Vitamin basics

The facts about vitamins in nutrition
DSM Overview

Our company
Royal DSM is a global science-based company active in health, nutrition and materials. By connecting its unique competences in Life Sciences and Material Sciences DSM is driving economic prosperity, environmental progress and social advances to create sustainable value for all stakeholders. DSM delivers innovative solutions that nourish, protect and improve performance in global markets such as food and dietary supplements, personal care, feed, pharmaceuticals, medical devices, automotive, paints, electrical and electronics, life protection, alternative energy and bio-based materials. DSM’s 22,000 employees deliver annual net sales of about €9 billion. The company is listed on NYSE Euronext. More information can be found at www.dsm.com

DSM Nutritional Products

Human Nutrition & Health largely addresses the nutritional ingredients part of the food market. Animal Nutrition & Health addresses the nutritional additives segment of the feed market. Personal Care is focusing on the actives and ingredients in the sun care, skin care and hair care industries.

DSM is the only producer who can supply the complete range of vitamins and carotenoids in the most suitable forms for all possible human and animal uses.

DSM has established leadership positions across all three areas of the ingredients business: food, feed and personal care. It is the world’s largest producer of vitamins. DSM is working from its strong basis as a global market leader in key value-added ingredients offered through an international infrastructure and reach unequaled by any competitor. DSM is uniquely involved in all three main steps of the value chain: the production of pure active ingredients, their incorporation into sophisticated forms, and the provision of tailored premixes. Being the only fully integrated player allows DSM to differentiate itself all the way through the chain.

Managing the interdependencies between active ingredients, forms and premixes, which have important implications for innovation, logistics, and value delivery, is a core competence of DSM.

A pioneer in innovation
Innovation is a survival skill. DSM Nutritional Products fosters this skill to the benefit of both the customers’ future and that of the company. Lateral thinking and innovative attitudes are valuable tools with which to secure that future. These lead to discoveries that DSM links to customers’ needs, extending the range of offering and creating new business opportunities.

Starting in 1935 with the chemical synthesis of vitamin C, Roche had always been a pioneer in the industrial synthesis of vitamins and carotenoids. Today DSM’s Nutrition cluster, which includes DSM Nutritional Products, maintains this tradition and invests more than 5% of its sales value in R&D, which is significantly higher than peers in the industry.

DSM’s Research & Development activities are concentrated in the regions of Switzerland, the Netherlands, China and the United States in six Research Centers: Human Nutrition & Health, Animal Nutrition & Health, Personal Care, Process Research and Development, Product Form Development and Analytics.
Global operations
With ten large production sites (excluding premix) in Europe, the United States and China, six R&D centers in Europe and China, over 45 premix plants for animal or human nutrition and over 40 sales offices across the globe, DSM Nutritional Products is never far away from its customers and can make products in response to specific customer needs.

Our customer focus
The customer is at the center of all our activities. Everything we do – and this not only in our Marketing & Sales organization – we do for our customers. Customer intimacy is a key word at DSM Nutritional Products: it means that we listen to our partners and develop solutions that add value to their businesses.

Customer intimacy
The basis of all activities for and with our customers is our new Dual Track Strategy, which involves sustaining our established products while boosting the full growth of our new business. Apart from applying the strategy to interactions with customers, we also attach great importance to certain behaviors and values, in particular customer intimacy. This is based on mutual trust and respect. We strive to build relationships with our customers in which all issues can be openly addressed. This is the foundation for jointly developing solutions which benefit our customers and add value to their business. Key Account Management and regularly updated Customer Action Plans ensure that we can anticipate our customers’ ever-changing needs.

Quality management and regulatory affairs
DSM Nutritional Products constantly strives to maintain and advance its own high quality standards. Our aim is not only to be the benchmark for the industry but also to give our customers the peace of mind that only comes with quality.

We apply industry-appropriate Good Manufacturing Practice to our production operations worldwide. Every part of the process – from sourcing through production, quality assurance and storage to delivery – is managed so as to ensure the highest levels of process safety and product quality. All processes are designed to anticipate customer requirements and market trends. Beside product specification, DSM Nutritional Products provides clear statements concerning, for example, GMO ingredients, BSE/TSE, residual solvents limits, and the absence of allergenic potentials. In many cases, Kosher and Halal certificates issued by highly reputed international organizations are available. These efforts are complemented by strict change control procedures and ISO 9001 certification for our entire business. Our dietary and food ingredients are manufactured following the HACCP concepts.

DSM Nutritional Products produces vitamins for use as active pharmaceutical ingredients, operating in compliance with the worldwide accepted GMP standard ICHQ7a. CEP (Certificate of Suitability to the European Pharmacopoeia) are available for our full-range of vitamin straight portfolio.
Dear Reader,

Vitamins are unique micronutrients: they are essential to human health, and are involved in many fundamental metabolic processes, but the body cannot produce them itself.

