.
Peer reviewers: Bryndis E Birgisdottir, Iceland, Maijaliisa Erkkola, Finland, Ulla Hoppu, Finland.

Protein experts: Agnes N Pedersen, Denmark, Jens Kondrup, Denmark, Elisabet Börsheim, Norway.
Peer reviewers: Leif Hambraeus and Ingvar Bosaeus, Sweden.

Vitamin D experts: Christel Lamberg-Allardt, Finland, Magritt Brustad, Norway, Haakon E Meyer, Norway, Laufey Steingrimsdottir, Iceland.
Peer reviewers: Rikke Andersen, Denmark, Mairead Kiely, Ireland, Karl Michaëlsson, Sweden, Gunnar Sigurdsson, Iceland.

Overviews

Alcohol experts: Anne Tjønneland and Janne Schurmann Tolstrup, Denmark.
Peer reviewers: Morten Grønbæk, Denmark and Satu Männistö Finland.

Fluid and water balance expert: Per Ole Iversen, Norway.

Vitamin B_6, Vitamin B_{12}: Chapters revised by the NNR5 working group.

Thiamin, Riboflavin, Niacin, Biotin, Pantothenic acid: Hilary Powers, United Kingdom. Evaluation of need for revision. Revised by the NNR5 working group.

Vitamin K expert: Arja T Erkkilä, Finland. Peer reviewer: Sarah L. Booth, USA.

Dietary Antioxidants expert: Samar Basu, France. Peer reviewer: Lars Ove Dragsted, Denmark.

Vitamin A: Håkan Melhus, Sweden. Evaluation of need for revision. Chapter revised by the NNR5 working group.

Vitamin E expert: Ritva Järvinen, Finland. Peer reviewer: Vieno Piironen, Finland.

Vitamin C expert: Mikael Fogelholm, Finland. Peer reviewer: Harri Hemilä, Finland.

Phosphorus expert: Christel Lamberg-Allardt, Finland. Peer reviewer: Susan Fairweather-Tait, United Kingdom.

Magnesium, Zink, Manganese experts: Ingibjörg Gunnarsdottir, Iceland, Helle Margrete Meltzer, Norway. Peer reviewer Lena Davidsson State of Kuwait.

Chromium, Molybdenum experts: Ingibjorg Gunnarsdottir, Iceland, Helle Margrete Meltzer, Norway.

Copper expert: Susanne Gjedsted Bügel, Denmark Peer reviewer: Lena Davidsson, State of Kuwait.

Sodium as salt and Potassium expert: Antti Jula, Finland. Peer reviewer: Lone Banke Rasmussen, Denmark.

Selenium experts: Antti Aro, Finland, Jan Olav Aaseth and Helle Margrete Meltzer Norway. Peer reviewer: Susanne Gjedsted Bügel, Denmark.

Fluoride expert: Jan Ekstrand, Sweden. Peer reviewer Pia Gabre, Sweden.

Physical activity experts Lars Bo Andersen, Danmark, Sigmund A Anderssen and Ulrik Wisløff, Norway, Mai-Lis Hellénius, Sweden. Peer reviewers Mikael Fogelholm, Finland, Ulf Ekelund, Norway.

Energy experts: Mikael Fogelholm and Matti Uusitupa, Finland. Peer reviewers: Ulf Holmbäck and Elisabet Forsum, Sweden.

Population groups in dietary transition expert: Per Wändell, Sweden. Peer reviewer: Afsaneh Koochek, Sweden.

Use of NNR experts: Inge Tetens, Denmark, Agneta Andersson, Sweden.

Sustainable food consumption expert: Monika Pearson, Sweden.

Librarians

The librarians have been responsible for literature searches in connection with the SRs, other database searches, and article handling.
Mikaela Bachmann, Sweden
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Introduction

For several decades, the Nordic countries have collaborated in setting guidelines for dietary composition and recommended intakes of nutrients. Similarities in dietary habits and in the prevalence of diet-related diseases, such as cardiovascular diseases, osteoporosis, obesity and diabetes, has warranted a focus on the overall composition of the diet, i.e. the intake of fat, carbohydrate, and protein as contributors to the total energy intake. 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 chronic 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 unsaturated fatty acids.

The Nordic Nutrition Recommendations (NNR) are an important basis for the development of food, nutrition, and health policies; for formulation of food-based dietary guidelines; and for diet and health-related activities and programmes. Previous editions mainly focused on setting dietary reference values (DRVs) for the intake of, and balance between, individual nutrients for use in planning diets for various population groups. The current 5th edition puts the whole diet in focus and more emphasis is placed on the role that dietary patterns and food groups play in the prevention of diet-related chronic diseases.

The NNR are intended for the general population and not for groups or individuals with diseases or other conditions that affect their nutrient requirements. The recommendations generally cover temporarily increased requirements, for example, during short-term mild infections or certain medical treatments. The recommended amounts are usually not suited for long-term infections, malabsorption, or various metabolic disturbances or for the treatment of persons with a non-optimal nutritional status. They are meant to be used for prevention purposes and are not specifically meant for treatment of diseases or significant weight reduction. The NNR do, however, cover dietary approaches for sustainable weight maintenance after significant and intentional weight reduction. For specific groups of individuals with diseases and for other groups with special needs or diets, dietary composition might have to be adjusted accordingly.

After a thorough revision in which experts have reviewed a vast amount of scientific publications, most of the recommendations from the 4th edition (2004) remain unchanged. However, the RIs for vitamin D in children older than 2, adults, and the elderly ≥75 years of age and for selenium in adults have been increased. An emphasis has been put on the quality of fat and carbohydrates and their dietary sources. The recommendation for protein has been increased for the elderly ≥65 years of age. No recommended intakes have been set for biotin, pantothenic acid, chromium, fluoride, manganese, or molybdenum due to insufficient data, and this represents no change from the 4th edition.

The primary aim of the NNR 2012 is to present the scientific background of the recommendations and their application. A secondary aim is for the NNR 2012 to function as a basis for the national recommendations that are adopted by the individual Nordic countries.

The NNR 2012 are to be used as guidelines for the nutritional composition of a diet that provides a basis for good health. The basis for setting recommendations is defined for each individual nutrient using the available scientific evidence. 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. As new scientific knowledge emerges with time, the NNR have to be reassessed when appropriate and should, therefore, not be regarded as definitive.

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

- satisfies the nutritional needs, i.e. covers the physiological requirements for normal metabolic functions and growth, and
- supports overall good health and contributes to a reduced risk of diet-associated diseases.

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

The NNR can be used for a variety of purposes:

- as guidelines for dietary planning
- as a tool for assessment of dietary intake
- as a basis for food and nutrition policies
- as a basis for nutrition information and education
- as guiding values when developing food products

15 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 intake level	LI	400	500			
Upper intake level	UL	3,000* 1,500*#				

* as preformed retinol.
\# Post-menopausal women.

Introduction

Vitamin A refers to any compound possessing the biological activity of retinol (1). The term 'retinoids' includes 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 compounds also occur in the all-trans configuration. Furthermore, the 11-cis aldehyde form, 11-cis retinal, is present in the retina of the eye, and several acid forms such as all-trans retinoic acid, 13-cis retinoic acid, and 9-cis retinoic acid can 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 to 60 members of a large class of naturally occurring compounds called carotenoids that have vitamin A activity. 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.

Recommendations on vitamin A include both vitamin A activity as retinol and some provitamin A carotenoids. The term 'retinol equivalents'

(RE) is used to convert all sources of preformed retinol and provitamin A carotenoids in the diet into a single unit. The conversion factors for the relevant carotenoids are based on human studies that showed that the absorption of a single dose of 45 mg to 39 mg β-carotene ranges from 9% to 22% (5). In addition, a number of factors such as protein-energy malnutrition, zinc deficiency, dietary fat, alcohol consumption, infections, and the degree of food processing and food matrix can affect the bioavailability and bioconversion of retinol and carotenoids (3–5). Based on these and similar studies, the US Institute of Medicine, IoM (5) 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)

The same factors are used in the NNR, but the term 'retinol equivalents' (RE) is maintained.

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 (6).

The mean intake of vitamin A in the Nordic countries varies from 960 to 1,240 RE/10 MJ. The corresponding range for preformed retinol is 740 to 1,100 µg/10 MJ. Icelanders used to have the highest intake followed by Norwegians. However, retinol intake in Iceland has decreased 31% between 2002 and 2010/2011 mainly as a result of changes in the vitamin A content of cod liver oil. Still, 4.6% of Icelanders exceed the upper limit of 3,000 mg/d of vitamin A when using the MSM method of estimating the distribution of intake (7). The main sources of retinol are liver and liver products, edible fat, milk, and dairy products including retinol-fortified margarine, spreads, and milk. The main sources of vitamin A-active carotenoids are vegetables and some fruits.

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, 8). When intake of vitamin A is inadequate to meet the body's needs, clinical vitamin A deficiency develops and is characterised by several ocular features (xerophthalmia) and a generalised impaired resistance to infection. A series of epidemiological and intervention studies in children living under poor socioeconomic 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 (9). Vitamin A deficiency is a public health problem in over 120 countries (10). The problem is probably uncommon in developed countries but might be under-diagnosed because there is a lack of simple screening tests to measure sub-clinical deficiency. Vitamin A might, however, be a double-edged sword because it has been suggested that intake even marginally above the recommended dietary intake is associated with embryonic malformations (8, 11), reduced bone mineral density, and increased risk for hip fracture (12).

The major dietary sources of vitamin A are provitamin A carotenoids from vegetables and preformed retinyl esters from animal tissues (3, 4, 13, 14). Carotenoids such as α- and β-carotene and β-cryptoxanthin are absorbed by passive diffusion, and the absorption of carotenoids can vary considerably depending on factors such as food matrix, preparation method, and processing (15). After entry into the enterocytes, provitamin A carotenoids are cleaved to yield 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. In general, 70% to 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% to 80% of the body's total retinol is stored in the hepatic stellate cells as retinyl esters, and the normal reserve of stellate cell retinyl esters is adequate to last for several months (16).

Retinol bound to retinol-binding protein is released from the liver and circulates in the plasma to ensure 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, 13, 14). Retinal functions as a chromophore in the visual process and retinoic acid activates specific nuclear retinoic acid receptors and thereby modulates gene transcription (4).

Requirement and recommended intake

Earlier recommendations have mainly been based on studies aimed at eliminating symptoms of vitamin A deficiency. In the Sheffield study (17), symptoms of vitamin A deficiency (reduced plasma retinol, reduced dark adaptation, dryness of the skin, and 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 somewhat improved the plasma retinol levels. Supplementation with 780 µg retinol per day for 45 days had little further effect on the subject's plasma retinol level. However, supplementation with 7,200 µg retinol per day increased his plasma retinol above his initial level of 1.2 mmol/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 (17) concluded that a daily retinol intake of 390 µg represents the minimum protective dose, but this figure should be raised to 470 µg to correct for an error in the conversion factor used in the analytical measurements (18).

Similar observations were obtained in the Iowa study (19) in which 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 mmol/L, impaired dark adaptation was observed at plasma retinol levels of 0.1–0.9 mmol/L, and follicular hyperkeratosis was found at plasma levels of 0.3–1.3 mmol/L. Plasma levels below 1.1 mmol/L were associated with a mild degree of anaemia that responded to retinol supplementation. The Iowa study also found that daily intake of 300 µg retinol partially corrected the abnormal electroretinograms, but supplements of 600 µg/d were needed to prevent eye changes in adult men.

Based on isotope-labelled retinol experiments it was calculated that the average rate of utilization of retinol during the state of vitamin A depletion was about 910 µg retinol per day. The study concluded that a daily retinol intake of 900 µg per day would maintain a plasma level of 1.1 mM in most adult men. For women, the requirement would be reduced in proportion to body weight.

The US Dietary Reference Intakes (5) for vitamin A were based on estimated requirements that assured adequate body stores of retinol and where no clinical signs of deficiency were observed, adequate plasma retinol levels were maintained, and there was protection against vitamin A deficiency for approximately 4 months on a vitamin A-deficient diet. The underlying evaluation assumed that the body turnover of retinol is 0.5%, the minimal liver reserve is 20 mg/g, the liver weight to body weight ratio is 1:33, the total body to liver vitamin A reserve ratio is 10:9, and that the efficiency of storage (i.e. retention of absorbed vitamin A in the liver) is 40%. Based on these assumptions (5), and using reference weights for US adults, the estimated average requirement (EAR) of preformed vitamin A required to assure an adequate body reserve in an adult male was 627 µg/d. The corresponding value for women was estimated to be 503 µg/d. Using a factor of 1.4 to account for variation in the population, the recommended daily allowance (RDA) was set to 900 µg/d for men and 700 µg/d for women above 19 years of age (5). These estimations are in general agreement with a large number of studies using functional criteria for vitamin A status, such as dark adaptation, papillary response test, conjunctival impression cytology, and markers of immune function (see (5) for a review of these studies).

In a more recent study (20), the estimated AR for vitamin A in adult males was studied using the deuterated retinol dilution technique in 16 men in Bangladesh. The results indicated that 254–400 µg/d was sufficient to assure an adequate body reserve (equivalent to 362–571 µg/d for a 70 kg man in the US), which is lower than the AR in the NNR 2004. Using the factor of 1.4 to cover the variation, this would result in a recommended intake of 500–800 µg/d. However, more studies of the variation in the AR are needed before a change in the current recommendations can be discussed.

Using the above factorial method for the Nordic reference subjects, the estimated AR for vitamin A would be very similar as for the US reference subjects, i.e. close to 600 µg/d and 500 µg/d for men and women, respectively. In NNR 2004, the recommended intakes (RI) for adults were based on these considerations and thereby set to 900 RE/d for men and 700 RE/d for women. There are limited scientific data to change the reference values from NNR 2004. Therefore, the RI of 900 RE/d for men and 700 RE/d for women are maintained. Also, the ARs of 600 RE/d for men and 500 RE/d for women and the lower intake levels (LI) of 500 RE/d for men and 400 RE/d for women are kept unchanged.

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. For non-breastfed infants, the vitamin A content of formula is sufficient. Therefore, no specific recommended intake of vitamin A for infants aged 0–6 months is given. Any contribution by carotenoids was not considered because the bioconversion of carotenoids in infants is not known.

Direct studies on the requirement for vitamin A are not available to estimate an AR 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 (5)).

Experimental data to estimate an AR 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 (5). The RI for pregnancy is set to 800 RE/d to cover 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/d. 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 (21). Some early studies (22) found an age-related trend toward higher serum retinol values with advancing age, but recent studies have found trends toward a slight decrease (23). None of these elderly subjects had retinol values below a cut-off value of 0.35 mmol/L. Using a cut-off value of 0.7 mmol/L as proposed by NHANES data from subjects ranging in age from 18 years to 74 years resulted in only very few subjects being at risk (23). In a Danish cross-sectional study of 80-year-old men and women, 10% had a dietary intake of vitamin A below the lower limit but only one subject had a retinol value below 0.7 mmol/L (24). Use of the same vitamin A-containing supplements has been linked to higher circulating retinyl ester levels in elderly subjects compared to younger subjects (25), and this is due, perhaps, to delayed plasma clearance in the elderly (26). An intervention study found an altered postprandial plasma retinol concentration in older subjects compared to younger, but the intestinal absorption and esterification were the same in the elderly compared to the younger subjects (27).

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 – but plasma β-carotene seems to be a possible biomarker of β-carotene status (28). Several studies (23, 29, 30) have found a positive relationship between plasma levels and the intake of β-carotene in elderly subjects. Consumption of fruits and vegetables rich in β-carotene is inversely related to overall mortality and cardiovascular mortality, even in the elderly (31, 32). 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.

Reasoning behind the recommendation

There are limited scientific data to change the reference values from NNR 2004. Therefore, the RIs of 900 RE/d for men and 700 RE/d for women are maintained. In addition, the ARs of 600 RE/d for men and 500 RE/d for women and the LIs of 500 RE/d for men and 400 RE/d for women are kept unchanged.

Upper intake levels and toxicity

Several studies have shown that doses up to 180 mg β-carotene per day as supplements can be used for many years with no evidence of vitamin A toxicity and without the development of abnormally elevated blood retinol concentrations. 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 the chapter on antioxidants).

Adverse effects of dietary retinol need to be considered in Nordic populations where the dietary intake of preformed retinol has been relatively high, especially in Iceland.

Vitamin D antagonism

Several studies have provided evidence of an antagonism between retinol and vitamin D both in animals (33–37) and humans (38). 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 (39). In a meta-analysis, which included all cases of retinol intoxication published in the scientific literature up to the year 2000 (40), it was found that the mean dose of retinol causing hypervitaminosis A was higher when the dose originated from a formula containing vitamin D. This observation implies that there is increased sensitivity for retinol toxicity among subjects with vitamin D insufficiency.

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 (41). Although adults in the Nordic countries have a generous intake of retinol, very few if any healthy individuals are likely to ingest amounts that might 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 (6, 40). Retinol in water-soluble, emulsified, or solid preparations generally seems to have more acute toxic effects than retinol in foods or oils (40). This might be relevant for potential hypervitaminosis A from supplements and from 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 might approach the upper safe levels. Therefore, oil-based retinol preparations should preferably be used in supplements and fortification of foods, and supplements and fortification with water-miscible and 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 the scientific literature in the Nordic countries up to 2003 (6). Chronic hypervitaminosis A is induced after daily doses of 2 mg/kg of retinol in oil-based preparations for many months or years (40). In contrast, only a few weeks of daily intake of doses as low as 0.2 mg/kg of retinol in emulsified/water-miscible and solid preparations caused hypervitaminosis A (6). 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 (40). 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 that can cause cellular toxicity, production of collagen, and eventually fibrosis and cirrhosis in the liver. 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 (42).

Risk of retinol-induced teratogenicity

Animal studies demonstrate that both retinol deficiency and retinol excess can give rise to embryonic malformations and that a single high dose of retinol or retinoic acid can be teratogenic if given at a susceptible stage of early embryonic development (see discussion in (6) 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. Because 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 (42). 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. Because the possible adverse effects of excess retinol intake appear very early during pregnancy, this advice applies to all women of childbearing age. Furthermore, due to high retinol content in liver, it is recommended that pregnant women should avoid eating whole liver as the main course of a meal.

Risk of retinol-induced osteoporosis

Results from animal experiments, in-vitro studies, pharmacological studies, and clinical observations have shown that retinol intoxication is associated with severe detrimental effects in the skeleton (see (6). Most human studies published during the last decade, however, have not shown any association between retinol intake and bone density (43–52), which is in line with animal data (53). In studies on rats, bone density was unaffected while bone diameter and strength were diminished. This seems to be related to increased periosteal bone resorption and reduced bone formation (54, 55). Observations on a human foetus have identified that a mutation in the enzyme CYP26B1 that specifically inactivates the bioactive vitamin A metabolite retinoic acid has effects resembling those seen when high retinol doses are administered to experimental animals, including a pronounced reduction in the diameter of the long hollow bones (56). In summary, most human studies have not found an association between retinol intake and bone density.

Retinol and fractures

A few prospective and case-control studies have found an increased risk for fractures in groups with retinol intakes from foods and supplements > 1.5 mg/d (e.g. (12, 57, 58). Caire-Juvera and coworkers (59) found no overall association between total retinol intake and the risk of hip or total fractures among 75,747 postmenopausal women from the Women's Health Initiative Observational Study. However, an increased risk for fracture was seen in the group with the highest quintile of total retinol intake (≥ 1.426 µg/d) among women with a vitamin D intake below the mean (≤ 11 µg/d), but the overall trend was not significant. In other studies, no associations between fractures and retinol intake from foods (47) or from foods and total intake (60) have been found. There are a few studies indicating associations between use of dietary supplements containing vitamin A and fractures (60, 61). Mean retinol intakes varied between studies, however, and some only measured retinol from foods (12, 47) while others report associations for both total and food retinol intake (57–60). There are also some studies showing an association between serum retinol levels and fractures (49, 58, 62). However, no retinol intake data were available in the studies by Barker et al (49) and Opotowsky et al (62).

In summary, results from some prospective cohort studies indicate that high intakes of retinol (> 1.5 mg/d) from foods and supplements might be associated with fracture risk, but others have shown no associations.

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.

Because of the low margin between the RI value and doses that might pose a risk to different groups of the population, setting an upper level (UL) of intake is not easy. In NNR 2004 the recommended maximum intake of 3 mg/ d of retinol supplements for women of childbearing age was chosen as the UL for the entire population. This level is 2.5 times below the level that might cause hepatotoxicity. This UL is kept unchanged in NNR 2012.

In NNR 2004, the UL of 1,500 µg/d was set for postmenopausal women in order to reduce the possible risk of osteoporosis. The results from the studies published after NNR 2004 are conflicting and do not give any clear indication as to what levels of intake increase the risk for fractures. Still, it cannot be ruled out that long-term intakes above 1,500 µg/d might increase the risk for fractures. Therefore, the previous recommendation that postmenopausal women who are at greater risk for osteoporosis and bone fractures should restrict their intake to 1,500 µg/d is maintained.

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16 Vitamin D

Vitamin D µg/d		Women	Men
Recommended intake	RI		
2–60 years		10	10
61–74 years		10	10
≥ 75 years		20	20
Average requirement	AR	7.5	7.5
Lower intake level	LI	2.5	2.5
Upper intake level	UL	100*	100*

* IoM 2010; EFSA 2012.

Introduction

Vitamin D_3 (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). Vitamin D_3 is also present in some animal foods, and vitamin D_2 (ergocalciferol) can be found in some mushrooms. The basic requirement for vitamin D_3 can be satisfied by exposing the skin to the sun. Experience demonstrates, however, that under the living conditions and at the latitude of the Nordic countries (55° N–72° N), vitamin D deficiency can occur if the diet is devoid of the vitamin. Infants can develop rickets and elderly people can develop osteomalacia, and for this reason vitamin D is considered a micronutrient. Vitamin D is also a pro-hormone because it is converted to a hormone, 1,25-dihydroxyvitamin D (calcitriol), in the body.

One IU (international unit) corresponds to 0.025 µg vitamin D.

Dietary sources and intake

Oily fish, edible fats, and milk products enriched with vitamin D are the major dietary sources. Certain lean freshwater fish might also contain high concentrations of vitamin D_3 (2, 3). Meat and eggs contribute some vitamin D_3, but they also contain $25OHD_3$ (4) that has a higher biopotency (4, 5). Vitamin D_2 is formed by UV irradiation of ergosterol present in some mushrooms and in yeast, and vitamin D_2 has a somewhat lower biopotency compared to vitamin D_3 (4, 5). Some plant-based milk substitutes contain added vitamin D.

Dietary survey data from the last decade show average intakes in both adults and children ranging from 3.5 µg/10 MJ in Denmark to12.8 µg/10 MJ in Finland (6–10).

Assessment of vitamin D status

The circulating serum 25OHD concentration is regarded as a good marker of vitamin D status (11). The reliability of the assays for serum 25OHD measurement has been questioned, however, and several studies have shown that different assays give different results (e.g. (12–15). In 1989, the Vitamin D External Quality Assessment Scheme was initiated (www.deqas.org) to provide laboratories with external control of accuracy. Later, standard reference material (SRM) of serum became available from the National Institute of Standards and Technology with an indicative value for 25OHD. Since 2009, a certified standard reference serum (SRM972) with assigned values for the content of $25OHD_2$, $25OHD_3$, and 3-epi-$25OHD_3$ has been available (www.nist.gov/mml/csd/organic/vitamindinserum.cfm). This material has been used in NHANES studies to standardize the values for vitamin D status obtained by various radioimmunoassay and LC-MS/MS methods (16). Currently, no standard method for measuring serum 25OHD concentrations has been selected but candidate methods based on LC-MS/MS techniques have been published (17, 18). An international standardization project (Vitamin D Standardization Program, VDSP) has the aim of standardizing serum 25OHD concentration measurements (19). Despite some methodological uncertainties, serum 25OHD concentration is so far the best available marker for assessing vitamin D status and sufficiency.

Vitamin D status in Nordic populations

Infants

As a consequence of a good public health service and because most infants receive vitamin D supplements, rickets has become very rare in the Nordic countries over the past 3–4 decades. In a review of studies on population groups in dietary transition in the Nordic countries published from 1990 to 2011, a high risk of vitamin D deficiency (serum 25OHD concentrations below 25 nmol/L) was evident among some ethnic groups (20). Among young children of immigrant parents, the risk of rickets was 50 times higher compared to children of indigenous parents (20).

Children and adults

Studies from Denmark show that serum 25OHD concentrations are generally low during wintertime with between 50% and 90% of the study population having insufficient status (< 50 nmol/L) (21, 22). Dietary vitamin D intake was low (median intakes were 2.4 µg/d to 3.4 µg/d). In a study on Icelandic adults aged 30 to 85 years, mean serum 25OHD concentrations were 43 nmol/L in the youngest age group (33–45 years) and 52 nmol/L in the oldest (70–85 years). Mean vitamin D intakes in the two respective age groups were 9.7 µg/d and 16.6 µg/d. Among those not taking vitamin D supplements, including cod liver oil, mean dietary intake was 5.2 µg/d and mean serum 25OHD levels were below 50 nmol/L throughout the year and reached a mean high of 45 nmol/L during the summer (23).

A systematic review (SR) by Holvik et al. (24) concluded that the vitamin D status in Norway was sufficient (serum 25OHD concentrations ≥ 50 nmol/L) for the majority of the general population and that available data suggested that the vitamin D status in Norway is better than more southerly locations in Europe. In Sweden, two small studies on children indicate adequate status during the summer season (25, 26). In a study of preschool children in northern Sweden (latitude 63–64 °N), 40% had insufficient vitamin D status during wintertime and the deficiency was greater in children with dark compared to fair skin pigmentation (26). In the other study conducted in southern Sweden (latitude 57–58° N), less than 10% of preschool children (4 years of age) had insufficient status during the winter. At 8 years of age, however, about 20% to 30% of the same children had insufficient status, and this difference was related to more frequent use of vitamin D supplementation at age 4. In a study of 116 Swedish women aged 61 to 86 years (mean age 69 years) and living at latitude 60° N, the mean serum 25OHD concentration during winter (January–March) was 69 nmol/L and about 20% had concentrations below 50 nmol/L. Estimated mean dietary intake of vitamin D, including vitamin D-fortified foods, was 6.0 µg/d (27). Previous studies in Sweden showed mean serum 25OHD concentrations of 50 nmol/L to 95 nmol/L among the general adult population (28).

Pregnant and lactating women

A study among 95 pregnant fair-skinned Swedish women living at latitudes from 57° N to 58° N found that the mean serum 25OHD concentration during gestational weeks 35 to 37 was 47.4 nmol/L (29). About 65% had concentrations below 50 nmol/L, and use of vitamin D supplements was linked to higher mean vitamin D status (55 nmol/L) compared to non-use (37.7 nmol/L). Mean dietary vitamin D intake was 6.1 µg/d. Milman et al. (30) measured vitamin D status during pregnancy and at 8 weeks postpartum in 141 healthy, ethnic Danish women with normal pregnancies who were residents of greater Copenhagen (latitude 55° N). Mean serum 25OHD concentrations were 77, 98, 91, and 73 nmol/L at 18, 32, and 39 weeks gestation and at 8 weeks postpartum, respectively. About 20% had concentrations below 50 nmol/L at each time point. Median dietary vitamin D intake was 2.4 µg/d, and about one third of the participants were taking multivitamin supplements at the time of inclusion in the study. Results from a longitudinal study in two cohorts of pregnant Finnish women showed that the mean intake of vitamin D increased from a range of 6.2 µg/d to 6.4 µg/d during the years 1997 to 2002 to 8.9 µg/d in 2003–2004 (31). This increase was mainly due to increased fortification of foods and to a lesser extent from supplements.

Physiology and metabolism

Skin synthesis

Exposure of the skin to sunlight (the UV-B band with wavelengths of 290 nm–315 nm) is needed for the photo-conversion of 7-hydroxy-cholesterol to pre-vitamin D_3, which is then converted to vitamin D_3. The amount of vitamin D_3 produced depends on several factors such as exposed skin surface, season, latitude, skin pigmentation, and age (32). Dermal production of vitamin D_3 is reduced by pigmentation of the skin and with increasing age.

During the summer months (June–July) at latitudes around 60° N, exposure of the face, arms, and hands (25% of body surface) to sunshine for 6–8 minutes 2 or 3 times a week is estimated to provide amounts equivalent to 5 μg/d to 10 μg/d vitamin D_3 in persons with fair skin pigmentation. About 10–15 minutes per day would be needed for persons with dark pigmentation (33). Datta et al. (34) investigated the effects of sun exposure on serum 25OHD concentrations from February to September in Danish subjects living at latitude 56° N. The results indicated that sun-induced changes in 25OHD concentrations might begin to occur already in early April and then peak by early August. The earliest period with a significant increase was seen at the beginning of May (weeks 17–19), which occurred after a mean of 2 days of exposing more than just the hands and face to the sun. Another one-year study among Danish adolescent girls and elderly women showed that the contribution from sun exposure to serum 25OHD concentration was considerable for both age groups (35).

Effect of Intake of vitamin D on serum 25OHD concentration

Absorption

The NNR SR (38) included one SR of good quality that evaluated the effect of supplements and foods fortified with vitamin D on serum 25OHD concentrations (39), one SR of low quality on fortified foods (40), and one SR of low quality on vitamin D supplementation (41).

Naturally occurring vitamin D is incorporated into chylomicrons and absorbed in the small intestine through the lymphatic system. It is estimated that about 80% of ingested vitamin D is absorbed via this route (1, 36). Experimental studies indicate that cholesterol transporters also play a role in vitamin D uptake (37).

Natural sources

There are limited data on the uptake of vitamin D from natural sources (36). No SR has been published on the relationship between dietary vitamin D from natural sources and serum 25OHD concentrations (38).

Fortified foods

The effect of intake of vitamin D from fortified foods on serum 25OHD concentration has been evaluated in an SR by Cranney et al. (39). Thirteen trials on food fortification and circulating serum 25OHD concentrations providing 5–25 μg vitamin D were included. Food fortification resulted in significant increases in serum 25OHD concentrations with the treatment effect ranging from 15 nmol/L to 40 nmol/L. The combined effect of fortified food from two trials with vitamin D_3 doses equivalent to 10–12 µg/d was an increase in serum 25OHD concentration of 16 nmol/L (95% CI 12.9–18.5). Similar results were obtained in the SR by Black et al. (40). This SR included 16 studies in which fortified foods provided 3–25 µg/d (mean 11 µg/d), often in combination with calcium, and the mean observed increase in serum 25OHD concentration was 19.4 nmol/L. However, heterogeneity was high due to variations in dose, latitude, and baseline serum 25OHD concentrations. Only one study, in which bread was used as the carrier, was carried out in Nordic countries.

Supplements

Children, adolescents, and adults younger than 50 years of age
The SR by Cranney et al. (39) included four randomised controlled trials (RCTs) carried out in Denmark, Finland, France, and Lebanon on the effect of vitamin D intake on serum 25OHD concentrations in children and adolescents. Doses ranged from 5 µg to 50 µg of vitamin D_3/d and were given for durations of one month to over one year. The results showed increases in serum 25OHD concentrations ranging from 8 nmol/L with a daily dose of 5 µg of vitamin D_3, to 16.5 nmol/L with a daily dose of 15 µg, and to 60 nmol/L with a daily dose of 50 µg.

The SR by Cashman et al. (41) included studies carried out or evaluated in the winter season at latitudes north of 49.5° N (northern Germany). The studies included in the SR reported large differences in response to the doses (which ranged from 5 µg/d to 20 µg/d). These responses included decreases in serum 25OHD concentrations of 5–20 nmol/L (42), increases of 10–38 nmol/L (43–45), or no change (46). The study durations varied from 8 weeks to 56 weeks.

Adults ≥ 50 years of age
The SR by Cashman et al. (41) included three studies of older adults and the elderly. Vitamin D_3 doses of 5–45 µg/d resulted in increases in serum 25OHD concentrations of 9–30 nmol/L. Responses varied with dose and baseline serum 25OHD concentrations, and a greater effect was seen at lower baseline levels. In an SR by Autier et al. (47), 76 studies were included with subjects > 50 years of age given vitamin D supplementation. Doses ranged from 5 µg/d to 250 µg/d (median 20 µg/d) and the duration was 1 month to 60 months (median 8.5 months). Meta-regression of studies without concomitant calcium supplementation showed an increase in

serum 25OHD concentration of 1.95 nmol/L per 1 µg vitamin D among community-dwelling populations.

Comments
The SRs show substantial heterogeneity for the effects of vitamin D supplementation on serum 25OHD concentrations. There are no straightforward explanations for this, but factors such as prior vitamin D status, compliance, latitude, and season might have played a role. Grossman et al. (48) evaluated the effects of vehicle substances in vitamin D supplements in five studies and found that vitamin D in an oil vehicle produced a greater response in serum 25OHD concentrations in healthy subjects than vitamin D in a powder form or in an ethanol vehicle.

Determinants of vitamin D status

Major external determinants of vitamin D status are intake from foods and supplements and sun exposure (including season, latitude, and travels to sunny climates) (35). Cultural habits such as clothing are mainly related to sun exposure (27, 49–51). Subject-specific determinants are skin pigmentation, age, and genetic factors (38). The reported data on the disappearance of 25OHD in the serum suggests a half-life of about 15 days to 50 days (52).

The association between vitamin D status and BMI and adiposity was reviewed in the NNR SR (38). In mainly cross-sectional studies, an inverse association was found between BMI and serum 25OHD, while some – but not all – supplementation studies showed a lower response in serum 25OHD concentrations in obese persons than in normal-weight subjects. Moreover, weight loss led to an increase in serum 25OHD concentrations in some studies. The NNR SR. (38) concluded that there are some indications that adiposity should be considered a determinant of serum 25OHD concentrations, although so far there is so no evidence that higher intakes are needed in obese persons than in those with normal weight.

Metabolism

The liver rapidly takes up vitamin D_3 formed in the skin or absorbed from the gut where it is hydroxylated to 25OHD. This metabolite is transported in plasma bound to the vitamin D binding protein (also known as the group-specific protein, Gc). 25OHD is further converted into 1,25-dihydroxyvitamin D (calcitriol) in the kidneys. This is a calcium-regulating hormone that becomes active after binding to a nuclear vitamin D receptor. Together with parathyroid hormone and calcitonin, 1,25-dihydroxyvitamin D ensures that the concentration of calcium and phosphate in the plasma is maintained 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 the maintenance of normal concentrations 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 leading to the development of rickets in children and osteomalacia in adults. The presence of vitamin D receptors in a number of tissues as well as epidemiological and experimental data indicate that vitamin D might play a role in cancer, autoimmune diseases, infections, and muscle strength in addition to the functions mentioned above.

Vitamin D and health

Total mortality

The NNR SR (38) included three SRs of high or good quality on the association between vitamin D status or intake and total mortality (39, 53, 54). The Cochrane Review by Avenell et al. (53) on vitamin D and fractures also included effects of interventions with vitamin D on total mortality as a secondary endpoint. Based on 23 trials, the relative risk (RR) of death was 0.97 (95% CI: 0.93–1.01) in those given vitamin D with or without calcium compared to those given placebo or calcium alone. However, in those given vitamin D plus calcium versus placebo or control (14 trials with 54,203 participants), the RR of death was 0.94 (95% CI 0.89–0.99).

Cranney et al. (39) included five prospective cohort studies. Four studies did not show an overall association between baseline serum 25OHD concentrations and total mortality, and one study reported a statistically significant inverse trend. In meta-analyses including four RCTs (13,899 participants), supplementation with vitamin D alone had no significant effect on all-cause mortality (RR = 0.97, 95% CI: 0.92–1.02), and supplementation with vitamin D and calcium (11 trials with 44,688 persons) did not show a significant reduction in mortality (RR = 0.93, 95% CI 0.86–1.0). The point estimate was, however, similar to that found by Avenell et al. (53).

The Cochrane Review by Bjelakovic (54) found that intervention with vitamin D_3 supplementation with or without calcium versus placebo or no intervention (32 RCTs with 74,789 participants) resulted in a 6% reduction in total mortality (RR = 0.94, 95% CI: 0.91–0.98). The effect was, however, only significant in RCTs giving vitamin D_3 in combination with calcium (range 300 mg to 1,600 mg, mean 929 mg, median 1,000 mg). Only eight trials tested supplementation with vitamin D_3 alone, and the difference between trials intervening with vitamin D_3 alone and trials intervening with vitamin D_3 and calcium was not significant. Significant effects were found in trials including participants with insufficient vitamin D status (serum 25OHD concentrations < 50 nmol/L) and in studies using daily doses lower than 20 µg/d. However, the differences from the other trials involving vitamin D adequacy (RR = 0.92, 95% CI: 0.7–1.07) and doses ≥ 20 µg (RR = 0.96, 95% CI: 0.92–1.01) were not statistically significant. Studies using vitamin D_2 (12 trials) did not show a significant reduction in mortality.

Based on the RCTs included in the aboveSRs, Lamberg-Allardt et al. (38) concluded that supplementation with vitamin D_3 at 10–20 µg/d combined with calcium significantly reduces total mortality. However, they note that it is still uncertain if co-supplementation with calcium is necessary to achieve this effect. Also, the threshold for serum 25OHD concentrations associated with reduced mortality is unclear.

In a meta-analysis of 18 RCTs (47), supplementation with vitamin D, often in combination with calcium, was associated with a 7% reduction in total mortality (95% CI: 1%–13%) compared to placebo. The mean study duration was 5.7 years and the mean vitamin D dose was 13 µg/d (range 7.5–50 µg/d). The calcium dose was generally around 1 g/d. A dose-response relationship could not be established. In studies where serum 25OHD concentrations were reported, these were generally below 50 nmol/L at baseline and increased to between 62 nmol/L and 105 nmol/L and a decrease was seen in the control group in several studies.

Larger prospective cohort studies, mainly from the US, generally show an increased risk of total mortality at serum 25OHD concentrations below 30–50 nmol/L (55–63). However, different categorization of serum 25OHD concentrations in the various studies precludes calculating a more precise cut-off for an increased risk.

In two cohort studies from Sweden and Denmark, both low and high serum 25OHD concentrations were associated with increased total mortality (60, 63). In the study by Michaëlsson et al. (60), increased all-cause mortality was observed among men with 25OHD concentrations < 46 nmol/L (10% of subjects) as well as among those with serum 25OHD concentrations > 98 nmol/L (5% of subjects). Follow-up time was 13.7 years on average. In a Danish cohort study among almost 250,000 subjects from the Copenhagen general practice sector followed for 3 years, a J-shaped relationship was found with the lowest mortality at serum 25OHD concentrations of 50–60 nmol/L (63).

In summary, there is *convincing* evidence that combined supplementation with vitamin D and calcium is associated with reduced total mortality, especially at low serum 25OHD concentrations less than 30 nmol/L to 50 nmol/L. The findings from some prospective cohort studies of an increased risk at concentrations at the higher end in the study populations warrant further investigations.

Bone health

Rickets

Low serum 25OHD concentration increases the risk of rickets. The precise threshold is uncertain, but a number of studies suggest increased risk at serum 25OHD concentrations below 27.5 nmol/L. However, many studies were conducted in developing countries with low dietary calcium intake. Low calcium intake might influence the relationship between serum 25OHD concentrations and rickets, and the serum 25OHD concentration threshold for rickets in populations with high calcium intake is unclear. Vitamin D supplementation has been used as a prophylaxis in the Nordic countries for decades, and the currently recommended daily dose of 10 µg has been effective in preventing rickets (64). Still, cases of rickets in children have been reported that have been ascribed to lack of vitamin D prophylaxis during prolonged breastfeeding or insufficient dietary intake (20, 65, 66).

Fractures

The NNR SR (38) included two SRs of high or good quality (53, 67) and one of low quality (68). The authors concluded that there is *convincing* evidence that supplementation with vitamin D at a dose of 10–20 µg/d combined with calcium reduces the risk of total fracture and hip fracture. The effect is more pronounced in institutionalized elderly, and supplementation with vitamin D alone has not shown a significant effect (38). A precise threshold for serum 25OHD concentrations associated with reduced incidence has not been established, but available data suggest that this might range from 40 nmol/L to 74 nmol/L (38). A Swedish cohort study in men found that a serum 25OHD concentration below 40 nmol/L was associated with an increased risk of fractures (69). Serum 25OHD concentrations were assessed at 71 years of age and the follow-up period was 11 years. However, only 5% of the cohort (20 subjects) had levels below 40 nmol/L.

Bone mineral density and bone mineral concentration

The NNR SR (38) included two SRs of high or good quality (67, 70) and one RCT (71). The overall conclusion was that supplementation with vitamin D alone in doses of 7.5 µg/d to 10 µg/d have no or limited effect on bone mineral density. Supplementation with vitamin D (10–50 µg/d) in combination with calcium (500–1,200 mg/d) prevented bone loss among Caucasians compared with placebo.

The serum 25OHD concentrations associated with maintaining adequate bone mineral density or bone mineral content varies among studies. The SR by Winzenberg et al. (70) found an effect of vitamin D supplementation only in children with low mean serum 25OHD concentrations at baseline (≤ 35 nmol/L). In observational studies among the elderly, bone loss at the hip was increased at serum 25OHD concentrations ranging from 30 nmol/L to 80 nmol/L in different studies.

Falls

The NNR SR (38) included four SRs of high or good quality (39, 67, 72, 73) and three of low quality (74–76). The definition of "falls" and "falling" varied among the included trials. It should be noted that the trials included in the different SRs were mostly the same with some variation due to different inclusion and exclusion criteria and timeframes.

The NNR SR (38) concluded that there is *probable* evidence that supplementation with vitamin D in combination with calcium is effective in preventing falls in the elderly, especially in those with low baseline serum 25OHD concentrations, among both community-dwelling individuals and those in nursing-care facilities. A dose greater than 20 µg/d in conjunction with calcium supplementation was effective in most cases. The threshold for an effect is unclear, but one study suggested that serum 25OHD concentrations below 39 nmol/L were associated with an increased risk of falls. The evidence for an effect of supplementation with vitamin D alone is *inconclusive*.

Muscle strength and function

The NNR SR (38) included two SRs of good quality regarding effects of vitamin D on muscle function in older subjects (77, 78). Stockton et al. (77) concluded that vitamin D supplementation does not have an effect at serum 25OHD concentrations above 25 nmol/L, but that vitamin D does have an effect in adults with vitamin D deficiency. Muir et al. (78) concluded that vitamin D doses of 20–25 μg/d showed beneficial effects on balance and muscle strength without taking baseline serum 25OHD concentrations into account. However, in most studies concentrations were 25–50 nmol/L.

There is *probable* evidence that vitamin D supplementation improves muscle function at low serum 25OHD concentrations, but the evidence for an effect at levels above 50–60 nmol/L is *inconclusive*.

Cancer

The association between vitamin D and cancer has been investigated in a number of cohort studies. Some RCTs have been performed, but they are secondary analyses of supplemental studies for the prevention of fractures (79, 80).

Total cancer. No consistent evidence was found for an association between vitamin D status and total cancer in SRs that include cohort studies and RCTs (38).

Colorectal cancer. The NNR SR (38) included four SRs of good quality (67, 81–83) that covered colorectal cancer. There is *suggestive* evidence based on prospective cohort studies of an inverse association between vitamin D status and risk of colorectal cancer (38), but the evidence for a causal relationship was judged as *limited*. A meta-analysis of observational studies by Touvier et al. (84) also assessed associations with vitamin D intake from foods and supplements. An increased intake of 2.5 μg/d from foods (10 studies) was associated with a small but significant reduction in risk (RR = 0.95, 95% CI: 0.93–0.98), but a corresponding increase in total intake (5 studies) did not have a statistically significant effect.