So far, we know of the existence of 13 vitamins; four fat-soluble (vitamins A, D, E and K) and nine water-soluble (vitamin C and the B-vitamins). The functions and metabolic roles of vitamins have been reviewed and summarized in a number of textbooks and new scientific reports on these essential micronutrients are published on a regular basis. However, it remains a challenge to convey the basic scientific facts to a broader audience of interested readers. The idea therefore arose to create a concise, easy to understand brochure which is still strictly science-based and therefore useful as a reference. For each vitamin there is information on the molecule, the dietary sources, intake recommendations, the known functions, the deficiency signs, as well as further implications to human health and a brief narrative of the key scientists involved in the discovery and investigation of these essential micronutrients. New published information appears regularly and this will also be touched upon. A good example is the interaction between micronutrients and genotype, where the data clearly shows that although vitamins have been an integral part of the human diet for centuries, new insights are still being unravelled, which will further clarify the understanding and role of these micronutrients in human health.

We hope you will find this brochure useful and enjoyable to read, and we hope you share it with interested friends and colleagues.

Best Wishes!

Peter Weber, MD, PhD
Professor of Nutrition
Corporate Scientist for Human Nutrition & Health
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Over the last century, vitamin research has significantly progressed, but a lot remains to be done!

Empirical observations helped to make the important connection between specific eating patterns or nutritional behavior and resultant diseases such as night blindness, scurvy or pernicious anemia. These diseases were treated with various approaches, for example, raw liver was used to treat night blindness long before any link to vitamins was known. Another good example of observation and treatment was the frequent coincidence of rickets and tuberculosis in children at the beginning of the 19th century. Exposure to sunlight or artificial sunlight resulted in an improvement of this bone disease and in more successful healing of tuberculosis. The link between vitamin D and its sun-induced synthesis had not yet been established.

100 years ago, the discovery was made that vitamin deficiency was the underlying cause of many diseases – a true breakthrough in nutrition and medicine. In the ensuing years, vitamins began to be commercially produced and thus became available to prevent and treat overt deficiencies. However, this was not the end of the story, and after 100 years of vitamins, the problem of ‘hidden hunger’ still persists.

Hidden hunger is a term which describes insufficient intake without visible symptoms of deficiency but with consequences for different diseases. Hidden hunger is not only a problem for developing countries but also in highly industrialized and developed regions including the U.S. and Europe. Several factors, including demographic shifts, increased life-expectancy and lifestyle changes contribute to the phenomenon that ‘Hidden Hunger’ persists, even in a world of affluence.

Current and future vitamin research has to address these issues. Researchers are investigating the actions of vitamins at a molecular level. For instance, in the case of vitamin D, an endogenous compound with antibiotic-like activities has been discovered. This compound, cathelicidin, is synthesised under the control of vitamin D, explaining the role of sunlight in helping with tuberculosis. Similarly, other molecular interactions of vitamins (Vitamin A, E, Folate) have been elucidated and help to explain the impact of low vitamin intake in human health. Low Vitamin D intake will have an impact on bone health and even other chronic diseases. As a consequence, its recommendation was increased and discussions are ongoing as to whether food fortification, which is already taking place in the U.S. and other countries, might be an approach to be taken more widely. A similar case can be made for folate fortification, which results in a decline of neural tube defects (NTD) in newborns.

These two cases are good examples of diseases occurring due to an intake below recommendations but without any typical signs of deficiencies. We must realize that recommendations in the 1940s were established to avoid overt clinical deficiencies because knowledge about these micronutrients was limited. Research over the last 20 years has uncovered many other vitamin functions beyond the mere prevention of deficiencies. In the future, vitamin research should look at the overall effect of vitamins on body function and health, including the consequences of marginal deficiencies.

Finally, the role of the interaction of micronutrients with the genome is another new chapter in the field of vitamin research. This will have to be explored further to better understand the fundamental role of vitamins in the human metabolism.

The future of vitamin research is bright and one key challenge of nutrition science will be to uncover the reasons and consequences of hidden hunger and the impact of an adequate vitamin supply to benefit human health!

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Head of Department
of Biological Chemistry and Nutrition,
University of Hohenheim, Stuttgart.

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Vitamin basics: the facts about vitamins in nutrition

Forewords
Introduction

The discovery that vitamins are an essential part of the diet was a scientific breakthrough that changed the world. It brought recognition that, given an adequate diet, it is possible to cure diseases such as scurvy, rickets, beriberi and pellagra. Prevalent for thousands of years, these diseases had made a dramatic impact on both societies and economic development. In the past, although they were rated as diseases, they were not related to malnutrition. Food was only viewed as a source of protein and energy. It therefore required a paradigm shift to recognize that poor nutrition can cause diseases.

Back in 1906, English biochemist Sir Frederick Gowland Hopkins indicated that: ‘No animal can live on a mixture of pure protein, fat and carbohydrate,’ thus initiating the search for ‘growth factors’ in food. However, it was Dutch physician Christiaan Eijkman who found that a constituent of rice bran could prevent a beriberi-like disease in chickens. It is to Gerret Gryns that credit is due for being the first scientist to adopt the deficiency theory for the etiology of this disease. Gryns stated that the disease broke out when food lacked a substance necessary for metabolism.

The birth of ‘vitamines’

In 1912, Polish biochemist Casimir Funk isolated a bioactive from rice bran, which was given the name thiamine. Funk realized that thiamine could cure chickens and patients of beriberi. Based on this finding, he published a landmark paper, ‘The etiology of deficiency diseases.’ He stated that all deficiency diseases ‘can be prevented and cured by the addition of certain preventive substances, the deficient substances,’ for which he proposed the name ‘vitamines.’

In 1916, American biochemist Elmer V McCollum introduced large letters in order to differentiate between vitamins A, B, C and D. Later on, vitamins E and K were added; it was also realized that a food containing vitamin B could contain more than one factor. This led to the application of further differentiation, into vitamins B₁, B₂ and so on. These observations and findings greatly facilitated experimental research in the years that followed. The next three decades were full of scientific breakthroughs in terms of understanding the role of vitamins. By 1941, all 13 vitamins had been discovered and characterized (Table 1).

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Table 1: The history of vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Alternative name</th>
<th>Discovery</th>
<th>Isolation</th>
<th>Structure</th>
<th>Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Retinol</td>
<td>1910</td>
<td>1931</td>
<td>1931</td>
<td>1947</td>
</tr>
<tr>
<td>Provitamin A</td>
<td>β-Carotene</td>
<td>1831</td>
<td>1831</td>
<td>1931</td>
<td>1950</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Calciferol</td>
<td>1919</td>
<td>1932</td>
<td>1936</td>
<td>1959</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Tocopherol</td>
<td>1922</td>
<td>1936</td>
<td>1938</td>
<td>1938</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Phylloquinone</td>
<td>1929</td>
<td>1939</td>
<td>1939</td>
<td>1939</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>Thiamine</td>
<td>1897</td>
<td>1912</td>
<td>1936</td>
<td>1936</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>Riboflavin</td>
<td>1920</td>
<td>1933</td>
<td>1935</td>
<td>1935</td>
</tr>
<tr>
<td>Vitamin B₃</td>
<td>Niacin</td>
<td>1936</td>
<td>1936</td>
<td>1937</td>
<td>1994</td>
</tr>
<tr>
<td>Vitamin B₅</td>
<td>Pantothenic acid</td>
<td>1931</td>
<td>1938</td>
<td>1940</td>
<td>1940</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>Pyridoxine</td>
<td>1934</td>
<td>1938</td>
<td>1938</td>
<td>1939</td>
</tr>
<tr>
<td>Vitamin B₇</td>
<td>Biotin</td>
<td>1931</td>
<td>1935</td>
<td>1942</td>
<td>1943</td>
</tr>
<tr>
<td>Vitamin B₉</td>
<td>Folic acid</td>
<td>1941</td>
<td>1941</td>
<td>1946</td>
<td>1946</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>Cobalamin</td>
<td>1926</td>
<td>1948</td>
<td>1956</td>
<td>1972</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
<td>1912</td>
<td>1928</td>
<td>1933</td>
<td>1933</td>
</tr>
</tbody>
</table>
Introduction

Nobel Prize-winning science
The following scientific breakthroughs were honored with 12 Nobel Prizes for 20 Nobel Laureates (Table 2). The Nobel Prize in Chemistry was awarded to Adolf Windaus in 1928 for his studies on the constitutions of steroids and their connection with vitamins. This was followed in 1929 by the Nobel Prize in Physiology and Medicine, steroids which was awarded to Christiaan Eijkman for the discovery of the anti-neuritic vitamin, and to Sir Frederick Gowland Hopkins for the discovery of growth-stimulating vitamins.

The understanding that micronutrients are essential for human and animal growth and health, and that they have to be part of the diet, was a major stimulus for nutrition science (Table 3). Subsequent to the recognition of vitamins, and the discovery of their function, it became clear that, in order for them to be used in humans and animals, it was necessary to achieve breakthroughs in terms of their production, formulation and application. This inspired scientists in pharmaceutical companies in both Europe and the U.S. to develop synthetic routes and formulation technologies.

The first production of a vitamin on a technical scale was achieved by Hoffmann-La Roche in 1934 for vitamin C. This was based on a combination of a fermentation and chemical transformation, developed by Tadeus Reichstein. In the years that followed, all vitamins were to become available via chemical synthesis, fermentation or extraction from natural materials, which offered opportunities to fortify diets or use as supplements.

An essential technology
By the 1940s, authorities had already started to establish dietary standards and nutrient requirements (such as recommended daily allowances) for the optimal and safe intake of vitamins, depending on age, gender and risk groups. In order to ensure that entire populations secured a sufficient intake of vitamins, a number of countries implemented staple food fortification programs. Today, staple food fortification has been established in more than 50 countries. Examples of this include the fortification of flour and sugar with vitamin A, especially in low income countries; the fortification of flour with folic acid in the U.S. and Canada; and the fortification of milk and juices with vitamin D. The World Bank commented on fortification that: ‘probably no other technology available today offers as large an opportunity to improve lives and accelerate development at such low cost and in such a short time.’