Breast cancer. The NNR SR included three SRs of good quality (67, 82, 83) that covered breast cancer. Based on these, there is *suggestive* evidence for an inverse association between vitamin D status and breast cancer risk, but good-quality studies are lacking and there is heterogeneity between studies (38).

Prostate cancer. The NNR SR included three SRs of good quality (67, 82, 83), and results of the studies showed that there is *inconclusive* evidence for an association between vitamin D and prostate cancer (38).

Some epidemiological studies indicate an increased risk of pancreatic cancer with high plasma 25OHD concentrations, but the overall evidence is *inconclusive* (38).

Hypertension and blood pressure

The NNR SR (38) included four SRs of good quality (67, 85) and two of low quality (86, 87) covering RCTs, one of which was of low quality, that investigated the relationship between vitamin D and blood pressure and hypertension. The results of that analysis showed that the evidence for an association is *inconclusive* (38). All SRs concluded that there was a need for further studies to explore this relationship for possible clinical significance.

The NNR SR (38) noted that low vitamin D status has been associated with a higher incidence of hypertension in population studies reviewed in two of the SRs (67, 85).

Cardiovascular disease (CVD)

The NNR SR (38) included two SRs of high or good quality (67, 88) and one of low quality (89) covering CVD outcomes and serum 25OHD concentrations. The SR of low quality focused on vitamin D supplementation and CVD (90) and one SR of good quality covered cardiometabolic outcomes (diabetes, hypertension, and blood pressure) and serum 25OHD concentrations (85).

The NNR SR (38) concluded that SRs based on cohorts or case-control studies have consistently found an association between low serum 25OHD concentrations, mostly below 37.5 nmol/L or below 50 nmol/L, and an increased risk of CVD. Evidence for an effect of vitamin D supplementation on CVD outcomes, however, is lacking because the trials in question were all designed for other health outcomes than CVD.

In a subsequent meta-analysis of 19 independent prospective cohort studies (6,123 CVD cases in 65,994 participants) an inverse association between serum 25OHD concentrations and risk of CVD outcomes was observed, but with considerable heterogeneity between studies. The pooled RRs, comparing the lowest serum 25OHD concentration categories with the highest, were 1.52 (95% CI: 1.30–1.77) for total CVD, 1.42 (95% CI: 1.19–1.71) for CVD mortality, 1.38 (95% CI: 1.21–1.57) for coronary heart disease, and 1.64 (95% CI: 1.27–2.10) for stroke (91). Associations remained significant when analyses were limited to studies that excluded participants with baseline CVD and were better controlled for season and confounding. The CVD risk tended to increase monotonically for decreasing serum 25OHD concentrations below about 60 nmol/L with an RR of 1.03 (95% CI: 1.00–1.06) per 25 nmol/L decrease in serum 25OHD concentrations.

In summary, there is *probable* evidence for an inverse association between low vitamin D status and increased CVD risk. However, data are insufficient to establish a precise cut-off for an increased risk.

Diabetes

Type-1 diabetes

The NNR SR identified one SR of good quality that investigated the relationship between vitamin D and type-1 diabetes (92). Five studies were included, one cohort study and four case-control studies. The results show some evidence that supplementation with vitamin D in early childhood might offer protection against type 1 diabetes. Randomized controlled trials are lacking thus far.

Type-2 diabetes

The NNR SR (38) included one SR of good quality (85) and one of low quality (89) and one RCT of high quality (93) on the relationship between vitamin D and the risk for type-2 diabetes. Pittas et al. (2010) included both cohort studies and RCTs, and the latter showed no significant clinical effect of vitamin D supplementation. The RCT (93) found no protective effect of 20 µg/d of a vitamin D supplement. Type-2 diabetes was, however, not the primary outcome in this high-quality study. The SR by Parker et al. (89) included a meta-analysis of prospective cohort studies and showed an overall decrease in the prevalence of diabetes associated with higher serum 25OHD concentrations. However, no grading of scientific quality of the included studies was given.

In summary, the evidence for a causal relationship or an association between vitamin D and type-1 or type 2-diabetes is *limited and inconclusive* (38).

Body weight

The NNR SR (38) included one SR of good quality (67) that evaluated RCTs on the effect of supplementation with vitamin D alone or with calcium on body weight. No significant effects were seen (38), and the evidence for an association between vitamin D intake and body weight is *inconclusive*.

Pregnancy outcomes

The NNR SR (38) included two SRs of high or good quality that covered maternal and neonatal outcomes (67, 94). The SR by De-Regil et al. (94) reviewed six RCTs including 1,023 pregnant women on the association between vitamin D supplementation and pre-eclampsia, preterm birth, low birth weight, and gestational diabetes and vitamin D status at term. In addition, there were a series of secondary intended outcome measures, including caesarean sections, maternal hypertension, and Apgar score. The vitamin D dose ranged from 20 μg/d to 30 μg/d, and three trials also included single high doses of 5,000 μg to 15,000 μg. Five of the studies (623 women) gave vitamin D alone, and one study (400 women) gave vitamin D in combination with calcium. Pre-eclampsia was reported by one study supplying both calcium and vitamin D, and no difference in risk was observed between the intervention and placebo groups. Vitamin D supplementation during pregnancy improved serum 25OHD concentrations at term. None of the included studies reported on gestational diabetes or preterm birth.

The SR by Chung et al. (67) included one small nested case-control study on the relation between vitamin D status and risk of pre-eclampsia. Lower adjusted mean serum 25OHD concentrations were associated with increased risk of pre-eclampsia, and early pregnancy serum 25OHD concentrations below 37.5 nmol/L were associated with a five-fold increased risk of pre-eclampsia.

In conclusion, vitamin D supplementation during pregnancy improves vitamin D status. There is *limited* evidence to assess the clinical significance at observed intakes.

Rickets

Two SRs of high or good quality were included in the NNR SR (38). The SR by Chung et al. (67) cited the previous SR by Cranney et al. (39) because no new studies were identified. The Cranney report included 13 studies regarding vitamin D status and rickets. In six studies, the mean or median serum 25OHD concentrations in children with rickets were < 27.5 nmol/L, but they were between 30 nmol/L and 50 nmol/L in the other seven studies. Most studies were conducted in developing countries with low calcium intakes. Low calcium intake can influence the relationship between serum

25OHD concentration and rickets, and the serum 25OHD concentration threshold for rickets in populations with high calcium intake (such as in North America) is unclear. Cranney et al. (39) concluded that there is fair evidence for an association between low serum 25OHD concentrations and the development of rickets, but that the evidence is inconsistent to determine a threshold serum 25OHD concentration above which rickets does not occur.

The SR by Lerch and Meissner (95) evaluated the effects of interventions for preventing nutritional rickets in children born at full term. The review was limited to studies performed in the last 50 years. Only four trials were included, of which three were carried out in China and Turkey and one among 10-year-old to 15-year-old children in France. The conclusions were focused on prevention of rickets in Africa, Asia, and the Middle East and in children who had migrated from these regions, and they stressed the importance of preventive measures including vitamin D supplementation.

In conclusion, risk of rickets increases with serum 25OHD concentrations below 50 nmol/L and the risk is high at concentrations below 27.5 nmol/L. The cut-off concentration depends on habitual calcium intake.

Other health outcomes

Infections

The NNR SR concluded that the evidence for an effect of vitamin D on infections is limited and that the reviewed trials were very heterogeneous (38).

Multiple sclerosis

The NNR SR concluded that there is insufficient data to draw any conclusion concerning the relation between vitamin D and multiple sclerosis (38).

Requirement and recommended intake

Criteria for setting reference values

In NNR 2004, serum 25OHD concentration < 40 nmol/L were judged to indicate moderate hypovitaminosis (96) and values > 50 nmol/L were considered to be desirable (97, 98).

The NNR SR on vitamin D found it difficult to establish an optimal serum 25OHD concentration or vitamin D intake based on the evaluated SRs (38) but noted that there is evidence that a concentration of 50 nmol/L would reflect a sufficient status. The IoM (32) considered calcium absorption together with bone mineral density, rickets, and osteomalacia to establish an optimal serum 25OHD concentration. The IoM found congruence among these outcomes with no additional benefits of serum 25OHD concentrations higher than 50 nmol/L and suggested that this level is consistent with an RDA-type reference value in that this level appears to cover the needs of 97.5% of the population. A serum 25OHD concentration < 30 nmol/L is regarded as indicating deficiency and between 30 nmol/L and 50 nmol/L is considered an insufficient vitamin D status (32).

The relationship between serum 25OHD and parathyroid hormone (PTH) concentrations has been considered in numerous studies, and based on these the proposed threshold for vitamin D sufficiency has varied between 25 nmol/L to 125 nmol/L. Using serum PTH as an outcome is difficult because the variation is large and because other factors have an effect on S-PTH. Sai et al. (99) concluded in a systematic review that "vitamin D insufficiency should be defined as serum 25OHD concentrations less than 50 nmol/L as it relates to bone". The IoM report stated that serum levels above 75 nmol/L are not consistently associated with increased benefit and that "there may be reason for concern at serum levels above 125 nmol/L" (32).

Assays for determining serum 25OHD concentrations might give different results, and this should be accounted for when interpreting results from studies linking status with health outcomes. Results from some immunoassay methods have been shown to give lower 25OHD values compared to, for example, HPLC or LC-tandem MS/MS (100, 101).

In NNR 2012, a serum 25OHD concentration of 50 nmol/L is used as an indicator of sufficiency, and a concentration of 30–50 nmol/L is considered to indicate insufficient status.

Infants

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 serum 25OHD concentrations to a level seen in rickets (102). Human milk does not contain sufficient vitamin D to prevent rickets even if the mother takes vitamin D supplements (103). Sun exposure has a marked effect on vitamin D status in infants, and vitamin D supplementation might not be required provided exposure is sufficient. At northern latitudes, as in the Nordic countries, however, vitamin D supplementation is required in order to ensure that no infant develops rickets. During the first 6 weeks after birth, the serum 25OHD concentration falls to a range where there is a high risk of rickets (< 27.5 nmol/L). Supplementation, therefore, should start during the first weeks of life.

A vitamin D intake of 2.5 µg/d might be sufficient to prevent rickets (16). In a Norwegian study by Markestad (102), a group of 8 infants were fed exclusively on milk formula containing 10 µg/L vitamin D. At 6 weeks of age, they all had serum 25OHD concentrations within the normal range (mean 92 ± 21 nmol/L), indicating that a mean intake of 7.5 µg (corresponding to an intake of 750 mL of formula) ensures satisfactory vitamin D status. In two previous Finnish studies, supplementation with 10 µg/d vitamin D from birth during wintertime resulted in mean serum 25OHD concentrations at 8 weeks (104) and 14 weeks (105) of age of about 45 nmol/L and >75 nmol/L, respectively, compared to about 14 nmol/L and 25 nmol/L in unsupplemented infants, respectively.

Based on these considerations, 10 µg/d is recommended for new-borns from the first weeks of age to 2 years.

Children and adults

Studies published during the last 10–15 years show large variations in vitamin D status among children and adolescents in the Nordic countries. These are mainly due to the degree of skin production of vitamin D from sun exposure during the summer and early autumn seasons, use of vitamin D supplements, and dietary intake. Serum 25OHD concentrations are generally lowest in late winter and early spring with exceptions due to occasional vacations to sunny climates.

The dietary requirement of vitamin D sufficient to maintain an adequate serum 25OHD concentration (≥ 50 nmol/L) throughout winter is partly dependent on the status in early autumn (35). Adequate sun exposure during the summer season is a suitable means to build up a body pool that can help maintain adequate status during the winter season. Engaging in outdoor physical activity in line with recommendations is one option.

Controlled intervention studies using vitamin D supplements carried out at latitudes covering the Nordic region show varying responses with respect to effects on serum 25OHD concentrations and the proportions of subjects below various cut-offs during the winter season.

In the NNR SR (38), results from an SR by Cashman et al. (41) were used as an approach to define dietary intakes that would maintain adequate serum 25OHD concentrations under various assumptions. The paper included meta-regressions of data from 12 intervention studies with vitamin D supplementation in children, adults, and the elderly carried out at latitudes between 49.5° N and 63° N and one study carried out in Antarctica at 78° S. Depending on the statistical model used, the vitamin D intake needed to achieve wintertime serum 25OHD concentrations of at least 50 nmol/L for 95% of the population varied between 9.0 µg/d and 12.0 µg/d. However, the estimations were dependent on the analytical model used, and the studies included in the analysis gave highly variable results.

Results from studies conducted at similar latitudes as the Nordic countries with children aged 8–12 years showed increases of 10–25 nmol/L (43, 71) or no change (41) after supplementation with 10 µg/d vitamin D and with total intakes of 12.5–15 µg/d during the winter season.

Studies with adults aged 18–49 years also show divergent results with increases of 30–38 nmol/L (44, 45) or decreases of 7–12 nmol/L (42) after supplementation with 10–20 µg/d vitamin D (total intakes of 13.5–29 µg/d) during the winter season. The responses seem to be associated with several factors, and baseline concentrations are important. Because different analytical methods were used to assess serum 25OHD concentrations, it is difficult to compare the actual status between studies.

A combined analysis of the supplementation studies on children and adults (up to about 60 years of age) that measured serum 25OHD concentrations during wintertime with limited sun exposure is presented in an Appendix (42–46, 71, 106). In this analysis the study from Antarctica was excluded because it was not considered representative for the Nordic setting and because it administered high doses of vitamin D. The studies were carried out at latitudes within the Nordic region (55° N to 61° N) or at latitudes somewhat further south (50° N to 55° N). The supplementation studies suggest that the response to supplementation was dependent on baseline serum 25OHD concentration with no or limited increase when baseline levels were above 50 nmol/L. The analysis shows that an intake of 7.2 µg/d would maintain a mean serum 25OHD concentration during the winter season of about 50 nmol/L (Appendix, Figure 16.1.). Based on this evaluation, the average requirement (AR) is set at 7.5 µg/d. Results from the studies indicated relatively large inter-individual variations in response that partly depended on baseline concentration. Using the lower 95% confidence interval in the graph, an intake of about 10 µg/d would be sufficient for the majority of the population.

In NNR 2004, the recommended intake (RI) was set to 7.5 µg/d for the age group of 2 years to 60 years in order to diminish the seasonal drop in serum 25OHD concentration. Based on the combined results from studies with supplements, a further increase of the RI is warranted and the RI is set to 10 µg/d. This RI considers some contribution of vitamin D from outdoor activities during the summer season (late spring to early autumn), which is compatible with normal, everyday life and also in line with recommendations on physical activity. Engaging in outdoor physical activity in line with recommendations is one option. A higher intake might be necessary for some parts of the population due to limited sun exposure or skin production related to cultural traditions, limited access to outdoor activities, or skin pigmentation.

Older adults and the elderly

Previous studies carried out in the Nordic countries showed that insufficient vitamin D status was common among the elderly, especially among those living in institutions (107–109). More recent studies give a more complex picture, and some studies have shown equal or higher serum 25OHD concentrations among the elderly (21, 23, 27, 110). However, elderly people living in institutions with little or no access to sun exposure are still at high risk of insufficient vitamin D status (24, 111). Apart from low dietary intake and limited time spent outdoors, 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). There is also some evidence that the PTH concentration tends to be higher among the elderly compared to younger adults at similar serum 25OHD concentrations. This might indicate less efficient bioconversion due to diminished kidney function resulting in secondary hyperparathyroidism (112–114).

A high incidence of osteoporotic hip fracture is seen in all Nordic countries, although a decrease has occurred in some countries in recent years (115–117). The more rapid bone loss and higher fracture rate in elderly women than in men is related to diminished oestrogen production in postmenopausal women.

The SR by Cashman et al. (41) included four RCT studies with mainly older adults (> 64 years of age) carried out at latitudes between 51° N (Ireland) and 63° N (southern Finland). In three studies, supplementation with 10–20 µg/d vitamin D (total intakes of 14–30 µg/d) resulted in increases in serum 25OHD concentrations of 12.5–39 nmol/L and mean concentrations of 60–88 nmol/L during wintertime (118–120). Supplementation with 5 µg/d (total intakes 9.1–14.7 µg/d) resulted in small changes (1–9 nmol/L) and mean concentrations of 53–55 nmol/L. In the fourth study, elderly women were given high doses of vitamin D (45 µg/d for a total intake of ~55 μg/d) in combination with calcium (121). Results were comparable with the above studies. In a repeated crosssectional study by Sem et al. (109), which was not included in the SR by Cashman et al. (41), a daily supplement of 11–15 μg/d (total intake 14–19 μg/d) among elderly people living in Oslo (60° N) maintained serum 25OHD concentrations of around 90 nmol/L during winter. Compared to subjects with no supplementation, wintertime serum 25OHD concentrations were 35–60 nmol/L higher in the supplemented groups. Based on a regression analysis, it was calculated that an intake above 5–6 μg/d was required to ensure serum 25OHD concentrations above 50 nmol/L.

A combined analysis of results from the above studies (109, 118–120) was performed in which the study by Honkanen et al. (121) was excluded due to the high vitamin D dosing. The result of that analysis indicated that an intake of about 5 μg/d would maintain a mean serum 25OHD concentration of about 50 nmol/L during wintertime (Appendix, Figure 16.2.). This estimate is lower than for children and younger adults. However, confidence intervals are wider, and based on the lower 95% confidence interval in the graph an intake of about 10–11 μg/d would be needed to cover the majority of the population.

The NNR SR concluded that data from the reviewed SRs show that there is *convincing* evidence of a protective effect of vitamin D on bone health, total mortality, and the risk of falling (38). The effect was often only seen in persons with low basal serum 25OHD concentrations (< 50 nmol/L). In intervention studies, effects were mainly seen for combined supplementation with vitamin D and calcium. There is, however, some epidemiological evidence that high concentrations of 25OHD are associated with increased total mortality. Low serum 25OHD concentrations (mainly < 37.5 nmol/L) were associated with increased risk of CVD in epidemiological studies, but data from intervention studies are lacking (38).

A vitamin D intake of 10 μg/d is recommended for individuals 61–74 years of age. For people with little or no sun exposure, an intake of 20 μg/d is recommended.

For those aged 75 years and older, a total intake of 20 μg/d is recommended.

Pregnancy and lactation

There is a marked increase in 1,25-dihydroxyvitamin D in plasma during pregnancy (32). A close correlation has also been found between vitamin D status of the mother and the new-born (102). Serum 25OHD concentrations have been found to be low during winter in pregnant women under normal circumstances in the Nordic countries (29, 30, 122, 123). A Norwegian study from the 1980s showed that a supplement of 10 µg/d to pregnant women resulted in a serum 25OHD concentration in the upper normal range (124).

The NNR SR (38) concluded that vitamin D supplementation during pregnancy improved vitamin D status as measured by serum 25OHD concentrations at term (39, 94). However, the clinical significance of this remains uncertain. Data on health outcomes are *limited and inconclusive*.

Because no new strong evidence has emerged, the recommendation from NNR 2004 is maintained. An intake of 10 µg/d is recommended during pregnancy and lactation.

Reasoning behind the recommendation

New scientific data has emerged since the NNR 2004 was published, and evidence has accumulated that vitamin D intake and status is associated with total mortality, fractures, falls, and CVD outcomes. As in NNR 2004, a serum 25OHD concentration of > 50 nmol/L is used as an indicator of sufficient vitamin D status. Intervention studies with various doses of vitamin D show that an intake of 10 µg/d is needed to maintain serum 25OHD concentrations around 50 nmol/L among the majority of the population during wintertime at latitudes within the Nordic region. This RI considers some contribution of vitamin D from outdoor activities during the summer season (late spring to early autumn), and this is compatible with normal, everyday life and is also in line with recommendations on physical activity. For people with little or no sun exposure, an intake of 20 µg/d is recommended. For the elderly (>75 years of age), an intake of 20 µg/d is recommended, and this is mainly to account for the more limited solar-induced vitamin D synthesis and the evidence for the protective effect of such an intake against mortality, fractures, and falls.

Lower intake level

In NNR 2004, the lower intake level (LI) was set to 2.5 µg/d and this was mainly applicable to individuals > 60 years of age. Results from the RCTs using various doses of vitamin D supplements indicate that an intake of 2.5–3 µg/d would maintain serum 25OHD concentrations of about 30 nmol/L during wintertime provided that there was sufficient sun exposure during the summer season (see Figures16.1. and 16.2.). However, for individuals with little or no sun exposure this intake might be insufficient to maintain this level. There is insufficient data to set an LI, for example, for infants under these circumstances. An intake below the LI might indicate deficient vitamin D status among children and adults especially in cases of little or no sun exposure.

Upper intake levels and toxicity

Large amounts of vitamin D are toxic and can lead to hypercalcaemia, nephrocalcinosis, and kidney failure. There are older reports of hypercalcaemia in connection with excessive supplementation of infant foods with vitamin D (125) and in connection with incidental over-enrichment of milk (126, 127). However, the exact ingested doses were not elucidated.

Relatively few RCTs included in the NNR SR report adverse effects of vitamin D (38). The adverse effects that were reported include milder transient and asymptomatic hypercalciuria or hypercalcaemia and gastrointestinal symptoms (67, 73, 128). Dose levels ranged from 10 µg/d to 143 µg/d vitamin D_3 and from 125 µg/d to 250 µg/d vitamin D_2. Other SRs found that vitamin D in doses of 10 µg/d given with calcium, but not vitamin D alone, moderately increased the risk of renal stones among postmenopausal women (39, 54). According to Bjelakovic et al. (54), the RR was 1.17 (95% CI: 1.02–1.34).

There are some observational studies suggesting that total mortality is increased at high serum 25OHD concentrations (60, 63, 83). Some studies have reported an increase in prostate cancer (129) or total cancer (60) at higher serum 25OHD concentrations ranging from 100 nmol/L to 150 nmol/L. A trial using large single yearly doses of vitamin D (corresponding to about 18 µg/d) reported increased incidence in fractures and falls in the elderly (130).

The EFSA (131) has set the tolerable upper level (UL) to 100 µg/d for adults and adolescents 11–17 years of age using hypercalcaemia as the criterion for adverse effects. For children 1–10 years of age, the UL was adjusted to 50 µg/d. For infants the UL of 25 µg/d was based on data relating high vitamin D intakes to impaired growth and hypercalcaemia.

The Institute of Medicine (32) also used onset of hypercalcaemia and related toxicity as basic criteria for setting ULs. For infants, the effects on growth were also included. The UL was set to 100 µg/d for adults and children older than 9 years of age. For younger children, ULs were extrapolated to 25 µg/d and 38 µg/d for infants aged 0–6 months and 6–12 months, respectively.

Taking the new studies mentioned above into consideration, the UL for adults and adolescents 11–17 years of age is set at 100 µg/d. For younger children, the UL is set at 50 µg/d and for infants (0–12 months) the UL is set at 25 µg/d.

Appendix:
Vitamin D intake and serum 25OHD – results from supplement studies

Results from randomized controlled intervention trials using vitamin D supplements at various levels and carried out at latitudes covering the Nordic region were used to estimate overall association between intake and serum 25OHD concentrations (Table 16.1.). The included studies were selected mainly from the systematic review by Cashman et al (41) and the NNR 2004 edition. The studies were conducted during winter with limited sunexposure. Studies that administered high doses of vitamin D (> 30 µg/d) were excluded (121).

Children and adults

Data from randomized controlled intervention studies on children and adults (up to about 60 years of age) where serum 25OHD concentrations were measured during wintertime were used to estimate overall association between intake and serum 25OHD concentrations. The studies are listed in table 16.1. (42–46, 71).The studies were carried out at latitudes within the Nordic region (55° N to 61° N) or at latitudes somewhat further south (50° N to 55° N). The study by Meier et al. (106) included subjects with a wide age range (33–78 years), but as mean age was 55–58 years it was also included.

The relationship between vitamin D supplementation intake and serum 25OHD concentrations (log transformed) was analysed using fitted line plot (Minitab® 15.1.0.). The supplementation studies suggest that the response to supplementation was dependent on baseline serum 25OHD concentration with no or limited increase when baseline levels were above 50 nmol/L. The results show that an intake of 7.2 µg/d would maintain a mean serum 25OHD concentration in half of the subjects during the winter season of about 50 nmol/L (Figure 16.1.). Based on these data, the average requirement (AR) is set at 7.5 µg/d. Results from the studies indicate relatively large inter-individual variations in response. Using the lower 95% confidence interval in the graph, an intake of about 10 μg/d was considered to be sufficient to ensure a serum 25OHD concentration in the majority of the population. Therefore the AR for these age groups was set at 7.5 μg/d and the RI at 10 μg/d.

Older adults and elderly

Data from randomized controlled intervention studies on older adults and elderly where serum 25OHD concentrations were measured during wintertime were considered too limited in number to use the same approach as above. The repeated cross-sectional study by Sem et al. (132) with elderly people living in their own homes or residents at old people's homes was therefore included as a basis to explore whether the same RI value would apply for the elderly as for the younger and adult groups. The studies were all carried out within latitudes 51° N to 61° N and listed in Table 16.1. (106, 109, 118–120). Based on the lower 95% confidence interval in the graph (Figure 16.2.) an intake of about 10–11 μg/d was considered to be sufficient to ensure a serum 25OHD concentration in the majority of this population group. Therefore the RI for this age group was set RI at 10 μg/d.

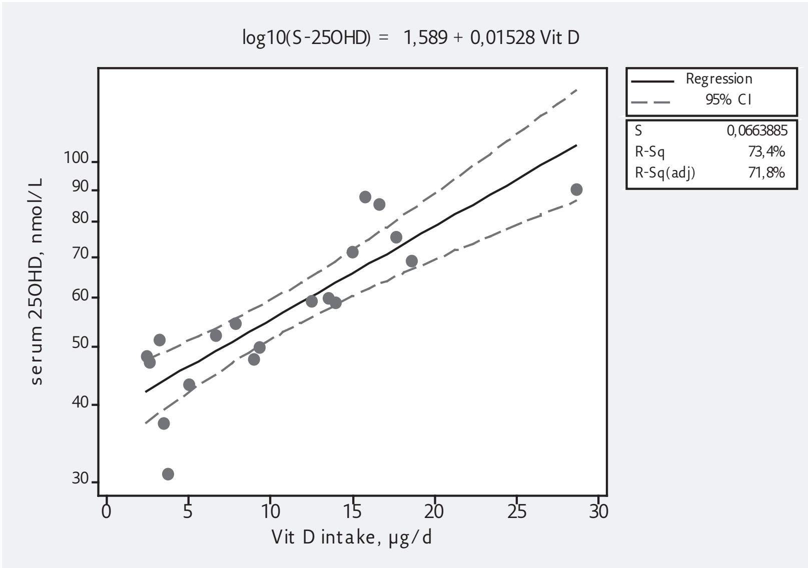

Figure 16.1. Effect of vitamin D supplementation on serum 25OHD concentrations during wintertime among children and adults up to about 60 years of age

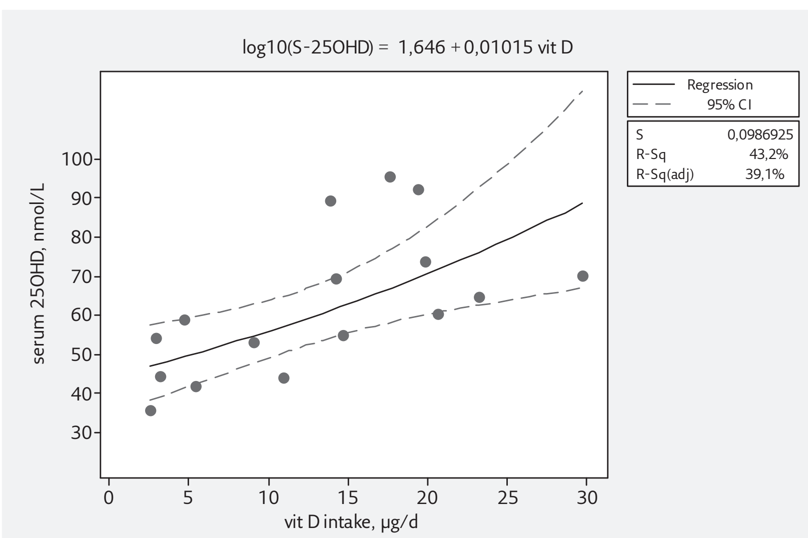

Figure 16.2. Effect of vitamin D supplementation on serum 25OHD concentrations during wintertime among the elderly (> 65 years of age)

Table 16.1. Vitamin D supplementation and serum 25OHD among children, adults and elderly during winter season

Reference	Country	Latitude	Season	Subject charac-teristics	Duration, wk	N	Suppl. Vit D_3 µg/d	Habitual vit D intake	Total vit D intake	Baseline S25OHD	Final S25OHD	SD	Mean change	Analytical method
Ala-Houhala et al. 1988 (43)	Finland	61 °N	Jan/Feb – Feb/ March	m, f, 8–10 y	56	24	10 µg D_2, 5–7 d/wk	5*	15	49.2	71.3 (77.9)	23.8	22.0 (28.7)	CPBA
						27	-	5*	5	45.9	43.2	19.5	-2.7	
Mølgaard et al. 2009 (133)	Denmark	55 °N	Nov-May	F, 11–12 y	~26	74	10	2.5	12.5	44.4	59.4	13.2	15.0	HPLC
						73	5	2.8	7.8	41.9	54.5	12	12.6	
						73	-	2.6	2.6	43.4	47.0	20	3.6	
Cashman et al. 2011b (46)	Finland, Denmark	61 °N, 55 °N	March – April	F, 11 y	52	49	10	3.9	13.9	58.4	58.8	10.9	0.4	HPLC
						49	5	3.9	8.9	57.4	47.6	8	-9.8	
						46	-	3.7	3.7	54.5	31.0	10	-23.5	
Barnes et al. 2006 (44)	UK	55 °N	March	M, f, 18–27 y	8	15	15	1.6	16.6	47.9	85.6	24.5	37.7	EIA
						12	-	2.4	2.4	55.5	48.3	16.8	-7.2	
Cashman et al. 2008 (42)	Ireland	51–55 °N	March	m, f, 20–40 y	22	53	15	3.6	18.6	75.9	69.0	18.6	-6.9	EIA
						57	10	3.5	13.5	72.2	60.0	13.4	-12.2	
						48	5	4.3	9.3	60.0	49.7	11.4	-10.3	
						57	-	3.4	3.4	65.7	37.4	12.2	-28.3	
Viljakainen et al. 2009 (45)	Finland	61 °N	April	m, 21–49 y	25	16	20	8.6	28.6	60.3	90.1	11.6	29.8	EIA
						16	10	7.6	17.6	62.3	75.6	13.6	13.3	
							-	6.6	6.6	64.7	52.2	18.5	-12.5	

Meier et al. 2004 (106)	Germany	49.5 °N	Feb/ March	m, f: 33–78 y	26	27	12.5	3.2	15.7	48.8	87.6	20.3	39.0	RIA
						16		3.2	3.2	61.5	51.2	21.3	-10.3	
Cashman et al. 2009 (119)	Ireland	51–55 °N	March	m, f, 64+	22	48	15	4.8	19.8	55.1	73.8	20	18.7	EIA
						53	10	4.2	14.2	54.3	69.5	17	15.2	
						48	5	4.1	9.1	51.8	53.2	17	1.4	
						55	-	4.7	4.7	58.8	58.8	17.1	0	
Viljakainen et al. 2006 (118)	Finland	61 °N	Jan -April	f, 65–85 y	12	13	20	9.7	29.7	44.1	70.2	13.5	26.1	HPLC
						11	10	10.6	20.6	46.5	60.4	10.2	13.9	
						13	5	9.7	14.7	46.0	55.0	10.2	9	
						12	-	10.9	10.9	52.2	43.9	19.9	-8.3	
Sem et al. 1987 (132) A=elderly homes B=free-living	Norway	60 °N	Feb – March	fA, 86 y		12		2.6	2.6	-	35.8	20.3		HPLC+ UV photo-metry
	Oslo			fA, suppl		23	15	2.6	17.6	-	95.5	30.3		
				mA, 80 y		13	21	-	5.4	-	42.0	13.8		
				mA, suppl		8	14	5,4	19.4	-	92.5	28.3		
				fB, 76 y		10	-	2.9	2.9		54.3	17.8		
				fB, suppl		14	11	2.9	13.9	-	89.3	20.0		
				mB, 79 y		2#	-	5.6	5.6		90.5	10.3		
				mB, suppl		4#	12	5.6	17.6	-	92.5	7.0		
Pfeifer et al. 2001 (120)	Germany	52 °N	March – May	f, 74 y	8	74	20 + Ca	3.2	23.2	25.7	64.8	25.8	39.1	RIA
						77	Ca	3.2	3.2	24.6	44.4	27.4	19.8	

* estimated # not included, too few subjects. CPBA: Competitive protein-binding assay, HPLC: High pressure liquid chromatography, EIA: Enzyme immunoassay, RIA: Radioimmunoassay.

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17 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 intake level	LI	3	4			
Upper intake level	UL	300*	300*			

* mg/d from supplement (EFSA 2003).

Introduction

Vitamin E has traditionally been used as the common term for four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and γ-tocotrienol) that have been shown to have varying levels of biological activity in experimental animal studies (1). However, α-tocopherol is the only form that is recognized to meet human requirements. α-tocopherol is a required nutrient for humans because it is needed for prevention of vitamin E deficiency symptoms including neuropathy and haemolytic anaemia (2). In NNR 2012, vitamin E activity is confined to α-tocopherol. Vitamin E activity has previously been expressed as α-tocopherol equivalents (α-TE) that include the small amounts of activity suggested by animal experiments to be provided by other tocopherols and tocotrienols.

The naturally occurring form of α-tocopherol is RRR-α-tocopherol. Synthetic α-tocopherol (also known as all-rac-α-tocopherol or dl-α-tocopherol) contains an equal mixture of eight different stereoisomers. All of the stereoisomers have equal antioxidative activities, but only those with the 2R-configuration (RRR-, RSR-, RRS-, and SRR) have biologically relevant activities. Due to the lower affinity that α-tocopherol transport protein (α-TTP) has for 2S-isomers, the relative bioavailability of the synthetic form of α-tocopherol is suggested to be only half that of the naturally occurring α-tocopherol (3). This means that only α-tocopherol in foods and 2R-α-tocopherols in vitamin E preparations contribute to vitamin E activity. For commercially available vitamin E preparations, the following conversion factors to α-TE 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 (4, 5). In older literature, vitamin E activity was expressed as IUs. One IU is equivalent to 0.67 mg of the natural form and 0.45 mg of the synthetic form of the vitamin (6).

Dietary sources and intakes

Vegetable oils, vegetable oil-based spreads, nuts, seeds, and egg yolk are good food sources of vitamin E. The α-tocopherol content is highest in sunflower oil followed by corn and rapeseed oil, olive oil, and soybean oil. In addition, vegetable oils contain variable amounts of other tocopherols and tocotrienols. Corn oil, soybean oil, and rapeseed oil are high in γ-tocopherol. On average, approximately half of the α-tocopherol in the diet of Finnish adults was provided by cereal and bakery products and fat spreads, oils, and dressings (7). Among the EPIC study participants from the Nordic countries, added fats contributed the most to vitamin E (α-TE) intake followed by cereal, cereal products, and cakes (8). Other important sources were fruits, vegetables, and fish and shellfish.

In recent dietary surveys from the Nordic countries, the mean dietary intake of vitamin E (α-tocopherol) among adult populations varied between 7 mg and 10 mg per day (9–14). When expressed in relation to energy intake, dietary intake of α-tocopherol by adults in the Nordic countries ranges from 8 mg/10 MJ to 16 mg/10 MJ. During pregnancy, intake of vitamin E is higher and most of women use supplements containing vitamin E (15–18). α-tocopherol intake by children ranges from 7 mg/10 MJ to 11 mg/10 MJ and does not differ greatly from that of adults (18–20).

Physiology and metabolism

The uptake, transport, and tissue delivery of α-tocopherol involves molecular, biochemical, and cellular processes that are closely related with overall lipid and lipoprotein metabolism (21). The presence of bile salts and pancreatic enzymes and the formation of micelles are prerequisites for vitamin E absorption. In order to obtain maximal absorption, vitamin E should be given at meals. However, knowledge of vitamin E absorption is incomplete, and both the amount of fat and the food matrix influence vitamin E absorption. In balance studies with small radioactive doses of α-tocopherol, absorption in normal subjects has ranged between 55% and 79% (2, 22), whereas a much lower figure of 33% was reported based on observed changes in plasma-labelled α-tocopherol after administration of a stable isotope-labelled dose of α-tocopherol (23). Large individual variation in vitamin E uptake has been reported.

Absorbed vitamin E is transported within chylomicrons or bound to HDL in the liver where α-TTP preferentially binds α-tocopherol and is essential for the selective resecretion of α-tocopherol (24). The metabolism of vitamin E is tightly regulated, and unlike other fat-soluble vitamins there is no toxic accumulation in the liver. α-tocopherol that is not released into circulation is excreted into the bile via transporters that are upregulated in the presence of α-tocopherol or it is metabolized via the cytochrome P450 system, also regulated with α-tocopherol, and excreted in bile or urine. The major route of excretion of α-tocopherol is in the faeces with small amounts excreted in urine (22). Turnover of vitamin E is slow; in a kinetic study with tracer-marked RRR-α-tocopherol tracked for 460 days in healthy men and women, the mean half-life of the dose was 44 days in plasma and 96 days in red blood cells (22).

Although no tissue serves to store vitamin E, depletion of body vitamin E takes decades rather than weeks (25). Non-α-tocopherols and tocotrienols are rapidly metabolized thereby preventing their tissue accumulation and limiting increases in their plasma concentrations (26). In human tissues, α-tocopherol is the most common tocopherol and contributes about 90% of the total amount of tocopherols and tocotrienols in plasma (27) and 50%–80% in other tissues (4). Recently, water-soluble α-tocopheryl phosphate has been shown to appear in minute amounts in foods and tissues (28).

The main biochemical function of α-tocopherol has been suggested to be its antioxidant activity. As a chain-breaking antioxidant, α-tocopherol might prevent the propagation of free radicals in membranes and in plasma lipoproteins (29). In addition, several other important biological functions, including modulation of cell signalling and gene expression, have been ascribed to vitamin E (30). α-tocopherol might modulate the activity of several enzymes. Most of these enzymes are membrane bound or activated by membrane recruitment, especially those affecting cell proliferation, membrane trafficking, and metabolism of xenobiotics (24). Genes involved in the metabolism and excretion of vitamin E are regulated by α-tocopherol itself. The ultimate biological function of vitamin E, however, remains to be elucidated (31).

Vitamin E is suggested to affect health through its antioxidant activity, immune system enhancement, inhibition of platelet aggregation, and anti-inflammatory function with much of this evidence coming from cell studies and the findings from animal experiments. Evidence for decreased oxidative stress with α-tocopherol supplementation in humans is inconsistent (32). The effect of vitamin E on biomarkers of oxidative stress appears to depend on the circumstances in which it is administered, most importantly on the level of baseline oxidative stress (33). Differences in the individual responses to α-tocopherol are also suggested to arise due to genetic factors (34, 35).

High vitamin E intake has been associated with prolonged bleeding suggesting that large amounts of vitamin E might interfere with the blood clotting system especially with simultaneous use of aspirin or treatment with anticoagulants (36, 37). It is hypothesized that vitamin E intake can affect vitamin K status because they share the same metabolic pathways (38, 39).

Vitamin E and chronic diseases

Vitamin E has been proposed to play a role in several chronic diseases such as cardiovascular diseases, cancer, dementia, and other diseases associated with increased oxidative stress and inflammation.

Observational studies have provided some evidence suggesting a lower risk of coronary heart disease with higher intake of vitamin E, but randomized clinical studies do not, in general, provide support for a significant or clinically important effect of vitamin E supplementation on coronary heart disease (40, 41) or stroke (42). However, some studies have reported specific beneficial and adverse effects. Supplementation of healthy women older than 45 years with 600 IU of a natural form of vitamin E every other day for 10 years did not affect the occurrence of cardiovascular events compared to placebo. It did, however, result in a significant 24% decrease in cardiovascular deaths in women over the age of 65 years at baseline (43). Results from the same study showed that women supplemented with vitamin E also had a 21% lower risk for vascular thromboembolism (44). Among women at high risk for cardiovascular diseases, such as those with prior cardiovascular disease, supplementation with 600 IU natural source vitamin E every other day for a mean of 9.4 years was associated with a marginal reduction of cardiovascular events (45). In another study of a high-risk population, daily supplementation with 400 IU natural α-tocopherol did not prevent major cardiovascular events, and instead an increased risk for heart failure was found (46). In line with the results from the ATBC study (47), increased risk for haemorrhagic stroke was reported among healthy physicians who received 400 IU synthetic α-tocopherol every other day for 8 years (48).

The significance of vitamin E in cancer prevention has been investigated in several clinical trials, none of which has provided evidence for overall protection from cancer (49). The decreased prostate cancer risk associated with a 50 mg daily dose of synthetic α-tocopherol among middle-aged Finnish male smokers (50) has not been supported by findings from other large-scale controlled trials (51). On the contrary, in post-trial analyses increased risk of prostate cancer was reported among men who had received an average daily supplement of 400 IU synthetic α-tocopherol for 5.5 years (52). There is also no evidence from randomized controlled trials that vitamin E supplementation would be effective against other type of cancer when given for 5 to 10 years to middle-aged or elderly men and women in doses ranging from 50 mg of synthetic α-tocopherol per day to 400 IU of natural-form α-tocopherol per day (43, 46, 53–57). Results from observational studies on α-tocopherol in cancer prevention are inconsistent (58).

There is some evidence from observational studies to indicate a putative role of vitamin E in preventing cognitive impairment, but findings from a few intervention studies have provided little support for this (59). In observational studies, the reduced risk of type 2 diabetes due to higher intake of antioxidants was mainly attributed to vitamin E (60), but such a beneficial effect of vitamin E supplementation has not been confirmed in randomized trials (61). Observational studies of vitamin E and the risk of cataracts and age-related maculopathy have shown mixed results. Only a very limited effect of vitamin E supplementation alone or in combination with other antioxidants on the incidence or progression of cataracts or age-related macular degeneration has been reported (62).

Supplementation with α-tocopherol above the recommended levels is suggested to improve immune function and decrease respiratory tract infections, especially in the elderly (63), but the results of a few randomized trials are inconsistent. Individual differences in the effects of vitamin E supplementation on respiratory tract infections are suggested to be due in part to genetic factors (64).

Requirement and recommended intake

Vitamin E deficiency due to low dietary intake has not been described in normal, healthy individuals. However, deficiency can be caused by prolonged fat malabsorption, genetic defects in lipoprotein transport, or genetic defects in the hepatic α-tocopherol transfer protein. In addition, premature and very low birth weight infants are in danger of deficiency, and neurological disorders due to protein and energy malnutrition are suggested to be related to vitamin E deficiency (25). In premature children, symptoms such as haemolytic anaemia, thrombocytosis, and oedema have been reported (65). 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 without any clinical symptoms (66) and this property can be used as a criterion of vitamin E adequacy.

A new approach to estimate the vitamin E requirement in humans was reported by Bruno et al. (23) based on a plasma α-tocopherol kinetics study among healthy adults given a 22 mg dose of stable isotope-labelled α-tocopherol with different amounts of fat. The estimated rate of α-tocopherol delivery to tissues was 5 mg per day. Using the observed absorption of 33%, the amount of dietary α-tocopherol needed daily to replace irreversible losses would be about 15 mg/d, which seems to support the current recommended daily allowance for vitamin E adopted by the US Institute of Medicine (3). However, absorption rates of 55–79% have been reported (2, 22), which, using the same approach, would lead to markedly lower estimates (6–9 mg/d).