As a consequence of the outcome of large epidemiological studies demonstrating the health benefits of vitamins beyond deficiency, large randomized human studies with high doses in risk groups were initiated. These stopped, however, when no effects or even signs of harm were identified. Today, when the results of various human studies of single vitamins and vitamin combinations are available, there is a wide consensus by scientists on safe intake and on the relationship of vitamins to health and healthy aging.

In addition, new data from national intake surveys are revealing that portions of the populations in industrialized countries in Europe, the U.S. and others parts of the globe also have a low status in terms of vitamins, such as vitamin D, folate and vitamin E.

A research renaissance
This century, there has been a renaissance in vitamin research, which continues to build on new science. Triggered by analysis of the human genome, it allows for the study of nutrient-gene interactions. With the discovery of polymorphism, specific requirements for vitamins have been identified.

After 100 years of vitamins, the ‘history of vitamins’ has still not come to an end. A constantly increasing number of studies and publications deal with vitamins’ relationship with risk reduction for non-communicable diseases (NCDs) and long-term health. We still need to ensure that everyone around the globe gets the vitamins they need, based on the relevant recommendations.

Science continues to provide fresh insights into the role of vitamins for health and healthy aging. Indeed, as stated by Sir Walter Norman Haworth, who won the Nobel Prize in Chemistry in 1937 for his investigations into carbohydrates and vitamin C, the identification of the role of vitamins ‘was one of the most important contributions of science to mankind.’

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Dietary reference intakes

From 1941 until 1989, RDAs (Recommended Dietary Allowances) were established and used to evaluate and plan menus to meet the nutrient requirements of certain groups. They were also used in other applications such as interpreting food consumption records of populations, establishing standards for food assistance programs and establishing guidelines for nutrition labelling. The primary goal of RDAs was to prevent diseases caused by nutrient deficiencies.

In the early 1990s, the Food and Nutrition Board (FNB), the Institute of Medicine (IOM), the National Academy of Sciences (NAS), with the involvement of Health Canada, undertook the task of revising the RDAs, and a new family of nutrient reference values was born – the Dietary Reference Intakes (DRIs). The primary goal of having these new dietary reference values was not only to prevent nutrient deficiencies, but also to reduce the risk of chronic diseases such as osteoporosis, cancer and cardiovascular disease.

Table 2: Vitamin-related Nobel Prize winners

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
<th>Field</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1928</td>
<td>Adolf Windaus</td>
<td>Chemistry</td>
<td>for his research into the constitution of the steroids and their connection with vitamins</td>
</tr>
<tr>
<td>1929</td>
<td>Christiaan Eijkman</td>
<td>Medicine and Physiology</td>
<td>for his discovery of the antineuritic vitamins</td>
</tr>
<tr>
<td>1934</td>
<td>Sir Frederick G. Hopkins</td>
<td>Medicine and Physiology</td>
<td>for his discovery of the growth stimulating vitamin</td>
</tr>
<tr>
<td>1937</td>
<td>Sir Walter N. Haworth</td>
<td>Chemistry</td>
<td>for his research into the constitution of carbohydrates and vitamin C</td>
</tr>
<tr>
<td>1938</td>
<td>Albert Szent-Györgyi</td>
<td>Medicine and Physiology</td>
<td>for his discoveries concerning liver therapy of anaemias</td>
</tr>
<tr>
<td>1938</td>
<td>Paul Karrer</td>
<td>Chemistry</td>
<td>for his research into the constitution of carotenoids, flavins and vitamins A and B2</td>
</tr>
<tr>
<td>1943</td>
<td>Edward A. Doisy</td>
<td>Medicine and Physiology</td>
<td>for his research into the constitution of carotenoids, flavins and vitamins A and B2</td>
</tr>
<tr>
<td>1943</td>
<td>Carl Peter Henrik Dam</td>
<td>Medicine and Physiology</td>
<td>for his discovery of vitamin K</td>
</tr>
<tr>
<td>1953</td>
<td>Fritz A. Lipmann</td>
<td>Medicine and Physiology</td>
<td>for his discovery of Coenzyme A and its importance for intermediary metabolism</td>
</tr>
<tr>
<td>1955</td>
<td>Axel H.T. Theorell</td>
<td>Medicine and Physiology</td>
<td>for his discoveries concerning the nature and mode of action of oxidation enzymes</td>
</tr>
<tr>
<td>1964</td>
<td>Konrad E. Bloch</td>
<td>Medicine and Physiology</td>
<td>for his discoveries concerning the mechanism and regulation of cholesterol and fatty acid metabolism</td>
</tr>
<tr>
<td>1966</td>
<td>Dorothy C. Hodgkin</td>
<td>Chemistry</td>
<td>for her structural determination of vitamin B12</td>
</tr>
<tr>
<td>1967</td>
<td>Ragnar A. Granit</td>
<td>Medicine and Physiology</td>
<td>for his research, which illuminated the electrical properties of vision by studying wavelength discrimination in the eye</td>
</tr>
<tr>
<td>1967</td>
<td>Halden K. Hartline</td>
<td>Medicine and Physiology</td>
<td>for his research on the mechanisms of sight</td>
</tr>
<tr>
<td>1967</td>
<td>George Wald</td>
<td>Medicine and Physiology</td>
<td>for his research on the chemical processes that allow pigments in the retina of the eye to convert light into vision</td>
</tr>
</tbody>
</table>
Introduction