In the absence of more specific measures, the plasma concentration of α-tocopherol is regarded as the most adequate indicator of vitamin E status (5, 67). Because the plasma lipid level influences the α-tocopherol concentration, correction for plasma lipids might be warranted in subjects with high lipid levels when assessing vitamin E status in populations. However, plasma levels might not necessarily display peripheral vitamin E status and might, therefore, be of limited validity (68).

The vitamin E requirement is partly related to the polyunsaturated fatty acids (PUFA) intake, because the antioxidant function of vitamin E is critical for the prevention of oxidation of tissue PUFA (69). In general, the need for greater amounts of vitamin E with higher intakes of PUFA is not a practical problem because most foods rich in PUFA are also rich in vitamin E.

Adults

Among adults, the criteria used for establishing the average requirements and recommended vitamin E intakes are the plasma concentration of α-tocopherol and the relationship to PUFA intake. Data from studies by Horwitt et al. (66) showed an increased haemolytic tendency in subjects with a plasma α-tocopherol concentration below 12 µmol/L, which corresponded to an α-tocopherol:total cholesterol ratio of 2.25 µmol/mmol (70). However, the in vitro haemolytic response was dependent on the PUFA content of the diet, and the limited number of subjects makes this limit uncertain. A plasma level above 16.2 µmol/L has been suggested as an indicator of acceptable vitamin E status (67).

Data from Nordic populations show that average α-tocopherol intakes of 6–10 mg per day are associated with mean plasma α-tocopherol concentrations of 23–28 µmol/L among adults (27, 71–73). Clearly higher concentrations with a range between 33–46 µmol/L have been reported among hyperlipidaemic subjects (74–78). Among a small group of sub-elite runners with irregular menstrual cycle, the serum concentration of α-tocopherol was low (15.7 µmol/L or 2.7 µmol α-tocopherol/mmol total lipids) apparently due in part to low vitamin E intake of only 5 mg/d (73). In these women, post-exercise osmotic erythrocyte fragility was increased at this low serum α-tocopherol concentration. Low vitamin E status has been observed in individuals who consume large amounts of alcohol (79), and occasional cases of neurological symptoms with ataxia due to vitamin E deficiency have been reported in the Nordic countries (80, 81). Other than these rare cases, the available data indicate that vitamin E status is sufficient in the Nordic populations at current vitamin E intakes. Results from a follow-up of the Finnish ATBC study among older male smokers showed that the lowest mortality during the 19 years of follow-up was among those whose serum α-tocopherol values, adjusted for cholesterol at baseline, were between 13 mg/L and 14 mg/L (30.2–32.5 µmol/L), and above this no further benefit was noted (82). These serum values correspond to a daily intake of approximately 13 mg vitamin E, and this might indicate the vitamin intake level that is sufficient to give protection from chronic diseases and protect from premature death (2). However, these findings might not be generalizable to other groups.

The US Institute of Medicine (3) derived an estimated average requirement (EAR) for adults on vitamin E intakes sufficient to prevent hydrogen peroxide-induced haemolysis mainly based on a study on men by Horwitt (66). However, the study diets contained high amounts of corn oil and estimates indicate that the proportion of linoleic acid was 11–12 E%, which is above the upper recommended range for PUFA in NNR 2012.

The relationship between vitamin E and PUFA intake could also be used as a criterion for the recommended intake. Based on a suggested requirement of 0.6 α-TE/g PUFA (69) and an average PUFA level of 5% of energy intake (E%), an intake of 7 and 9 mg α-tocopherol/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 (70) for adults provided vitamin E does not fall below 4 mg/d for adult men and 3 mg/d for adult women. Based on this ratio, the estimated average requirement would thus be 5 and 6 mg α-tocopherol/d for women and men, respectively. These values are used as average requirements (ARs) in NNR 2012.

In the absence of signs of vitamin E inadequacy in the general Nordic population and because no new data supporting changes have emerged, the recommended intake (RI) from 2004 is maintained in NNR 2012. The RI of vitamin E is set to 8 α-TE/d for women and 10 α-TE/d for men. Because 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. A number of studies suggest that besides α-tocopherol, other tocopherols and tocotrienols might have important functions and beneficial effects (58, 83) but thus far evidence of their importance in human health is limited.

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 (84). The Scientific Committee on Food considered a ratio of 0.4 α-TE/g total PUFA to be adequate also for children (70). In NNR 2012, the recommended intakes are based on a ratio of at least 0.6 α-TE/g total PUFA and a mean intake of PUFA corresponding to 5 E%.

Pregnancy and lactation

The recommended intake value for pregnancy is set to 10 α-TE/d, which is applicable in the last two trimesters and covers the increased intake of energy and PUFA. The recommended intake during lactation is set to 11 α-TE/d and also includes the extra requirement to cover secretion in breast milk.

Reasoning behind the recommendation

In the absence of signs of vitamin E inadequacy in the general Nordic population, and because no new strong evidence supporting changes since NNR 2004 have emerged, the recommended intakes of vitamin E remain unchanged.

Upper intake levels and toxicity

The toxicity of natural vitamin E is low, and this is apparently due to efficient metabolic control that prevents any excess accumulation of the vitamin in the body. No adverse effects have been described from intakes provided by food sources. In a review of 24 clinical trials with vitamin E supplementation published between 1974 and 2003, Hatchcock et al. (85) concluded that few adverse effects have been reported and that doses ≤1,600 IU (1,073 mg RRR-α-tocopherol) were suggested to be safe for most adults. This amount corresponds to the upper safe limit set at 1,000 mg α-tocopherol per day by the US Institute of Medicine (3). The Scientific Committee on Food (86) 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.

Concern regarding potential harm of long-term vitamin E supplementation was initially raised by findings from the ATBC study suggesting increased mortality due to haemorrhagic stroke (47). A meta-analysis of nine RCTs that investigated effects of vitamin E supplementation on stroke risk, which included the ATBC study, showed no association for risk of total stroke (seven studies), a reduced risk for ischaemic stroke (five studies), and an increased risk for haemorrhagic stroke (five studies) (87). Doses ranged from 50 to 300 mg/d. The effects were mainly seen in men, and no significant association for haemorrhagic stroke was seen in the one study of women. Five of the RCTs were secondary prevention studies and two included subjects with high cardiovascular disease risk. Thus, high intakes of supplemental vitamin E might interfere with the blood clotting system, especially with simultaneous use of aspirin.

A small but statistically significant increase in mortality was seen among those supplemented with vitamin E in two meta-analyses of randomized clinical trials (88, 89). Increased mortality was suggested among subjects supplemented with doses of 400 IU per day or higher, and a dose-response analysis showed progressively increased all-cause mortality even at vitamin E doses of 150 IU/d (88). However, generalization of these findings, which were largely based on studies in patients with chronic diseases compared to healthy adults, is uncertain. Although the causal relationship between vitamin E supplementation and increased mortality remains unclear, this possibility is a reason to be cautious in relation to vitamin E supplementation.

Taken together, the available scientific data suggest that there are no overall benefits of prolonged high intakes of supplemental vitamin E in the general population. The UL established by EFSA for vitamin E as supplement, 300 mg/d, was included in NNR 2004. In the absence of clear risks associated with long-term high supplemental intakes of vitamin E this UL is maintained in NNR 2012. However, additional, long-term studies are warranted.

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18 Vitamin K

No recommendation given due to lack of sufficient evidence

Introduction

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 primarily produced by bacteria. Both forms are found in animal tissues.

Dietary sources and intake

Leafy green vegetables, vegetable oils, and vegetable oil based fat spreads are the main sources of phylloquinone (1–3). Menaquinones, especially MK-4, are found in liver, meat, egg yolk, and dairy products (4). Natto, a fermented soybean preparation, is particularly rich in menaquinone-7. Based on HPLC analyses of vitamin K in a large number of food products and food intake data from various sources in Finland, an average intake of 120 µg/d has been calculated (2, 5). In a nationally representative nutrition monitoring study in Finland, it was estimated that the mean vitamin K intake is 90 µg/d in women and 100 µg/d in men aged 25–64 years (6). In the Norwegian Hordaland study, it was estimated that intake of phylloquinone is 130 µg/d and that of menaquinones is 15–20 µg/d in women and men aged 47–50 years based on food frequency data (7). Using food records, smaller phylloquinone intakes (60–70 µg/d) have been reported in Danish women aged 45–58 years (8).

Physiology and metabolism

Compounds with vitamin K activity are required as cofactors for the carboxylation of glutamic acid to γ-carboxyglutamic acid (Gla) that is needed for the synthesis of factors II (prothrombin), VII, IX, and X, and proteins C, S, and Z that are involved in the coagulation of blood (9). The presence of Gla in these proteins enables them to bind calcium. Several Gla-containing proteins have been identified in bone, including osteocalcin, matrix Gla protein, protein S, and growth-arrest-specific gene (Gas6) protein. Osteocalcin is most likely involved in the regulation of bone mineral maturation, but otherwise the exact function of these proteins in bone is not known. Matrix Gla protein is involved in regulation of soft-tissue calcification. In addition, a number of other Gla-containing proteins with unknown functions have been identified in several tissues (3, 10, 11).

Vitamin K is absorbed in the jejunum and ileum, and it is estimated that 80% of purified phylloquinone is absorbed (12). However, bioavailability from food sources is considerably less, and absorption of phylloquinone from food sources was found to be 10–15% that of phylloquinone absorbed from tablets or suspensions (13, 14). Bioavailability of phylloquinone from kale was approximately 5% as assessed using a stable isotope (15). Fat malabsorption decreases the absorption of vitamin K significantly, and bleeding is an early sign of secondary vitamin K deficiency.

Absorbed vitamin K is transported by chylomicrons in the lymph and is taken up primarily by the liver. In addition to the liver, vitamin K is also stored in bone tissue, the heart, the pancreas (3), and fat tissue (16). Compared to other fat-soluble vitamins, the total body pool is small. Turnover of phylloquinone is rapid, but the turnover of most menaquinones is somewhat slower. Hepatic reserves are rapidly depleted when dietary vitamin K is restricted, and a more or less continuous supply is required to maintain satisfactory body stores.

Because of poor placental transport of vitamin K and consequent deficiency in new-borns, haemorrhage, sometimes intracranial, can occur during the neonatal period.

Vitamin K and bone health

The association between phylloquinone intake or status and the risk of fracture has been investigated in several observational studies, and the majority of them show an inverse association (11). The association between phylloquinone intake and bone mineral density has been less consistent. Several randomized clinical trials have assessed the effect of phylloquinone supplementation with doses ranging from 200 μg/d to 5 mg/d on bone mineral density and hip fracture, and the majority have reported no effect of the supplementation (1, 17–19). A three-year RCT found that supplementation with phylloquinone, in combination with a mineral + vitamin D supplement, reduced bone loss of the femoral neck in postmenopausal women (20). A one-year RCT in post-menopausal women found that supplementation with calcium and vitamin D alone or in combination with either phylloquinone or menaquinone-7, increased total-body BMD, while significant increases in lumbar spine BMD were only seen in the groups receiving additional vitamin K (21). Earlier interventions using pharmacological doses of menaquinone-4 carried out in Japan supported prevention of fractures, but the quality of those trials has been criticised (22). Recent trials in other populations have not indicated a significant effect of menaquinones on bone mineral density (18, 23, 24). In a meta-analysis of the effect of long-term treatment with oral anticoagulants on bone density, no differences were found at any site other than lower bone density in the ultradistal radius (25). Furthermore, poorer health of the anticoagulant users as compared to non-users could be an important confounder in the association between oral anticoagulants and bone health (26).

Vitamin K and atherosclerosis

Vitamin K-dependent matrix Gla protein inhibits vascular calcification suggesting a role for vitamin K in atherosclerosis. However, human data from observational studies have been inconsistent (27, 28). High phylloquinone intake can reflect a generally heart-healthy diet instead of a direct effect. A randomized clinical trial has suggested that phylloquinone supplementation slows the progression of coronary artery calcification among healthy adults who have existing calcification (29), but no effect has been observed on carotid intima-media thickness (30). A few prospective cohort studies have reported a reduced risk of coronary heart disease at higher dietary menaquinone intakes (31, 32), but more studies are needed before recommendations can be made based on cardiovascular health outcomes.

Vitamin K and other health effects

Anticarcinogenic effects of vitamin K have been reported in animal and cell studies, and an observational study has suggested an association between menaquinone intake and reduced risk of cancer (33). In addition, a role for vitamin K against insulin resistance has been proposed, but human data are still limited (34, 35). Vitamin K is also suggested to reduce inflammation (3, 11).

Requirement and recommended intake

Clinical deficiency is normally not detected after the first few months of life in otherwise healthy individuals. 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 because it is not possible to induce clinical deficiency symptoms with a vitamin K depletion diet. Bacterial synthesis in the intestine is not sufficient, however, to maintain normal serum levels of vitamin K. The traditional, insensitive method to evaluate vitamin K status has been to determine the concentration of coagulation factors, most often measured with the prothrombin time test. Newer biomarkers of vitamin K status include serum concentrations of phylloquinone, the degree of carboxylation of vitamin K-dependent proteins, and urinary vitamin K metabolites (1, 11, 36). The U.S. Institute of Medicine (37) determined, however, that these methods could not be used in the assessment of requirements because of uncertainty surrounding their true physiological significance and the lack of sufficient dose-response data. Therefore, the U.S. Institute of Medicine set an adequate intake (AI) of 120 and 90 μg/d for men and women, respectively, based on self-reported median vitamin K dietary intakes in apparently healthy population groups (37).

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 for 3 weeks resulted in reduced plasma phylloquinone, an increase in undercarboxylated prothrombin in plasma, and reduced urinary excretion of Gla (38). Supplementation with 50 μg/d reversed these changes. However, in another study a similar supplementation did not bring the plasma phylloquinone levels back to original levels after the depletion diet (39). Healthy young individuals with intakes of about 60 to

80 µg/d (corresponding a daily intake of 1 µg/kg) have shown no signs of clinical deficiency, indicating that this intake is adequate for the majority of individuals based on our current understanding of vitamin K's function in blood coagulation (38, 40–42). However, studies indicate that this amount might be insufficient to support adequate carboxylation of extrahepatic vitamin K-dependent proteins (43–48). However, available data to base recommendations are not sufficient.

In NNR 2004 a provisional recommended intake of 1 µg/kg body weight per day was given for both children and adults. This level is maintained in NNR 2012, since no strong scientific evidence for change has emerged.

Breastfed new-borns are at risk of haemorrhage. Vitamin K concentrations in human breast milk have ranged from 0.85 µg/L to 9.2 µg/L with a mean of 2.5 µg/L (37). Using the average concentration as a basis, and average intake of milk, the U.S. Institute of Medicine set an AI at 2 µg/d for infants 0–6 months of age. It is recommended that all new-borns should routinely be given vitamin K (as a 1 mg intramuscular dose or as weekly oral doses) to avoid haemorrhage during the neonatal period, and oral prophylaxis should be continued for the first three months (49, 50).

Upper intake levels and toxicity

No evidence of toxicity associated with high intakes of any 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 (51). This is supported by Cheung and co-workers (19) who reported no increased adverse effects in women receiving 5 mg phylloquinone daily for 4 years. Synthetic analogues such as menadione have been associated with liver damage and haemolytic anaemia and should not be used therapeutically.

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19 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 intake level	LI	0.5*	0.6*			
Upper intake level	UL	– **	– **			

* 0.8 mg at energy intakes <8 MJ/d and 1.0 mg/d for elderly.
** Not established.

Introduction

Thiamin (vitamin B_1) is essential for the utilisation of carbohydrates and branched-chain amino acids in the body. Thiamin participates in metabolism in the form of thiamin pyrophosphate (TPP, also known as thiamine diphosphate) as a coenzyme for pyruvate dehydrogenase, transketolase, and α-ketoglutarate dehydrogenase in the oxidative decarboxylation of α-keto acids to aldehydes and in the utilisation of pentoses (1, 2). TPP is also a coenzyme for keto acid dehydrogenase in the metabolism of branched chain amino acids (1). Thiamin triphosphate is involved in nerve and possibly muscle function (1).

Dietary sources and intakes

Major food sources of thiamin in the Nordic diet are cereals and cereal products, meat and meat products, and milk and dairy products. The dietary supply of thiamin in the Nordic countries is 1.4–1.7 mg/10 MJ (see chapter on intake of vitamin and minerals in the Nordic countries).

Physiology and metabolism

In vegetable foods, thiamin occurs mainly in the free form and in animal foods mainly in phosphorylated forms that are converted to free thiamin prior to absorption (3, 4). Absorption takes place in the small intestine, generally via an active, carrier-mediated system involving phosphorylation. At high intakes, passive diffusion also takes place (5, 6). Thiamin is also obtained from the normal bacterial microflora of the large intestine and it is absorbed in that region of the gut (4), but the quantitative importance of this source is uncertain. Studies with ^{14}C-labelled thiamin in young men (7) showed that more than 95% of the vitamin was absorbed at intakes of 1–2 mg/d. At intakes above 5 mg/d, the relative absorption rapidly decreases.

After absorption, thiamin is transported to the liver where it is converted to its biologically active form, TPP (2). The total body pool of thiamin in an adult is about 30 mg and most of this is found in the muscles and liver (2, 7). 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 (7).

Thiamin deficiency causes beriberi. In adults, symptoms include disturbances in the peripheral nervous system and heart function. Early deficiency symptoms can include anorexia, weight loss, mental changes, and muscle weakness. In alcoholics, conditions such as Wernicke's encephalopathy and Korsakoff's psychosis can occur and these are strongly related to insufficient thiamin intake and/or malabsorption (8). Among children, symptoms appear more quickly and are generally more severe and can lead to heart failure.

Commonly used indicators of thiamin status include the enzymatic activity of transketolase in the erythrocytes (ETK_{AC}). The NNR reference values for thiamin consider urinary excretion relative to ETK_{AC} and to thiamin intake. The activity coefficient represents the degree of enzyme activity stimulation in vitro, and the activity of this enzyme depends not only on TPP availability but also on glucose phosphate availability. An activity coefficient below 1.15 is regarded as an indicator of sufficient status, and an activity coefficient of 1.15–1.25 indicates marginal status (9). The concentration of free thiamin and its phosphate esters in blood or erythrocytes has been shown to be a good indicator of thiamin status (10), especially among subjects at risk for thiamin deficiency (10, 11). 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 erythrocyte thiamin (12).

Thiamin and chronic diseases

Several epidemiological studies have investigated the relationship between intake of thiamin and other B-vitamins (folate, riboflavin, vitamin B_6, and B_{12}) and various cancers, particularly colorectal and breast cancer. No clear evidence for a relation between thiamin intake and different cancer forms has been found (13–15). Thiamin has also been related to neurodegenerative disorders in the elderly such as Alzheimer's disease (16), but evidence for a role in preventing neurological disorders is limited (17).

Requirement and recommended intake

The requirement of thiamin has been related to energy and carbohydrate intake (18, 19), and a clear relationship was shown by Sauberlich and co-workers (20). The current U.S. dietary reference intakes are, however, based on absolute intakes (21). Generally, thiamin intakes are related to energy and protein intakes in the 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, which corresponds to 0.05 mg/MJ (0.2 mg/1000 kcal) (18, 21). In other studies, thiamin excretion in the urine and ETK_{AC} were normalised at intakes of 0.07–0.08 mg/MJ (0.30–0.33 mg/1000 kcal).

In the absence of new data, the reference intakes set in NNR 2004 (22) are kept unchanged. The average requirement for adults and children is thus set at 0.10 mg/MJ and the recommended intake is set 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/d. The recommended intake for infants 0–12 months old is set at 0.10 mg/MJ. The lower limit of intake is estimated at 0.05 mg/MJ.

Studies on pregnant and lactating women indicate a higher requirement as assessed by biochemical parameters (21). 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 (23). Therefore, when planning diets for elderly with energy levels below 8 MJ/d, the thiamin content should be at least 1.0 mg/d.

Reasoning behind the recommendation

The reference intakes set in NNR 2004 (22) are kept unchanged. A few studies have explored relationships between thiamin intake and function and a few studies have examined the effects of supplements on various clinical or biochemical outcomes, but these studies do not make a useful contribution to understanding requirements in healthy populations (24). Thus, there is no strong evidence to support a change of the recommendation.

Upper intake levels and toxicity

The EU Scientific Committee for Food (25) concluded that it was not possible to set a safe upper intake level for thiamin due to a lack of data. Habitual thiamin intakes up to 6–7 mg/d have not been associated with negative effects. Oral intakes up to 500 mg/d for periods up to one month have not been associated with toxic effects (25).

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20 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 intake level	LI	0.8	0.8			
Upper intake level	UL	– *	– *			

* Not established.

Introduction

Riboflavin, formerly known as vitamin B_2, functions as a precursor for the coenzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) and as covalently bound flavin. These are necessary components of a number of oxidative enzyme systems and participate in electron transport pathways (1).

Dietary sources and intake

Major sources of riboflavin in the Nordic diets are milk and dairy products, meat, and meat products. The average dietary intake according to national dietary surveys is in the range of 1.8 mg to 2.4 mg/10 MJ (see chapter dietary intake in Nordic countries).

Physiology and metabolism

Riboflavin occurs in foods as a free molecule or as FAD or FMN complexed with proteins. Protein-bound riboflavin is hydrolysed to free riboflavin in the gastrointestinal tract and absorbed via a specific transport mechanism (1–4). This mechanism is saturated at doses of about 30–50 mg. Absorption rates of free riboflavin are reported to be 50–60% at doses of 2–25 mg (4), and studies on whole foods have shown absorption rates of 60–70% (5).

Riboflavin is mainly stored in the body as flavoproteins and to a lesser degree as free riboflavin. As a consequence, urinary excretion can be affected by changes in nitrogen balance. The urinary excretion of riboflavin can increase under conditions of negative nitrogen balance or during infections, but the opposite can be seen during periods of rapid growth (6). No consistent relationship 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 folate metabolism because FAD is a co-enzyme for methylenetetrahydrofolate reductase (MTHFR), which influences the metabolism of homocysteine. Low riboflavin status, assessed by erythrocyte glutathione reductase activity (EGRAC), has been associated with increased plasma homocysteine levels in subjects with a specific genotype of MTHFR (7).

Biomarkers of riboflavin status include the activity coefficient of EGR (EGRAC) and the urinary excretion of riboflavin (8). At habitual intakes, urinary excretion is proportional to the intake because the body has a limited ability to store riboflavin (4). Depletion-repletion studies show that the urinary excretion of riboflavin increases gradually with increasing intakes, and a sharp increase at intakes of about 1 mg/d indicates tissue saturation. The EGRAC represents the degree of enzyme stimulation in vitro after addition of FAD. A ratio of 1.0 indicates an absence of stimulation. There is a general relationship between the activity coefficient and the riboflavin intake, which is clearest at intakes up to about 1 mg/d. Different criteria for normal EGR activity have been suggested, and this can complicate the interpretation of results from studies on riboflavin status (1, 8, 9). EGRAC ratios above 1.3–1.4 have been suggested to indicate deficiency and ratios of 1.0 to 1.2 indicate adequate status (1).

Although the metabolic effects of riboflavin deficiency are profound, there are only a few clear-cut clinical symptoms. These include various skin changes, including angular stomatitis and seborrheic dermatitis, and glossitis. Severe riboflavin depletion has been associated with impaired iron status, anaemia, and mental disturbances (13). Isolated dietary riboflavin deficiency does usually not occur, and 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, which corresponds to 0.06 mg/MJ (0.25 mg/1000 kcal) (1, 10–13). In a long-term study, consumption of a diet containing

0.75–0.85 mg/d (0.3–0.4 mg/1000 kcal (4.2 MJ) for up to two years resulted in certain clinical symptoms in one subject.

Powers and co-workers (14) found that supplementation with riboflavin improved some markers of iron status in women with biochemical signs of riboflavin deficiency based on EGRAC (>1.40). However, significant improvements were seen only in those women with EGRAC ratios above 1.65 indicating that the upper threshold for inadequacy might be too low.

Riboflavin and chronic diseases

Several epidemiological studies have investigated the relationship between intake of riboflavin and other B-vitamins (folate, vitamin B_6, and B_{12}) and various cancers – in particular colorectal and breast cancer. Results from prospective or nested case-control studies published between 2000 and 2012 have found no association (15–21). A few retrospective case-control studies have found an inverse relationship (22, 23), but others have found no association (15, 24–27). Results of studies using biomarkers of riboflavin intake are inconclusive (28–32).

Requirement and recommended intake

In setting dietary reference values, previous expert groups have related riboflavin intake to either energy or protein intake (10, 12, 13). The U.S. dietary reference intakes are based on absolute intakes (1). Generally, riboflavin metabolism and intake are related to energy and protein intake at normal intake ranges in populations such as those of the Nordic countries. However, at low energy intakes (below 8 MJ/d) the requirement expressed per MJ might be higher and the opposite might be the case at energy intakes well above 12 MJ/d.

In NNR 2004 (33) the average requirement (AR) was estimated to be 0.12 mg/MJ based on older studies in which riboflavin status was assessed using primarily urinary excretion of riboflavin and to a lesser extent using the EGRAC. There are limited new scientific data for setting reference values for riboflavin.

Few controlled studies in healthy adults in Western populations, and generally with few subjects, have assessed the effects of graded intakes of riboflavin on EGRAC ratios (9, 34, 35). Cut-off ratios used to estimate adequacy were around 1.2–1.25, and recent data indicate that the commonly used EGRAC ratio threshold for inadequacy (1.3–1.4) might be too low (14).

There is insufficient data to change the reference values from NNR 2004 (33). Thus, an RI of 0.14 mg/MJ is maintained, and this applies to both children and adults. This level corresponds to an intake of about 1.5–1.6 mg/d for adult men and 1.2–1.3 mg/d for adult women with moderate physical activity. However, when planning diets the riboflavin content should not be lower than 1.2 mg/d, even at energy intakes below 8 MJ/d (8). For pregnant and lactating women, an extra 0.3 and 0.4 mg/d, respectively, is recommended.

The lower intake level (LI) is estimated to be 0.8 mg/d based on earlier depletion-repletion studies.

Reasoning behind the recommendation

In NNR 2004 (33) the average requirement (AR) was estimated to be 0.12 mg/MJ based on older studies in which riboflavin status was assessed using primarily urinary excretion of riboflavin and to a lesser extent using the EGRAC. There are limited new scientific data for setting reference values and the values from NNR 2004 are therefore maintained.

Upper intake level 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). There are insufficient data to set a UL for riboflavin.

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21 Niacin

Niacin NE/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y girls/boys
Recommended intake	RI	15	18	9	12	14/16
Average requirement	AR	12	15			
Lower intake level	LI	9*	12*			
Upper intake level	UL	35**	35**			

* 8 NE/d at intakes < 8 MJ/d.
** as nicotinic acid.

Introduction

Niacin is the common term for nicotinic acid, nicotinamide, and derivatives that exhibit the biological activity of nicotinamide. The main function of niacin is in the form of the coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) that are involved in a number of redox reactions in the metabolism of glucose, amino acids, and fatty acids.

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 33–43 niacin equivalents (NE)/10 MJ (see chapter on dietary intake in Nordic countries).

Physiology and metabolism

In foods, niacin occurs mainly as NAD and NADP, and these are effectively hydrolysed and absorbed in the intestine (1, 2). Data from human studies indicate near complete absorption of up to 3 grams of nicotinic acid.

In cereals such as maize, niacin can be esterified to polysaccharides and this form is considered to be less available (3). Alkaline treatment during preparation of these foods releases much of the niacin.

In the body, niacin can be formed from the conversion of tryptophan. On average, 60 mg of dietary tryptophan is estimated to yield 1 mg niacin (60 mg tryptophan = 1 niacin equivalent (NE)). Niacin status can be measured by urinary excretion of certain metabolites, including N'-methylnicotinamide and methyl pyridone carboxamides. The body has a limited capacity for storing niacin nucleotides and deficiency symptoms can occur after 50–60 days of consuming a low-niacin, corn-based diet (4).

Niacin deficiency results in pellagra and is mainly observed in populations consuming a diet predominantly based on maize or other cereals with a low protein content and low bioavailability of niacin. Few controlled studies, with few subjects, have investigated the effects of niacin-restricted diets (4, 5). In one controlled study, pellagra developed at an intake of 8.8 NE/d (4). In two other studies, no clinical symptoms were seen in subjects with an intake of 9.2–12.3 NE per day, which is the equivalent to about 1 NE/MJ (4).

Requirement and recommended intake

In the absence of new scientific data, the reference values for niacin given in NNR 2004 remain unchanged. The average requirement is set at 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). The recommended intake is set at 1.6 NE/MJ. This corresponds to an intake of 16–19 NE/d for adult men and 13–15 NE/d for adult women. However, when planning diets the niacin content should not be lower than 13 NE/d, even at an energy intake below 8 MJ/d. For pregnant women and lactating women, an extra 1–2 NE/d and 4–5 NE/d, respectively, is recommended. This 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 limit of intake is estimated to be 1 NE/MJ, and at energy intakes below 8 MJ/d the lower limit is estimated to be 8 NE/d.

Reasoning behind the recommendation

The focus of interest for niacin requirements over the last decade has been as a "drug" for the treatment of various dyslipidaemias. The reference values for niacin given in NNR 2004 (6) are kept unchanged because there are no new scientific data to suggest a change.

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 of 30 mg/d to 1000 mg/d can result in mild symptoms such as flushing. Higher intakes have been reported to induce liver damage. The U.S. Food and Nutrition Board (4) set an upper limit of 30–35 mg/d for adolescents and adults based on the risk of flushing. For children 1–3 years old, they set the UL to 10 mg/d, for children 4–8 years old they set the UL to 15 mg/d, and for children 9–13 years old they set the UL to 20 mg/d. The EU Scientific Committee for Food (1) has proposed an upper limit for nicotinic acid of 10 mg/d and for nicotinamide of 900 mg/d for adults. These levels are also used in the NNR 2012.

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22 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	1.5	0.7	1.0	1.1/1.3
Average requirement	AR	1.0	1.3			
Lower intake level	LI	0.8	1.0			
Upper intake level	UL	25	25			

Introduction

Vitamin B_6 is the common term for pyridoxine, pyridoxal, and pyridoxamine. Pyridoxal phosphate (PLP) and pyridoxamine phosphate function as coenzymes for a number of enzymes that mainly participate in amino acid metabolism (1, 2). PLP is regarded as the most biologically active form and is a co-enzyme for glycogen phosphorylase.

Dietary sources and intake

Important sources of vitamin B_6 are fish, meat, offal, potatoes, and milk and dairy products. The average content in the Nordic diet is 1.6–2.5 mg/10 MJ (see chapter on dietary intakes in Nordic countries).

There is limited information on vitamin B_6 status in Nordic populations (3). Studies on the elderly show good status on average, but up to 30% of 80-year-old Danes had plasma PLP levels below 20 nmol/L. This indicates insufficient intake despite an acceptable reported dietary intake.

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 (4). Studies indicate that pyridoxal and pyridoxamine raise the PLP concentration by about 10% less than pyridoxine. In most fruits, vegetables and grains, a portion of the pyridoxine occurs as a glucoside, which is considered to be less bioavailable than other non-glucoside forms (4, 5). The content of pyridoxine-glucoside in a mixed American diet has been estimated to be about 15% of the total vitamin B_6 content (6). The bioavailability of vitamin B_6 in a mixed American diet (as assessed by plasma PLP levels and urinary pyridoxine excretion) was estimated to be 71%–79% of the ingested pyridoxine (as hydrochloride), but the design of the study was not optimal (7).

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 the muscles. The turnover of the vitamin is relatively fast with a half-life of 25–33 days for PLP in plasma (1).

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 (8, 9). PLP makes up 70%–90% of the total vitamin B_6 in plasma, and this level reflects both the tissue stores and intake of vitamin B_6. PLP levels might also be affected by factors independent of the dietary supply such as age, pregnancy, and physical exercise.

The PLP concentrations among adult subjects with clinical symptoms of vitamin B_6 deficiency have been reported to be less than 10 nmol/L. PLP levels indicative of adequate tissue stores and enzyme functionality have been suggested to be 20 nmol/L or 30 nmol/L (8, 9). 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 such as 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 body weight resulted in approximately 40% lower PLP levels than a protein intake of 0.5 g/kg (10, 11). However, in another study the PLP levels did not vary systematically with the protein intake (12).

Some studies (12, 13) indicate that the PLP level decreases with age, which would suggest an increased vitamin B_6 requirement among elderly subjects. However, some recent longitudinal studies of 2–5 years duration actually found a weak increase in the PLP status of elderly Europeans (14, 15) despite observation of an apparent decrease in vitamin B_6 intake (16). In a study by Pannemans et al. (12), 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 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 (17, 18), although these were of short duration. Thus, the relationship between protein and vitamin B_6 intake in the elderly has so far been difficult to estimate, and the data to establish a vitamin B_6 requirement are conflicting.

The intake of riboflavin might also influence the vitamin B_6 status because flavin enzymes are involved in the formation of PLP. Severe riboflavin deficiency might, therefore, affect PLP levels.

Vitamin B_6 intake might influence the plasma homocysteine level via the trans-sulphuration pathway, and this has been proposed to be a risk factor for coronary heart disease (19). However, homocysteine levels are more strongly influenced by folate status (19, 20). In a randomized controlled trial (RCT) (20) on healthy elderly subjects (mean age 70 years) with PLP levels of 28–30 nmol/L, six weeks supplementation with 1.6 mg vitamin B_6 per day only marginally reduced homocysteine levels after repletion of folate and riboflavin status. Metabolic studies in young, healthy adults using controlled diets with restricted vitamin B_6 contents (<0.5 mg/d) for 30 days did not influence homocysteine levels, although these levels were already low (7 µmol/L) at baseline (21, 21a). In another study, serum concentrations of the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decreased somewhat, but whether this effect was due to the vitamin B_6 restriction or to changes in dietary intake is not clear (21).

Deficiency

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, gastro-intestinal problems, and microcytic anaemia (8, 22). Experimentally induced deficiency among adults leads to various mental disturbances, abnormal EEGs, 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 of vitamin B_6 (8). Insufficient intake of vitamin B_6 also induces certain biochemical disturbances such as increased excretion of xanthurenic acid in the urine and decreased transaminase activity in the erythrocytes.

Health effects

Several clinical and epidemiological studies have been published during the last decade on the relationships between vitamin B_6 intake or biomarkers of intake and various health outcomes including cardiovascular disease (CVD), cancer, and cognitive function.

CVD

Low vitamin B_6 status has been associated with low-grade inflammation and risk of cardiovascular outcomes, including coronary artery disease, myocardial infarction, and stroke (23–27). Results from more recent epidemiological studies in healthy populations are conflicting. No association between dietary vitamin B_6 intake and stroke was found in prospective cohort studies on men (28, 29). An inverse association between plasma PLP and myocardial infarction was found in the US Nurses Health Study (30), but no association was found in men or women in a Dutch nested case-control study (31). PLP levels in the lowest reference quartile of both studies were <27.9 nmol/L with a mean of 16 nmol/L.

Reported intake ranges for vitamin B_6 vary considerably in the literature, mainly due to the use of supplements and, to some extent, to the dietary assessment method used. Although reported intakes from dietary sources seem to be relatively similar in Europe and the US, total intakes in several US studies are higher due to the use of supplements. Inclusion of supplements introduces uncertainties in the interpretation of results because most supplements contain a variety of B-vitamins and other micronutrients. Results from RCTs with supplements containing different combinations of large doses of vitamin B_6, B_{12}, and folic acid or vitamin B_6 alone are mostly negative (23, 32, 33). Study populations have usually included subjects with prior heart disease or who are at high risk for CVD. Studies in which baseline intakes of vitamin B_6 or plasma PLP levels were reported have indicated high or adequate vitamin B_6 intakes (2.5 mg/d) and PLP levels (30–50 nmol/L).

Cancer

Colorectal cancer

Several studies have investigated an association between vitamin B_6 intake or status and the risk of colorectal cancer. Vitamin B_6 is involved in cell proliferation, oxidative stress response, and angiogenesis. A systematic review (SR) and meta-analysis of nine prospective studies from Europe, the US, and Asia (including both cohort and nested case-control studies) showed a marginal and non-significant risk reduction when extreme intakes (quartiles or quintiles) were compared. Heterogeneity among the studies was large, but one Dutch study showed an increased risk of colon cancer among women in the highest intake category (34). Exclusion of this study yielded a pooled risk reduction of 20% (95% CI: 8–31%) (35). In five studies reporting both age-adjusted and multivariate risk estimates, the relative risks (RRs) were 0.79 (95% CI: 0.70–0.90) and 0.85 (95% CI: 0.68–1.07), respectively. Subsequent cohort studies from the US and Europe have either found an inverse association between vitamin B_6 intake (36) or found no significant associations (37–39).

The SR by Larsson et al. (35) also included four studies on the association between blood PLP levels and colorectal cancer and reported a pooled relative risk reduction of 48% (95% CI: 29–62%) when highest intake categories (quartiles or quintiles) were compared to the lowest intake categories. Results from a nested case-control study with the EPIC cohort including 1,365 cases and 2,319 controls showed a risk reduction with increasing quintiles of PLP concentrations (RR per quintile = 0.89, 95% CI: 0.84–94) (40). However, the mean follow-up time was relatively short at 3.6 years.

An RCT from the US in which female health professionals with high risk of CVD were given a daily supplement with folic acid (2.5 mg/d), vitamin B_{12} (1 mg/d), and vitamin B_6 (50 mg/d) did not find any difference compared to placebo in colorectal adenoma incidence during 9 years of intervention (41).

Other cancers

Results from recent prospective studies do not support an association between vitamin B_6 intake or status and breast cancer (42–48), endometrial cancer (49), prostate cancer (50–52), or pancreatic cancer (53, 54). Results from a nested case-control study within the EPIC study found a reduced risk of gastric adenocarcinoma (RR per quartile = 0.78 (95% CI: 0.65–0.93)) with increasing levels of the vitamin B_6 plasma markers (plasma PLP, pyridoxal, and 4-pyridoxic acid) (55). The association with PLP alone was non-significant.

Cognitive function

A Cochrane Review identified only two RCTs investigating the association between vitamin B_6 and cognitive impairment or dementia, and these studies were only among healthy elderly men and women (55a). The authors found no evidence for any short-term benefit from vitamin B_6 supplementation in improving cognitive functions or mood. This conclusion is supported by prospective cohort studies that found no statistically significant associations between risk of dementia or Alzheimer's disease and total intake of dietary vitamin B_6 and supplements (56–58) or between cognitive function and plasma PLP (59). Only one study (60) found higher plasma PLP to be related to better cognitive performance (memory). Thus, at present there is no evidence for an association between cognitive function and vitamin B_6.

Other health effects

There are some observations implying a role for vitamin B_6 in diabetes (2). An RCT from the US in which female health professionals with high risk of CVD were given a daily supplement with folic acid (2.5 mg/d), vitamin B_{12} (1 mg/d), and vitamin B_6 (50 mg/d) did not find any difference in type-2 diabetes incidence compared to placebo during 7.3 years of intervention (61). However, the reported baseline mean intake of vitamin B_6 was high, about 5 mg/d.

Requirement and recommended intake

In infants, symptoms such as convulsions have been seen with the consumption of formula containing vitamin B_6 at a concentration of 0.06 mg/L (22). Among adults, clinical symptoms of deficiency have not been observed at intakes above 0.5 mg/d. The available controlled studies suggest that PLP levels are related to the level of protein intake in both men and women, but the effect is estimated to be rather limited within the usual range of protein intakes (1.0–1.5 g/kg body weight) seen in the Nordic countries.

Results from epidemiological studies indicate that vitamin B_6 status might be related to certain cancers, especially colorectal cancer, but associations with reported dietary intakes are less conclusive. RCTs have shown that supplements including high doses of vitamin B_6 do not consistently reduce the incidence of CVD outcomes or colorectal adenomas. Reported intakes and plasma PLP levels at baseline have varied considerably between studies, and this limits the interpretation of their results.

In NNR 2004, the estimated average requirement (AR) of vitamin B_6 for adult men and women was set at 0.013 mg/g dietary protein. This value was based on the results from depletion-repletion studies with controlled intakes of vitamin B_6 (expressed as free pyridoxine) that indicated that PLP levels above 20 nmol/L could be reached at intakes of 0.6–1.0 mg/d or around 0.01 mg/g dietary protein (62–67).

The recommended daily intake (RI) is maintained at 0.015 mg/g protein. However, when planning diets the daily intake of vitamin B_6 should not be lower than 1 mg/d. The values for the RIs for each sex and age group, up to 60 years of age, are calculated based on the reference value for energy intake and the assumption that the protein content of the diet provides 15 E%. For older age groups, RIs are calculated assuming a protein content of 18 E%.

The basic requirement for vitamin B_6 is increased for pregnant women, especially during the last trimester, to cover the extra needs 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 recommended. Although there are data indicating that plasma PLP levels decrease throughout pregnancy, there are insufficient data to support higher intakes (68–70).

For infants and older children, the reference intakes are based on the same value as for adults due to a lack of scientific data to suggest otherwise.

The lower intake level (LI) for adults is set to 0.01 mg/g protein. However, the scientific basis for this LI is weak.

In earlier studies, consumption of high-dose oral contraceptives was found to influence biochemical vitamin B_6 status in some women due, for example, to increased excretion of xanthurenic acid after a tryptophan load (8). The clinical relevance of this effect is uncertain, however, and this observation has not been included when setting the reference values.

Some epidemiological studies have suggested an association between low vitamin B_6 status and/or intake and increased risk of CVD and some cancers, especially colorectal cancer. However, most RCTs using supplements with high doses (50 mg/d) have not shown any major protection against these diseases. The available data are considered insufficient as a basis for setting reference values.

Reasoning behind the recommendation

In NNR 2004, the recommendations were based on depletion-repletion studies among adults using a cut-off for PLP levels of 20 nmol/L. Results from epidemiological studies indicate that low vitamin B_6 status might be related to increased risk of certain cancers. RCTs with supplements including vitamin B_6 have shown no benefits with respect to CVD or cognitive function. The recommendations from NNR 2004 are maintained.

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 of months to years. Symptoms include minor neurological symptoms and, at higher levels of 500 mg/d or more, neurotoxicity (71). 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 (71). This level is also adopted in the NNR 2012.

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23 Folate

Folate µg/d		Women	Men	Children		
				2–5 y	6–9 y	10–13 y
Recommended intake	RI	300 (400[1])	300	80	130	200
Average requirement	AR	200	200			
Lower intake level	LI	100	100			
Upper intake level	UL	–	–			

1 Women of reproductive age are recommended to consume 400 µg/d.
2 There is no evidence for adverse health effects associated with high intakes of folates from natural sources.

Introduction

Folate is a generic term for a group of compounds that includes folic acid and derivatives having nutritional properties similar to folic acid. Folacin is sometimes used synonymously with folate. Folic acid (pteroylmonoglutamic 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 consist of the pteroylpolyglutamates that contain one to six additional glutamate units.

Dietary sources and intake

Folate is present in most foods, and high concentrations are found in liver, green vegetables, and legumes. The most important food groups contributing to folate intake are cereal products (including bread) and vegetables, but dairy products, fruits, and berries are also significant sources. The folate content in foods might be underestimated in food composition databases and food tables mainly because common methods of analysis fail to open up the food matrix and liberate all of the folate (1).

Folates are labile, and significant losses in the cooking process are common. However, the cooking losses are dependent on both the food in question and method of processing (2).

The average dietary intake in the Nordic countries is 280–350 μg/10 MJ (see the chapter on dietary intakes in Nordic countries).