The first report, DRIs for calcium, phosphorus, magnesium, vitamin D and fluoride, was published in 1997. Since then, additional vitamin related reports have been released, addressing folate and other B-vitamins, dietary antioxidants (vitamins C, E, selenium and the carotenoids), and vitamins A, K and trace elements such as iron and iodine. The DRIs are a comprehensive scientific source primarily for nutrition scientists (see References).

There are four types of DRI reference values: the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), the Adequate Intake (AI) and the Tolerable Upper Intake Level (UL).

**Estimated Average Requirement (EAR)**
the amount of a nutrient that is estimated to meet the requirement of half of all healthy individuals in a given age and gender group. This value is based on a thorough review of the scientific literature.

**Recommended Dietary Allowance (RDA)**
the average daily dietary intake of a nutrient that is sufficient to meet the requirement of nearly all (97 – 98%) healthy persons. This is the number to be used as a goal for individuals. It is calculated from the EAR.

**Adequate Intake (AI)**
only established when an EAR (and thus an RDA) cannot be determined because the data are not clear-cut enough; a nutrient has either an RDA or an AI. The AI is based on experimental data or determined by estimating the amount of a nutrient eaten by a group of healthy people and assuming that the amount they consume is adequate to promote health.

**Tolerable Upper Intake Level (UL)**
the highest continuing daily intake of a nutrient that is likely to pose no risks of adverse health effects for almost all individuals. As intake increases above the UL, the risk of adverse effects increases. Consistently consuming a nutrient at the upper level should not cause adverse effects. Intake levels at the UL can be interpreted as a ‘warning flag’, not as reason for alarm.

### Table 3: Biochemical function of vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Main functions</th>
<th>Deficiency disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Visual pigments in the retina; cell differentiation</td>
<td>Night blindness, xerophthalmia; keratinization of skin</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Antioxidation</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Maintenance of calcium balance; enhances intestinal absorption of Ca⁺⁺ and mobilizes bone mineral</td>
<td>Rickets (poor mineralization of bone); osteomalacia (demineralization of bone)</td>
</tr>
<tr>
<td>E</td>
<td>Antioxidant, especially in cell membranes</td>
<td>Extremely rare: serious neurological dysfunction</td>
</tr>
<tr>
<td>K</td>
<td>Coenzyme in formation of γ-carboxyglutamate in enzymes of blood clotting and bone matrix</td>
<td>Impaired blood clotting, hemorrhagic disease</td>
</tr>
<tr>
<td>C</td>
<td>Coenzyme in hydroxylation of proline and lysine in collagen synthesis; antioxidant; enhances absorption of iron</td>
<td>Scurvy; impaired wound healing, loss of dental cement, subcutaneous hemorrhage</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Coenzyme in pyruvate and 2-keto-glutarate dehydrogenases, and transketolase; poorly defined function in nerve conduction</td>
<td>Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke–Korsakoff syndrome)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Coenzyme in oxidation and reduction reactions; prosthetic group of flavoproteins</td>
<td>Lesions of corner of mouth, lips, and tongue; seborrheic dermatitis</td>
</tr>
<tr>
<td>B6</td>
<td>Coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase; role in steroid hormone action</td>
<td>Disorders of amino acid metabolism, convulsions</td>
</tr>
<tr>
<td>B12</td>
<td>Coenzyme in transfer of one-carbon fragments</td>
<td>Pernicious anemia (megablastic anemia with degeneration of the spinal cord)</td>
</tr>
<tr>
<td>Niacin</td>
<td>Coenzyme in oxidation and reduction reactions, functional part of NAD and NADP</td>
<td>Pellagra: photosensitive dermatitis, depressive psychosis</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>Functional part of coenzyme A and acyl carrier protein</td>
<td>Peripheral nerve damage (burning foot syndrome)</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>Coenzyme in transfer of one-carbon fragments</td>
<td>Megablastic anemia</td>
</tr>
<tr>
<td>Biotin</td>
<td>Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis</td>
<td>Impaired fat and carbohydrate metabolism, dermatitis</td>
</tr>
</tbody>
</table>
Vitamin A crystals in polarized light

Synonyms:
Retinol, axerophthol

Chemistry:
Retinol and its related compounds consist of four isoprenoid units joined head to tail and contain five conjugated double bonds. They naturally occur as alcohol (retinol), as aldehyde (retinal) or as acid (retinoic acid).