Physiology and metabolism

The metabolically active forms of the vitamin are reduced folate – THF – coupled to additional glutamate molecules. These serve as coenzymes in the transport of one-carbon units in amino acid metabolism and nucleic acid synthesis. The carbon can be carried as a methyl group (CH_3-THF), a formyl group (CHO-THF), a methylene group (–CH_2-THF), a methenyl group (–CH^+-THF), or a formimino group (CHNH-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 re-methylation of homocysteine to methionine. The methyl donor is 5-CH_3-THF, the synthesis of which is catalysed by the enzyme methylenetetra-hydrofolate reductase (MTHFR) and requires vitamin B_{12}. Homocysteine is also removed by a reaction that requires vitamin B_6. Elevated levels of homocysteine in serum can indicate low folate status, thus a normal serum homocysteine level is a measure of adequate folate supply. Other markers of folate status are erythrocyte (or RBC) folate and serum folate concentrations. Erythrocyte folate levels reflect tissue stores and are an indicator of long-term dietary intake. Unlike serum folate, erythrocyte folate is not affected by recent or transient changes in intake and thus might reflect actual intake. However, serum folate is strongly correlated to intake in population studies (3).

Bioavailability

Food folates must be hydrolysed by brush border folate conjugase to mono-glutamates prior to absorption in the upper part of the small intestine (4). 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 (5, 6). It is not possible to predict the overall bioavailability of folates from the composition of a diet (4), and there are few studies on absorption of food folate from composite meals. Bioavailability of folates has traditionally been estimated to be approximately 50% (7), but this level should only be considered a rough estimate. The value differs among populations and ethnic groups depending on the dietary composition, genetics, and a variety of other factors (1). Furthermore, natural food folate from a diet rich in fruit, vegetables, and liver products was estimated to have a bioavailability of 80% compared to supplemental folic acid (8).

Very few data are available from well-controlled intervention trials with diets naturally rich in folate or with foods fortified with folic acid. Interventions with folic acid-fortified breads or breakfast cereals (9, 10), as well as with meals and foods high in natural folate (11), have resulted in improvement of folate status indicators. In intervention trials with synthetic folate compounds, the natural folate form 5-methyl tetrahydrofolate was shown to be similarly effective or even more effective than the common fortificant folic acid in improving folate status (12, 13). Consumption of leafy green vegetables in the NHANES cohort (prior to mandatory fortification) was associated with higher serum folate levels, and promoting the consumption of fruits and vegetables was considered an effective strategy for enhancing nutritional adequacy (14).

Not enough data exist comparing the bioavailability of different forms of natural folate with each other or estimating folate bioavailability from different food matrices or meals. One study showed that female volunteers with a normal folate status could maintain their status during a 12-week intervention by adding five slices of wholegrain bread to their habitual diet. The women could also improve their status by adding a breakfast meal containing an additional 125 µg of folate for a total folate intake of about 310 µg/d, and such an intake complied with the recommended intake for folate (11). However, no additional improvement in folate status was observed during a 4- to 8-week intervention with an additional 150 µg folate from food (11, 15).

Based on the available literature, it can be concluded that long-term interventions with folates from natural foods and folic acid from supplements or fortified foods in physiological doses (up to 400 µg/d) improve folate status. However, due to a lack of studies, inconsistencies in the available data, and possible limitations in the experimental designs (e.g., unsuitable reference doses) there is not enough evidence for a quantitative estimation of the bioavailability of natural folates and synthetic folic acid (16).

Health outcomes

Pregnancy outcomes

There is convincing evidence that maternal folate status and intake is inversely associated with neural tube defects (NTD). A Cochrane Review found consistent results showing prevention of NTD from daily folic acid supplementation (alone or in combination with other vitamins and minerals) compared with no intervention/placebo or to vitamin and mineral supplements without folic acid. However, there was no statistically significant evidence for any effects on prevention of cleft palate, cleft lip, congenital cardiovascular defects, miscarriages, or any other birth defects (17). With respect to non-neural birth defects, the evidence is *inconclusive*.

In addition, low maternal folate status or inadequate use of folic acid supplements during early pregnancy has been reported to be associated with a higher risk of behavioural problems in the offspring (18–20). Use of folic acid supplements during pregnancy was associated with improved neurodevelopment in 4-year-old Spanish children when adjusting for socio-demographic and behavioural factors (20). The absence of folic acid supplementation in early pregnancy was associated with a higher risk of behavioural problems in the offspring at 18 months of age (18). In Swedish adolescents, higher folate intake and lower homocysteine status have been associated with improved achievement in school and this effect was consistent after correcting for parental education and other confounders (21).

Neurological outcomes in adults

There is *probable* evidence that an adequate folate intake or folate status (i.e. according to the recommendations) protects against poor cognitive function and potentially against some of the neurological disorders that tend to develop in the elderly. However, individuals deficient in vitamin B_{12} might be at risk for cognitive impairment even when serum folate levels are elevated (16).

Cancer

The NNR systematic review (SR) concluded that there is *limited-suggestive* evidence for a protective association between dietary folate at recommended intake levels (>300 µg/d) and risk of colorectal cancer (16). Similar conclusions are found in the report by the WCRF/AICR (22) regarding colorectal cancer. That report also found *limited* evidence that foods containing folate protect against cancers of the pancreas and the oesophagus.

In contrast, the NNR SR identified too few studies to draw any conclusions on associations between folate intake or folate status and cancers of the pancreas, the bladder, or the prostate (16). According to the NNR SR, the overall evidence regarding dietary folate and breast cancer risk is *inconclusive*, and most studies reported no significant associations (16). Although a meta-analysis of epidemiological studies reached similar conclusions regarding overall risk for breast cancer, that report concluded that adequate folate intakes might protect against breast cancer in women with moderate to high alcohol intakes (23). In addition, women of the Malmö Diet Cancer cohort with high folate intakes (i.e. in the highest quintile of the population and at the recommended level of folate intake) had a lower risk of postmenopausal breast cancer compared to the lowest quintile of folate intake (24).

Folate plays an important role in the proliferation of both normal and tumour cells. This has been demonstrated by animal experiments using very high doses (megadoses) of folic acid that resulted in enhancement of tumour formation from pre-carcinogenic stages (25). Because intake levels are generally higher in populations with mandatory folic acid fortification compared to populations with no fortification, there are concerns that the use of folic acid supplements and fortification might have population-wide adverse health effects (26, 27). However, the NNR SR identified too few studies regarding folic acid supplementation and cancer risk, and thus the evidence was graded *insufficient* (16).

Because the need for folate (and methyl groups) is greatest in rapidly growing cells, the potential influence of high folic acid doses on protection from, or stimulation of, tumour formation varies between different types of cancer. Also, inconsistencies and non-significant findings across epidemiological studies might depend not only on differences in study design but also on population differences regarding background diet, lifestyle, and genetic predisposition. For instance, recent SRs and meta-analyses from China, Norway, and the US regarding folic acid supplementation and risk of total cancer separately concluded that there is no significant association (28), a borderline increased risk (29), or a significantly increased cancer risk (30). One of these studies also reported protection of folic acid supplementation against melanoma skin cancer (28), but another reported an increased risk of prostate cancer (29).

Many studies have examined variations in the genes coding for enzymes of key importance for the metabolism of folate such as MTHFR. A meta-analysis that found support for folate in protecting against cancers of the oesophagus, pancreas, and stomach also indicated that the MTHFR 677TT genetic variant was linked to reduced risk for several cancers (31).

Cardiovascular disease

An adequate dietary folate intake (i.e. according to the recommendations) has been shown to be inversely associated with both severe and subclinical cardiovascular disease (CVD) outcomes (16). Elevated concentrations of homocysteine in the blood have been associated with increased risk of CVD (32, 33). Mild hyperhomocysteinaemia 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 if folate status is low homocysteine is elevated in the less common homozygous TT genotype compared to the heterozygous CT genotype and the more common homozygous CC genotype. If plasma folate is in the upper range of intake, there are little or no differences between the three genotypes (34–38). The frequency of TT-homozygotes in studies of Nordic populations is 5%–8.4% (34, 39). These frequencies are below earlier reported figures – average ≈12% (range 5.4%–16%) – in the Caucasian population (40). In general, men have higher concentrations of homocysteine than women, and the plasma homocysteine concentration tends to increase with age in both sexes (34, 41). The cause and significance of homocysteine increasing with age is not well understood, but the physiological decline in renal function might partly explain this age effect (42).

The effects on disease rates of supplementation with folic acid to lower plasma homocysteine levels are uncertain. A review by Clarke and coworkers (43) showed that dietary supplementation with folic acid (doses ranged from 0.8 mg/d to 5.0 mg/d except for one trial in which 40 mg/d was given) had no significant effects within five years on cardiovascular events or on overall cancer or mortality in the populations studied. A meta-analysis by Clarke et al. (44) further confirmed that available evidence does not support the routine use of B-vitamins to prevent CVD.

Requirement and recommended intake

Adults

The minimum dietary requirement to prevent folate deficiency anaemia in adults has been estimated to be 50–100 µg/d (45), or 50 µg/d of absorbed folate as judged from a daily parenteral dose of this amount (46).

On a virtually folate-free diet, the daily losses from stores in the liver and extrahepatic tissues are about 60 μg/d (47). The daily excretion of folate catabolites is around 0.3%–0.8% of the folate body pool (48, 49). Excretion of intact folate in the urine is between 1% and 5% of the ingested folates (7, 48, 49), but excretion increases at higher intakes (48, 49). Well-nourished individuals excrete 5–40 μg/d in the urine, and the losses from the enterohepatic circulation are similar (47). Based on these criteria, the lower intake level (LI) of dietary folate for adults is set to 100 μg/d.

Assessment of average requirements (AR) and recommended intakes (RI) are based on a combination of indicators reflecting folate status including the concentrations of serum or plasma folate, erythrocyte folate, and serum or plasma homocysteine. Serum and erythrocyte folate concentrations below 6.8 nmol/L and 317 nmol/L, respectively, are considered low (50). Because deficiency of folate is one of several causes of hyperhomocysteinaemia, the total plasma homocysteine concentration is regarded as a functional index of folate status. There is no consensus with regard to the definition of normal or elevated plasma homocysteine levels. The upper limit of the reference range is proposed to be 12 μmol/L (41, 51).

Results from well-controlled intervention trials with diets naturally rich in folate or with foods fortified with folic acid have shown that intakes of 200–300 μg/d are associated with adequate folate status, i.e., erythrocyte or plasma folate levels above the cut-off values (9–11).

Intervention with 120–400 μg/d supplemental folic acid or folic acid fortified foods over a period of 4 weeks to 14 weeks resulted in improvements of several or all folate status indicators, including the concentrations of plasma folate, erythrocyte folate, and plasma/serum homocysteine (9, 52–57). However, while reduced 5-methyl-THF is the dominant folate form in circulating blood, un-metabolized folic acid was detected in circulating plasma after ingestion of folic acid doses of around 400 μg but not doses of 150–200 μg (54). No studies regarding the implications of un-metabolised folic acid on human health are available (16).

An average dietary intake of 200 μg folate every day for 6–8 months maintained normal levels of serum and erythrocyte folate in adult men staying in a metabolic unit and consuming a controlled diet (58). In a study of women aged 17–40 years (n = 45), a calculated mean intake of 190–200 μg/d was sufficient to maintain levels within the normal reference range of serum and erythrocyte folate concentrations over a period of 12 weeks (59).

Results from a depletion-repletion study in 10 women housed in a metabolic unit for 92 days found that a daily intake of 200 μg/d from food folates resulted in erythrocyte folate levels of above 300 nmol/L, and an intake of 300 μg/d resulted in an additional small rise in plasma folate levels (7).

Observational studies linking dietary intake and levels of plasma and erythrocyte folate are less precise because of possible underestimation of intake due to misreporting and/or inadequate food composition data (1, 60). However, this is counteracted if intake estimates are not corrected 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.

Results from dietary surveys indicate that reported mean or median intakes of folate of 270–316 μg/d were associated with erythrocyte folate levels above 676–959 nmol/L (41). Intakes of 240–325 μg/d were associated with plasma folate levels of 11.3–14.2 nmol/L (34, 61, 62). In the study by Brussaard and co-workers (62), the prevalence of serum folate levels below 7 nmol/L (value indicating adequate folate status (63)) was 6% among those with a mean intake of 325 μg/d and 13% among those with a mean intake of 270 μg/d.

The above-cited studies indicate that intakes around 300 μg/d are sufficient to keep serum and erythrocyte folate levels well above cut-off values.

Keeping the possible underestimation of folate intake in mind and the fact that only a few of the cited studies found individuals with very low intakes, the AR with respect to maintaining normal blood levels is estimated to be 150–200 μg/d. An intake of 300 μg per day should keep folate concentrations in the blood above accepted cut-off values and homocysteine concentrations below accepted cut-off values. Therefore, the estimated AR for adults in the NNR is set to 200 μg/d and the RI to 300 μg/d.

Women of reproductive age represent a specific challenge because there is convincing evidence that an adequate supply of folate before and up to 12 weeks after conception reduces the risk of NTD. However, far from all pregnancies are planned. Therefore, an RI of 400 μg/d for all women of reproductive ages should provide adequate folate supply to women experiencing unplanned pregnancies. Currently, most women in the Nordic countries do not meet the RI for folate. In the Swedish national survey "Riksmaten" 2010–2011, less than 20% of the adult female population reached the RI (64). About 10% of women of childbearing age in Iceland have intakes lower than the AR of 200 μg/d. The average daily intake is 304 μg for men and 248 μg for women. Finnish studies report mean folate intakes in women of reproductive ages ranging from 215 μg/d to 230 μg/d (65, 66).

Pregnancy and lactation

Because folate requirements increase during pregnancy, especially in the last trimester, the risk of deficiencies in women with low folate stores also increases. When folate intake is inadequate, maternal serum and erythrocyte folate concentrations decrease and megaloblastic anaemia can develop. Caudill and colleagues (67) compared pregnant (second trimester) and non-pregnant women on controlled intakes 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 erythrocyte folate concentrations were high at the end of the 12-week study indicating that a lower intake might be sufficient. In NNR 2004, the recommended intake during pregnancy was set to 500 µg per day. This was based on previous studies indicating that 400–500 µg/d is considered sufficient to meet the increased requirement from fast growing tissues during pregnancy (67). Because there are no new scientific data, this recommendation is kept unchanged in NNR 2012.

The concentration of folate in human milk varies throughout the lactation period and is highest between 3 and 6 months (68). Smith and co-workers (69) reported the average concentration of folate in human milk to be 85 µg/L. Based on a daily 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/d, and this amount will allow replenishment of stores before a possible new pregnancy.

Infants and children

In NNR 2004, infants were recommended to consume 5 µg folate 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 over a period of 6–9 months (70). Slightly higher serum and erythrocyte folate concentrations were found in infants at the upper end of the intake range. Because no new data on the requirements in children have been identified, the recommendations for children in the age group 1–14 years are kept unchanged in NNR 2012 and are based on 5 µg folate per kg body weight.

Reasoning behind the recommendation

According to the NNR SR, there is *probable* evidence that an adequate folate intake or status protects against poor cognitive function and *limited-suggestive* evidence for a protective effect against some neurological disorders. Folate intake and folate status have also been shown to be inversely associated with CVD outcomes. Folate intake from foods and folate status are also inversely associated with the risk for colon cancer, but findings are less clear regarding other cancers. Long-term intervention trials with natural food folate and folic acid from supplements and fortified foods improve folate status. However, studies and data on quantitative estimation of the bioavailability of various natural folates and synthetic folic acid are sparse and inconsistent. This is partly due to limitations in experimental design.

No evidence emerged from the NNR SR to prompt a change in the current recommendations of 300 µg folate per day for adult women and men and 400 µg/d for women of reproductive age (16). No published data provide evidence regarding the specific folate intake level required to maintain an optimal folate status. There is also no evidence in the evaluated articles to motivate changing any of the other folate dietary reference values (DRV).

Upper intake levels and toxicity

There is no evidence for adverse health effects associated with high intakes of folates from natural sources. However, a high intake of folic acid (either supplemental or from fortification) can mask haematological symptoms caused by deficiency of vitamin B_{12}. The Scientific Committee on Food (71) has set the upper level of intake (UL) of folic acid to 1000 µg/d for adults. The UL for children and adolescents is adjusted on the basis of bodyweight and is 200, 300, 400, 600, and 800 µg per day for children aged 1–3, 4–6, 7–10, 11–14, and 15–17 years, respectively (71). The NNR SR found no evidence to support a change to the UL (16).

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24 Vitamin B_{12}

Vitamin B_{12} µg/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 intake level	LI	1	1			
Upper intake level	UL	[a]	[a]			

[a] Not established.

Introduction

Vitamin B_{12} is the common term for a group of cobalt-containing compounds (corrinoids) that are biologically active in humans. Cobalamin can be used synonymously with vitamin B_{12}. Inactive compounds analogous to vitamin B_{12} are found in the diet, especially in plant foods (1).

Dietary sources and intake

Vitamin B_{12} is mainly found in foods of animal origin. Plant foods might contain trace amounts from bacterial contamination or as a result of fermentation, but the adequacy of these sources is questionable (2). Meat, liver, dairy products, fish, and shellfish, are particularly good sources and are main sources in the average diet (3, 4). Seaweeds and algae contain biologically inactive vitamin B_{12} analogues as well as a few active vitamin B_{12} compounds. However, the adequacy of these sources is uncertain (5). Some seaweeds and seaweed products also contain high amounts of iodine, and this can lead to excessive intake of dietary iodine with adverse effects on thyroid function (2, 6–8). Some plant-based milk substitutes (e.g. soy milk, oat milk, and rice milk) might be enriched with vitamin B_{12} and, therefore, might be an important source of vitamin B_{12} in vegans. The diet in the Nordic countries has a mean vitamin B_{12} content of 6–8 mg/10 MJ.

Vegetarian diets, especially vegan diets, tend to contain low or minimal amounts of vitamin B_{12} (9–11).

Physiology and metabolism

Absorption of vitamin B_{12} is a multistep process. Protein-bound vitamin B_{12} in foods is cleaved from the protein through the action of hydrochloric acid and pepsin in the stomach and then re-bound to haptocorrin (transcobalamin I, TC I). The absorption requires a glycoprotein – intrinsic factor – secreted by the parietal cells of the stomach. In the small intestine, vitamin B_{12} is released from haptocorrin and binds to the intrinsic factor and the resulting complex is absorbed via special receptors in the ileum. Vitamin B_{12} is linked to transcobalamin II in the enterocytes, and the TC II-vitamin B_{12} complex, also called holotranscobalamin (holoTC), enters the blood circulation and is rapidly taken up by the liver, bone marrow, and other tissues (12, 13). Most of the circulating vitamin B_{12} is bound to transcobalamin I (TC I) and has a half-life of several days compared to the half-life of about an hour for holoTC (13). The ileal receptors are saturated at intakes of 1.5 µg to 2.0 µg per meal (12, 14). As the intake increases, the percentage of absorbed vitamin B_{12} decreases. Bioavailability of vitamin B_{12} from various foods, as assessed by whole-body retention or faecal excretion, ranges from about 20% up to 90% at single doses of 0.25 µg to 5 µg (12). It is estimated that approximately 50% of dietary vitamin B_{12} is absorbed by healthy adults with normal gastric function (15, 16).

The function of vitamin B_{12} is related to the metabolism of methyl groups. Methylcobalamin is a cofactor for methionine synthase – the enzyme that catalyses 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. Intracellular deficiency of vitamin B_{12} results in increased plasma concentrations of methylmalonic acid (MMA) and homocysteine.

Total body stores of vitamin B_{12} are reported to be 2–5 mg, of which about a half is in the liver (13). The daily loss of vitamin B_{12} is about 0.1% of the total body pool (14, 17). Clinical symptoms of vitamin B_{12} deficiency generally develop only after several years of insufficient dietary intake or decreased absorption (13).

The most common biochemical markers for assessing vitamin B_{12} status are the mean corpuscular volume of red blood cells (MCV) and the concentrations of plasma vitamin B_{12}, holoTC, and serum MMA. Serum homocysteine can also be used, but this is more strongly influenced by folate status and only to some extent by vitamin B_6 and riboflavin status. A plasma vitamin B_{12} concentration below 150 pmol/L is considered an indicator of vitamin B_{12} deficiency, although levels between 150 pmol/L and 220 pmol/L can indicate insufficient supply. A decreased level of plasma holoTC is an early sign of negative vitamin B_{12} balance and has been used as a complementary indicator of vitamin B_{12} status together with an increased MMA concentration (13). Both holoTC and MMA levels are increased when kidney function is impaired. During pregnancy, plasma holoTC is considered to be the most suitable marker of maternal supply of vitamin B_{12} to the foetus (18).

An adequate supply of vitamin B_{12} is essential for normal blood formation and neurological function. Vitamin B_{12} deficiency results in macrocytic, megaloblastic anaemia and/or neurological symptoms due to degeneration of the spinal cord, brain, and optic and peripheral nerves. Deficiency caused by inadequate dietary intake is only observed in adults who have been eating vegan diets for many years without taking vitamin B_{12} supplements or including products enriched with vitamin B_{12}, or in infants and children from families following such a dietary pattern (2, 19–21).

Elderly people frequently have low vitamin B12 levels (22) that cannot be attributed to poor intake of vitamin B_{12} (23). A major cause of vitamin B_{12} deficiency is vitamin B_{12} malabsorption, which usually results from atrophic gastritis and hypochlorhydria. This disorder is defined as the inability to absorb protein-bound vitamin B_{12} by a person who is fully capable of absorbing free vitamin B_{12}. 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 vitamin B_{12} concentrations (22).

A systematic review found inconclusive evidence for an association between subnormal blood levels of vitamin B_{12} and anaemia (24). The result was weakened by the fact that the included studies used different tests to measure the blood levels and used different cut-off points to define vitamin B_{12} deficiency. However, even the studies with the lowest cut-off points for deficiency in which the strongest associations would be expected did not report clear associations.