Introduction
Although it has been known since ancient times that certain foods, such as liver, would cure night blindness, vitamin A per se was not identified until 1910. Its chemical structure was defined by Paul Karrer in 1931. Professor Karrer received a Nobel Prize for his work because this was the first time that a vitamin’s structure had been determined.

Vitamin A is a generic term for a group of lipid-soluble compounds related to retinol. Retinol is often referred to as preformed vitamin A. It is found only in animal sources, mainly as retinyl esters and in food supplements. Many cultures have used ox liver as an excellent source of vitamin A to cure night blindness. The liver was first pressed to the eye and then eaten; the Egyptians described this cure at least 3,500 years ago.

β-Carotene and other carotenoids that can be converted to vitamin A by an enzymatic process in the body are referred to as provitamin A. They are found only in plant sources.

Functions
Retinal, the oxidised metabolite of retinol, is required for the process of vision. Retinoic acid, another vitamin A metabolite, is considered to be responsible for nearly all non-visual functions of vitamin A. Retinoic acid combines with specific nuclear receptor proteins which bind to DNA and regulate the expression of more than 500 genes, thereby influencing numerous physiological processes and having hormone-like activity.

Vision
Receptor cells in the retina of the eye (rod cells) contain a light-sensitive pigment called rhodopsin, which is a complex of the protein opsin and the vitamin A metabolite retinal. The light-induced disintegration of the pigment triggers a cascade of events which generate an electrical signal to the optic nerve. Rhodopsin can only be regenerated from opsin and vitamin A. Rod cells with this pigment can detect very small amounts of light, making them important for night vision.

Cellular differentiation
The many different types of cells in the body perform highly specialised functions. The process whereby cells and tissues become ‘programmed’ to carry out their special functions is called differentiation. Through the regulation of gene expression, retinoic acid plays a major role in cellular differentiation. Vitamin A is necessary for normal differentiation of epithelial cells, the cells of all tissues lining the body, such as skin, mucous membranes, blood vessel walls and the cornea. In vitamin A deficiency, cells lose their ability to differentiate properly.

Growth and development
Retinoic acid plays an important role in reproduction and embryonic development, particularly in the development of the spinal cord and vertebrae, limbs, heart, eyes and ears.

Immune function
Vitamin A is required for the normal functioning of the immune system in a number of ways. It is essential in maintaining the integrity and function of the skin and mucosal cells, which function as a mechanical barrier and defend the body against infection. Vitamin A also plays a central role in the development and differentiation of white blood cells, such as lymphocytes, killer cells and phagocytes, which play a critical role in the defence of the body against pathogens.
Vitamin A

Dietary sources
The richest food source of preformed vitamin A is liver, with considerable amounts also found in egg yolk, whole milk, butter and cheese. Provitamin A carotenoids are found in carrots, yellow and dark green leafy vegetables (e.g. spinach, broccoli), pumpkin, apricots and melon. Until recently, vitamin A activity in foods was expressed as international units (IU). This unit is still the measurement generally used on food and supplement labels. In order to standardise vitamin A measurement, it has now been internationally agreed to state vitamin A activity in terms of a new unit called the retinol activity equivalent (RAE), which accounts for the rate of conversion of carotenoids to retinol.

Absorption and body stores
Vitamin A is absorbed in the upper part of the small intestine. Pro-vitamin A carotenoids can be cleaved in the intestine and other organs into retinol via an enzymatic process. Preformed vitamin A occurs as retinyl esters of fatty acids. They are hydrolysed and retinol is absorbed into intestinal mucosal cells (i.e. enterocytes). After re-esterification, the retinyl esters are incorporated into chylomicrons, excreted into lymphatic channels, delivered to the blood and transported to the liver. Vitamin A is stored in the liver as retinyl esters; stores are enough for one to two years in most adults living in industrialised countries.

Recommended daily intakes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&gt;16 months</td>
<td>400 µg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>500 µg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>300 µg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>400 µg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>600 µg</td>
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<tr>
<td>Males</td>
<td>14 years</td>
<td>900 µg</td>
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<tr>
<td>Females</td>
<td>14 years</td>
<td>700 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 18 years</td>
<td>750 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>&gt;18 years</td>
<td>770 µg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 18 years</td>
<td>1,200 µg</td>
</tr>
<tr>
<td>Lactation</td>
<td>&gt;18 years</td>
<td>1,300 µg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** As retinol activity equivalents (RAEs)
AI Adequate Intake

Measurement
Vitamin A can be measured in the blood and other body tissues by various techniques. For rapid field tests, a method has been developed recently using dried blood spots. Typical serum concentrations are \(1.1 - 2.3 \mu mol/L\). According to the World Health Organization (WHO), plasma concentrations of <0.35 µmol/L indicate a vitamin A deficiency.

Stability
Vitamin A is sensitive to oxidation by air. Loss of activity is accelerated by heat and exposure to light. Oxidation of fats and oils (e.g. butter, margarine, cooking oils) can destroy fat-soluble vitamins including vitamin A. The presence of antioxidants such as vitamin C and E therefore contributes to the protection of vitamin A.

Interactions

Positive interactions
- Retinoic acid has a fundamental role in the regulation of vitamin A target genes. The biologically active metabolite retinoic acid (RA) binds via nuclear hormone receptors (RARs, RXRs) to the promoters of more than 500 genes. The products arising from these genes are necessary for many different pathways

Negative interactions
- Chronic liver and kidney diseases can impair storage and transport of vitamin A
- Protein malnutrition, general malabsorption and infectious diseases decrease the uptake of vitamin A in the intestine and lower the vitamin A status due to impaired binding protein synthesis

Deficiency
Vitamin A deficiency has a high impact on morbidity and mortality, especially in infants, children and pregnant and lactating mothers. Worldwide, 250 million pre-school children are subclinically (missing clinical signs) vitamin A deficient. Vitamin A deficiency is rare in the Western world, but in developing countries it is one of the most widespread, yet preventable, causes of blindness. The earliest symptom of vitamin A deficiency is impaired dark adaptation, or night blindness. Severe deficiency causes a condition called xerophthalmia, characterised by changes in the cells of the cornea that ultimately result in corneal ulcers, scarring and blindness. The appearance of skin lesions is also an early indicator of inadequate vitamin A status. Growth retardation is a common sign in children. Because vitamin A is required for the normal functioning of the immune system, even children who are only mildly deficient in vitamin A have a higher incidence of respiratory disease and diarrhoea, as well as a higher rate of mortality from infectious diseases, than children who consume sufficient vitamin A. Some diseases may themselves induce vitamin A deficiency, most notably liver and gastrointestinal diseases, which interfere with the absorption and utilisation of vitamin A. Vitamin A deficiency during pregnancy leads to malformations during foetal development.

Vitamin basics: the facts about vitamins in nutrition
Groups at risk of deficiency
- Pregnant and lactating women (additional demands)
- Infants and children, adolescents
- Alcoholics (alcohol hampers the capacity of vitamin A storage)
- Chronically ill people
- People with protein malnutrition and malabsorption
- Vegetarians and vegans with additional polymorphisms in the BCMO1 gene

Disease prevention and therapeutic use

Studies have shown that vitamin A supplementation given to children aged over 6 months reduces all-cause mortality by 23% to 30% in low income countries. The beneficial effect is assumed to be due to the prevention of vitamin A deficiency. The WHO recommends that supplements should be given when children are vaccinated. The currently recommended doses are 100,000 IU at age 6 – 11 months and 200,000 IU at age >12 months every 3 – 6 months.

Xerophthalmia is treated with high doses of vitamin A (50,000 – 200,000 IU according to age). In developing countries, where vitamin A deficiency is one of the most serious health problems, children under the age of 6 years and pregnant and lactating women are the main vulnerable groups.

Since vitamin A can be stored in the liver, it is possible to build up a reserve in children by administration of high-potency doses. In regular periodic distribution programmes for the prevention of vitamin A deficiency, infants < 6 months of age receive a dose of 50,000 IU of vitamin A, and children between six months and one year receive 100,000 IU every 4 – 6 months, while children > 12 months of age receive 200,000 IU every 4 – 6 months. A single dose of 200,000 IU given to mothers immediately after delivery of their child has been found to increase the vitamin A content of breast milk. However, caution is necessary when considering vitamin A therapy for lactating women, otherwise a co-existing pregnancy may be endangered: during pregnancy, a daily dose of 2,800 – 3,000 µg retinol per day (9,300 – 10,000 IU) should not be exceeded.

Administration of high doses of vitamin A to children with measles complications, but no overt signs of vitamin A deficiency, decreases mortality by over 50% and significantly lowers morbidity. Natural and synthetic vitamin A analogues have been used to treat psoriasis and severe acne.

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin A varies according to age, sex, risk group and other criteria applied in individual countries.

Safety

Because vitamin A (as retinyl ester) is stored in the liver, large amounts taken over a period of time can eventually exceed the liver’s storage capacity and produce adverse effects (liver damage, bone abnormalities and joint pain, alopecia, headaches, vomiting and skin desquamation). Hypervitaminosis A can occur acutely following very high doses taken over a period of several days, or as a chronic condition from high doses taken over a long period of time. Thus, there is concern about the safety of high intakes of preformed vitamin A (retinol), especially for infants, small children and women of childbearing age. Normal foetal development requires sufficient vitamin A intake, but consumption of excess retinol during pregnancy is known to cause malformations in the newborn. Several recent prospective studies suggest that long-term intakes of pre-formed vitamin A in excess of 1,500 µg/day are associated with increased risk of osteoporotic fracture and decreased bone mineral density in older men and women. Only excess intakes of preformed vitamin A, not β-Carotene, were associated with adverse effects on bone health. Current levels of vitamin A in fortified foods are based on RDA levels, ensuring that there is no realistic possibility of vitamin A overdosage in the general population. In the vast majority of cases, signs and symptoms of toxicity are reversible upon cessation of vitamin A intake.