There are limited data on vitamin B_{12} status in Nordic populations. Results from population surveys among adults have shown a prevalence of low plasma vitamin B_{12} concentrations (<150 pmol/L) of 1%–6% at mean dietary intakes of 5–7 µg vitamin B_{12} per day (25–27). A higher prevalence of low plasma vitamin B_{12} concentrations was found in an earlier study on a population of Danish 80 year olds (28). In the study by Vogiatzolou et al. (26), 5% had levels below 200 pmol/L and in the study by Loikas et al. (27) among persons aged 65–100 years 32% had concentrations of 150–250 pmol/L.

Health effects

Several randomized controlled trials (RCTs) have tested the effect of supplemental intake of B-vitamins (folic acid, vitamin B_6, and B_{12}) on the risk of cardiovascular disease (CVD) and cancers. Doses of vitamin B_{12} in the studies ranged from 0.4 mg/d to 1 mg/d (29, 30). In addition, cohort studies have investigated the association between vitamin B_{12} intake or status and CVD and cancers.

CVD

Results from a few recent cohort studies show no consistent association between CVD and dietary intakes (31, 32) or plasma concentrations of vitamin B_{12} (33). In the study by Weikert et al. (33), a significantly increased risk of ischaemic stroke (RR = 1.57, 95% CI: 1.02–2.43) was found in the lowest tertile of plasma vitamin B_{12} (median 191 pmol/L) compared to the upper tertile (median 394 pmol/L) in the German cohort of the EPIC study. Among elderly subjects, no association was found between plasma concentrations of vitamin B_{12} and coronary heart disease mortality or all-cause mortality (34).

RCTs using supplements containing combinations of folic acid, vitamin B_6, and vitamin B_{12} (with doses of vitamin B_{12} ranging from 0.4 mg/d to 1 mg/d) did not show significant reductions in the risk for myocardial infarction or angina pectoris, but one of the four studies showed a reduced risk of stroke (35, 36).

Cancer

Results from cohort and nested case-control studies have generally shown no associations between intake or status of vitamin B_{12} and breast cancer (37–40), endometrial cancer (41, 42), or colorectal cancer (43–47). A positive association between plasma levels of vitamin B_{12} and the risk for prostate cancer has been indicated, and a meta-analysis of five cohorts found an odds ratio of 1.10 (95% CI: 1.01–1.19) for each 100 pmol/L increase in plasma vitamin B_{12} concentrations (48). Results from the Finnish ATBC study among heavy smokers (47) found a significantly increased risk of prostate cancer in the highest quintile of total vitamin B_{12} intake (>14 µg/d) after up to 17 years follow-up.

Cognitive function

Some prospective cohort studies have examined the relationship between cognitive function and vitamin B_{12} intake and/or status among the elderly. The Chicago Health and Aging Project included men and women aged 65 years and older and found that total dietary and supplementary intake of vitamin B_{12} (49) as well as plasma levels of vitamin B_{12} (50) were inversely related to cognitive decline, but the study found no relation between total intake and risk of Alzheimer's disease (51). A prospective study from Oxford University also found that vitamin B_{12} status was inversely associated with cognitive decline (52), but other prospective cohort studies found no association between cognitive decline and vitamin B_{12} status (53–55) or total vitamin B_{12} intake (56).

Intervention studies that have examined the relationship between vitamin B_{12} and cognitive function have used a combination of vitamin B_6, vitamin B_{12}, and folate (57, 58), and a Cochrane Review found that the evidence for any effect of vitamin B_{12} on improving the cognitive function of people with dementia and low serum vitamin B_{12} levels was insufficient (59). Thus, the evidence based on intervention studies of the effect of vitamin B_{12} *per se* among the healthy elderly is limited, and the results from prospective cohort studies are inconclusive.

Requirement and recommended intake

The requirement for vitamin B_{12} to prevent anaemia can be estimated from studies of patients with pernicious anaemia (60). In a study on 20 patients, an intramuscular dose of 0.5–2.0 µg vitamin B_{12} per day was needed for normalizing and maintaining haematological status, and 0.5–1.0 µg was sufficient for most subjects (60). Because these patients are unable to reabsorb vitamin B_{12} excreted in the bile, the physiological requirement for healthy individuals is somewhat lower.

In NNR 2004, an average physiological requirement of vitamin B_{12} was set to 0.7 µg/d based on the above and other studies (1, 15). With correction for absorption losses (50%) the average requirement (AR) was 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 for adults was set to 2 µg/d.

The US IoM used a similar approach for setting the DRIs. The estimated AR was set to 2.0 µg/d and the RDA to 2.4 µg/d assuming a coefficient of variation of 10%. Herbert (1) calculated the average dietary requirement to be 1.4 µg/d based on the assumptions that adequate body stores were 1,000 µg, the mean half-life was 1,000 days, and the mean absorption was 50%.

In NNR 2004, the recommended intake (RI) for the elderly was the same as for younger adults. Increasing the RI is not considered likely to overcome malabsorption of food-bound vitamin B_{12} or lack of intrinsic factor.

Pregnant women usually have adequate stores to cover the estimated additional requirements of 0.1–0.2 µg/d (15). In NNR 2004, the RI was the same as for non-pregnant women. The RI is maintained in NNR 2012 because there are no new scientific data to suggest a change.

Lactating women are recommended an additional 0.6 µg/d to compensate for the content of vitamin B_{12} in breast milk. This recommendation is maintained in NNR 2012.

For children, the recommended intake in NNR 2004 was based on 0.05 µg/kg body weight, and this is also used as the basis for setting the RI in NNR 2012.

Results from cross-sectional population studies have shown that biochemical indicators of vitamin B_{12} status are stabilised at intakes of about 4–10 µg/d among adults (26, 61, 62). Whether intakes in the above range are associated with long-term benefits is unclear.

The lower dietary intake needed to prevent anaemia is 1 µg/d.

Reasoning behind the recommendation

The reference values for vitamin B_{12} are kept unchanged from NNR 2004 because there are no strong scientific data to suggest that changes are needed. The AR and RI are based on studies on patients with pernicious anaemia. Results from intervention and epidemiological studies do not support benefits of higher intakes for the prevention of common diet-related diseases such as cancer, CVD, or cognitive impairment. There is insufficient evidence for an association between subnormal blood levels of vitamin B_{12} and anaemia among the elderly. However, many elderly persons with impaired gastric function might need supplementing with vitamin B_{12} due to food-vitamin B_{12} malabsorption.

Upper intake levels and toxicity

There are no clearly defined adverse effects of excess vitamin B_{12}, and data are insufficient to establish an upper intake level (UL). There is no evidence that intakes up to 100 µg/d from foods and supplements represent a health risk (63).

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25 Biotin

No recommendation given due to lack of sufficient evidence

Introduction

Biotin is a water-soluble heterocyclic compound formerly known as vitamin H that belongs to the group of B-vitamins. Biotin is essential to all known organisms and is synthesized by plants and microorganisms, but animals, including man, lack the ability to synthesize biotin (1). Biotin in foods exists in free or protein-bound forms.

Dietary sources and intake

Biotin is found in most foods at low concentrations. Offal meats such as liver and kidney, egg yolks, rolled oats, and wheat bran are rich sources (2). The 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 (2).

Physiology and metabolism

Protein-bound biotin is digested in the gut prior to absorption and requires the enzyme biotinidase (1) to cleave the covalent bond between the biotin and the protein (1). Bioavailability of biotin in different foods varies from very low to almost complete utilisation. In general, however, less than half of the biotin in foods is available (3). Raw egg white contains the glycoprotein avidin that binds to biotin and prevents its absorption, but the biotin binding capacity of egg white is lost upon cooking. A potential source of biotin is microbial synthesis in the large intestine, but the quantitative contribution of this source to biotin metabolism is unclear (4).

Biotin functions as a cofactor in carboxylation reactions and assists in the transfer of one-carbon units in the form of activated carboxyl groups during intermediary metabolism. These reactions are important in fatty acid synthesis, in the conversion of pyruvate into oxaloacetate (an intermediate in the citric acid cycle), and in degradation of branched amino acids and odd-chain fatty acids.

When the activity of 3-methylcrotonyl-CoA carboxylase decreases, its substrate is shunted to an alternate metabolic pathway that produces 3-hydroxyisovaleric acid (3-HIA). 3-HIA is excreted in the urine, and an elevated urinary concentration of 3-HIA is regarded as an early and sensitive indicator of biotin deficiency (1, 5). Dietary deficiency of biotin is rare and has only been conclusively demonstrated in individuals on parenteral nutrition without biotin or on diets with chronic ingestion of raw egg white. Biotin deficiency has also been demonstrated in cases of inherited biotinidase deficiency (1). Increased excretion of 3-HIA is frequently seen during normal pregnancy and is reflective of reduced biotin status (6). However, no untoward effects of a marginally reduced biotin status in pregnancy have been documented (7).

Requirement and recommendation

Data providing an estimate of biotin requirements are scarce, and no recommendation is given in NNR 2012. The U.S. Food and Nutrition Board set an adequate intake (AI) for adults of 30 μg/d (8). This reference intake is based upon the intake of biotin in breast-fed infants and is extrapolated to adults by body weight.

Upper intake levels and toxicity

Data on adverse effects from high biotin intake are not sufficient to set a tolerable upper intake level (UL). Although no numerical UL can be established, existing evidence from observational studies indicates that current levels of biotin intake from all sources do not represent a health risk for the general population (9).

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26 Pantothenic acid

No recommendation given due to lack of sufficient evidence

Introduction

Pantothenic acid belongs to the group of B-vitamins. The vitamin is water-soluble and has an important role in intermediary metabolism as part of coenzyme A (1, 2). Pantothenic acid is widely distributed in nature as its name implies (from the Greek *pantos* meaning "everywhere").

Dietary sources and intake

Pantothenic acid is found in many foods. Rich sources of pantothenic acid are offal, dried legumes, and wholegrain products. Data on dietary intakes in the Nordic countries are scarce. The content of pantothenic acid in the average Danish diet is estimated to be approximately 5 mg per 10 MJ (3). The majority (~ 75%) of this amount comes from milk and cheese, cereal products (including bread), meats, and vegetables (3).

Physiology and metabolism

As a constituent part of coenzyme A and acyl-carrier protein, pantothenic acid plays a central role in both catabolism and anabolism as a carrier of acyl groups. The bioavailability of pantothenic acid from foods is 40%–60% (1).

Deficiency of pantothenic acid is rare because of the widespread nature of the vitamin, and deficiency has only been observed in individuals on a diet free of pantothenic acid or given an antagonist to pantothenic acid (4). Deficiency-induced greying of the 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 (5, 6).

Requirement and recommended intake

There is insufficient information for estimating the requirement of pantothenic acid, and no recommended intakes are included in NNR 2012. In the U.S., the recommendation for an adequate intake (AI) for adults was set to 5 mg/d (4). This reference intake is mainly based upon estimated usual intakes of pantothenic acid in the US 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, and due to a lack of systematic oral intake dose-response studies no upper intake level can be derived. The evidence available from clinical studies using high doses of pantothenic acid indicates that intakes considerably in excess of current levels of intake from all sources do not represent a health risk for the general population (7).

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27 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 intake level	LI	10	10			
Upper intake level	UL	–	–			

Introduction

The term vitamin C refers to both ascorbic acid and dehydroascorbic acid because both forms have an anti-scorbutic effect. 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 research into daily vitamin C allowances.

Dietary sources and intake

The concentration of vitamin C is high in many vegetables, berries, and fruits (especially citrus fruits). Moreover, intake from vitamin C-enriched products (e.g. juices) can be considerable. The average intake of vitamin C in the Nordic countries is 123–152 mg/10 MJ. The plasma level of vitamin C is a biomarker of fruit and vegetable consumption (1), and the observed associations between plasma (and dietary) vitamin C and health might at least partly reflect other health-enhancing components in fruit and vegetables or even other lifestyle variables.

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 a 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 neutrophils, semen, and plasma (e.g. protection against Low density lipoprotein (LDL) oxidation) (2, 3). Ascorbic acid can also regenerate other antioxidants such as vitamin E. As a reducing agent, ascorbic acid can 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 (the efficiency of gastrointestinal tract absorption) is at least 80% for doses of 100 mg or less, 60–70% for doses of 200–500 mg, and less than 50% for doses exceeding 1,000 mg (3). Unabsorbed ascorbate is degraded in the intestine and this process can lead to diarrhoea and intestinal discomfort that are 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, the remaining vitamin C is excreted in the urine. For doses up to 60 mg no ascorbic acid is excreted (3), for doses of 100 mg about 25% is excreted, for doses of 200 mg about 50% is excreted, and for doses of 500 mg about 80–90% is excreted. The estimated threshold for excretion is about 80 mg/d meaning that essentially no vitamin C is excreted in the urine if the daily intake is lower than the threshold (5).

The body pool of ascorbic acid increases up to a daily intake of approximately 100 mg (6) at which point neutrophils, monocytes, and lymphocytes become saturated (3, 7). When white blood cells become saturated, the plasma ascorbic acid concentration is approximately 50–60 mmol/L but very large doses (2,500 mg/d) are capable of increasing plasma levels up to 80 mmol/L (3, 7). However, above a daily intake of about 100 mg ascorbic acid, further increases in vitamin C intake lead to gradually smaller increases in plasma vitamin C levels (9). Plasma ascorbic acid concentrations below 23 mmol/L are indicative of marginal vitamin C status (8). This level is reached with an estimated daily intake of 41 mg, but this exact value depends on body size (8). Marginal status can present as decreased antioxidant capacity, fatigue, and irritability (3). Symptoms of scurvy are observed when plasma levels are below 11 mmol/L (8) or the total body pool is below 300 mg (9). Scurvy is very uncommon, but cases have been reported even in Nordic countries (10).

Prospective cohort studies

One way to study the associations between vitamin C and chronic diseases is to use longitudinal population samples, or cohort studies. Unfortunately, these are not ideal for many reasons, the most important of which is that it is almost impossible to make precise estimations of vitamin C intake by using the methods available in studying large populations (mainly food-frequency questionnaires).

Another approach is to study the association of plasma ascorbic acid concentration and disease outcomes. The advantage of this approach is that plasma vitamin C measurements are more accurate and reliable than estimates of dietary vitamin C intake. The drawback to this approach is that plasma vitamin C levels reflect many other dietary and lifestyle variables than just vitamin C intake from the diet. For example, consumption of fruits and vegetables correlates with plasma ascorbic acid concentration (1) but fruits and vegetables also have positive health effects that are not explained by their vitamin C content. In addition, even after multiple adjustments a high intake of fruits and vegetables can still be associated with some unmeasured lifestyle variables that are positively related to health (11, 12).

Eight large prospective studies have found an inverse association between plasma ascorbic acid concentration and cardiovascular and/or all-cause mortality (13–20). Moreover, five prospective cohort studies, all using the EPIC data (European Prospective Investigation into Cancer and Nutrition), have reported on associations between plasma ascorbic acid concentration and type 2 diabetes (21), coronary artery disease (22), stroke (23), blood pressure (24), and heart failure (25). All of these studies showed that the risk for mortality and morbidity was highest in subjects with the lowest plasma concentrations. In contrast, Lawlor et al. (12) did not find an association between plasma vitamin C concentration and coronary heart disease even after adjustment for socioeconomic conditions.

The relationship between plasma vitamin C concentration and morbidity was curvilinear in most of the above studies, that is, the largest decrease in risk (compared to, for example, the adjacent lower quartile), was observed for those between the 20th and 40th percentile. Studies with cancer mortality as the outcome have also identified the lowest plasma ascorbic acid category as being clearly associated with increased risk (20, 26). However, in some studies (13, 14, 17, 18, 20), 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. above 40th percentile). The same variation was seen in studies using disease incidence as outcome: in some cases, those above the 25th percentile had similarly reduced risk ratios (22, 25), but other reports showed that the risk was still reduced at least up to the median plasma ascorbic acid concentration (21, 23, 24).

Supplementation studies

Supplementation studies are controlled interventions. The definite advantage – compared to observational cohort studies – is that the additional intake of vitamin C is known. However, the estimation of dietary intake (without supplements) is as difficult to assess as in observational studies. Another more principal problem is that the amount of supplemented vitamin C is often significantly higher than the assumed average and recommended intakes (27). Therefore, these studies do not provide much information about variations of intakes that are closer to what can be achieved from ordinary diets.

Bjelakovic and co-workers (27) published a meta-analysis on mortality in randomized trials of antioxidant supplements for the prevention of diseases. They identified only three trials with vitamin C as the single supplement, and only one of these trials (28) had an outcome with major relevance to the NNR. Although Salonen and co-workers (28) reported that vitamin C slowed down atherosclerotic progression in hypercholesterolemic persons, the overall conclusion in the meta-analysis was that vitamin C alone or in combination with other antioxidants had no significant effect on mortality (27).

More recently, two papers based on the Physicians' Health Study II (a randomized controlled trial) concluded that vitamin C did not reduce the risk of prostate or total cancer (29) or cardiovascular disease (30) in middle-aged and older men. In contrast, a meta-analysis of clinical trials concluded that vitamin C supplementation (median dose 500 mg/d) lowered blood pressure in both hypertensive and normotensive participants (31). However, most trials were short in duration (median 8 weeks) and the trial sizes were rather small and ranged only from 10 to 120 participants. Therefore, larger studies of longer duration are needed to get more insight into the potential blood pressure lowering effects of vitamin C supplementation.

Dietary micronutrient recommendations are typically based on data on deficiency symptoms (lower intake level) and on associations with, and effects on, chronic diseases such as cardiovascular disease, type 2 diabetes, cancer, and osteoporosis. In addition to chronic diseases, vitamin C has a potential effect in the prevention and treatment of the common cold. However, a meta-analysis has concluded that there is no scientific evidence supporting a protective role of vitamin C supplementation in reducing the incidence of colds in normal populations (32). In contrast, randomized trials suggest that vitamin C supplementation might reduce the incidence of the common cold in athletes and other individuals who are under extreme physical stress (33, 34). Moreover, high daily intakes of vitamin C (200–1,000 mg) might reduce the duration of the common cold by approximately 10% (32).

Requirement and recommended intake

Earlier Nordic recommendations (35), as well as the U.S. RDIs from 1989 (9), were based on an estimated adequate body pool (1,500 mg) that would give an ample safety margin against scurvy (36). 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 (9). This intake would also lead to plasma ascorbic acid concentrations above 23 mmol/L (8). By assuming a large inter-individual variation (50%) and to ensure adequate iron absorption, the NNR 1996 was set at 60 mg for both men and women.

Based on the increased recognition of the antioxidant property 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 (3). Based on these arguments, the recommendations for vitamin

C intake in NNR 2004 (37) were grounded mostly on the role of ascorbic acid in preventing morbidity and mortality from chronic diseases such as cancer and cardiovascular diseases (37). This reasoning could obviously be challenged because it is mostly based on population studies with the limitations noted earlier in this chapter.

By using the cut-off points in population studies for clearly lowered risk to morbidity and mortality from chronic diseases, such as cancer and cardiovascular diseases (in relation to the lowest 20%), the mean cut-off point was an ascorbic acid concentration of 32 mmol/L (unweighted mean of the eight studies with mortality as outcome) (13–20). This plasma level was chosen as the basis for the average requirement in NNR 2004 (37). The more recent studies on morbidity could indicate a slightly higher optimal level of roughly 40–50 mmol/L as a basis for the average requirements (21–25). However, the evidence might be biased due to the fact that all identified cohort studies relied on the same data.

Using the pharmacokinetic data of Levine et al. (3, 7), a 32 mmol/L concentration of ascorbic acid in the plasma corresponds to a daily vitamin C intake of approximately 60 mg/d in men and 50 mg/d in women. This is close to the intake at which vitamin C begins to be excreted in the urine (3) and corresponds to a body pool of approximately 1,000–1,200 mg (36). By giving a conservative 25% allowance for the inter-individual variation, the daily recommendation is set to 75 mg. Hence, this recommendation can be seen as the meeting point of two approaches: one from population studies and the other from pharmacokinetics (the excretion of vitamin C into the urine). In addition, an intake of 75 mg/d would lead to a plasma vitamin C concentration of around 40 mmol/L (3, 38), which is a level that has already been associated with inhibition of LDL oxidation in vitro (39).

The pharmacokinetics of vitamin C in women appear to be similar to those in men (7). However, at daily intakes below 100 mg, women have slightly higher plasma vitamin C concentrations for a given level of intake. These data suggest that the average requirements are slightly lower in women, which might be due to their smaller body size (9). However, to ensure adequate non-haem 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. Smokers might need about 30 mg more vitamin C daily to reach plasma vitamin C levels comparable to non-smokers (40).

The recommended intake is increased by 10 mg/d during pregnancy to cover the increased needs due to the growth of the foetus and catabolised vitamin C (9). Breast milk contains approximately 30 mg vitamin C per litre (9). 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 during pregnancy to 85 mg/d and during lactation to 100 mg/d.

The average requirements for children were extrapolated from the adult values by assuming growth factors of 1.3 for children younger than 2 years old and 1.15 for children 2 to 13 years old. The recommended intake was calculated as 1.25 times the estimated average requirement.

Reasoning behind the recommendation

The 2004 Nordic Nutrition Recommendation (37) was based on a mean ascorbic acid cut-off point of 32 mmol/L, which was the unweighted mean of the eight studies with mortality as the outcome. The average dietary vitamin C intake leading to this plasma ascorbic acid concentration was estimated to be 60 mg. When adding an estimation for the intra-individual variance (2 SD = 15 mg), the recommendation was set at 75 mg/d for adults. The more recent studies on morbidity could indicate a slightly higher optimal level, roughly 40–50 mmol/L, as a basis for the average requirement. However, the evidence for this might be biased due to the fact that all of the identified cohort studies relied on the same data. Therefore, these data were not regarded as sufficient for raising the average requirement or the recommended intake.

Upper intake levels and toxicity

There is no evidence that high intakes (>1,000 mg/d) of vitamin C are carcinogenic or teratogenic (40). However, high intakes might cause diarrhoea and other gastrointestinal disturbances and possibly also increased oxalate formation and kidney stone formation in susceptible individuals.

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