The Food and Nutrition Board of the Institute of Medicine (IOM, 2001) and the E.C. Scientific Committee on Food (2002) have set the tolerable upper intake level (UL) of vitamin A intake for adults at 3000 µg RE/day with appropriately lower levels for children.

Supplements and food fortification

Vitamin A is available in soft gelatine capsules, as chewable or effervescent tablets, or in ampoules. It is also included in most multivitamins. Retinyl acetate, retinyl palmitate and retinal are the forms of vitamin A most commonly used in supplements. Margarine and milk are commonly fortified with vitamin A. β-Carotene is added to margarine and many other foods (e.g. fruit drinks, salad dressings, cake mixes, ice cream) both for its vitamin A activity and as a natural food colourant.

Industrial production

Nowadays vitamin A is rarely extracted from fish liver oil. The modern method of industrial synthesis of nature-identical vitamin A is a highly complex, multi-step process.
Vitamin A

History

1831 Wackenroder isolates the orange-yellow colourant from carrots and names it ‘carotene’.

1876 Snell successfully demonstrates that night blindness and xerophthalmia can be cured by giving the patient cod liver oil.

1880 Lunin discovers that, besides needing carbohydrates, fats and proteins, experimental animals can only survive if given small quantities of milk powder.

1887 Arnaud describes the widespread presence of carotenoids in plants.

1909 Stepp successfully extracts the vital liposoluble substance from milk.

1915 McCollum differentiates between ‘fat-soluble A’ and ‘water-soluble B’.

1929 Thomas Moore demonstrates that β-Carotene is converted into the colorless form of vitamin A in the intestine. This is the proof that plant derived carotenoids serve as precursor for vitamin A.

1931 Karrer isolates practically pure retinol from the liver oil of a species of mackerel. Karrer and Kuhn isolate active carotenoids.

1946 Otto Isler was the first to synthesize Vitamin A (pure all-trans retinol) at Hoffmann-La Roche. He then developed an efficient and economical industrial synthesis for Vitamin A.

1947 Karrer was honored with the Nobel prize for chemistry.

1984 Sommer demonstrates that vitamin A deficiency is a major cause of infant mortality in Indonesia.

1987 Chombon in Strasbourg and Evans in San Diego, and their respective coworkers, simultaneously discover the retinoic acid receptors in cell nuclei.

1997 UNICEF, the World Health Organisation (WHO), and the governments of countries including Canada, the United States and the United Kingdom, as well as national governments in countries where vitamin deficiency is widespread, launch a global campaign to distribute high-dose vitamin A capsules to malnourished children.
β-Carotene is one of more than 600 carotenoids known to exist in nature. Carotenoids are yellow, orange and red pigments that are widely distributed in plants. In 1831, β-Carotene was isolated by Wackenroder. Its structure was determined by Karrer in 1931, who received a Nobel prize for his work. About 50 of the naturally occurring carotenoids can potentially yield vitamin A and are thus referred to as provitamin A carotenoids. β-Carotene is the most abundant and most efficient provitamin A in our foods. Currently available evidence suggests that in addition to being a source of vitamin A, β-Carotene plays many important biological roles that may be independent of its provitamin status.

**Functions**

β-Carotene is the most important safe dietary source of vitamin A. Vitamin A is essential for normal growth and development, immune system function and vision. β-Carotene can quench singlet oxygen, a reactive molecule that is generated, for instance, in the skin by exposure to ultraviolet light, and which can induce precancerous changes in cells. Singlet oxygen is capable of triggering free radical chain reactions.

β-Carotene has antioxidant properties that help neutralise free radicals – reactive and highly energised molecules which are formed through certain normal biochemical reactions (e.g. the immune response, prostaglandin synthesis), or through exogenous sources such as air pollution or cigarette smoke. Free radicals can damage lipids in cell membranes as well as the genetic material in cells and the resulting damage may lead to the development of cancer.

**Dietary sources**

The best sources of β-Carotene are yellow/orange vegetables and fruits and dark green leafy vegetables:

- **Yellow/orange vegetables** – carrots, sweet potatoes, pumpkins, winter squash
- **Yellow/orange fruits** – apricots, cantaloupes, papayas, mangoes, carambolas, nectarines, peaches
- **Dark green leafy vegetables** – spinach, broccoli, endive, kale, chicory, escarole, watercress and beet leaves, turnips, mustard, dandelion
- **Other good vegetable and fruit sources** – summer squash, asparagus, peas, sour cherries, prune plums

The β-Carotene content of fruits and vegetables can vary according to the season and degree of ripening.
Absorption and body stores

Bile salts and fat are needed for the absorption of β-Carotene in the upper small intestine. Many dietary factors, e.g. fat and protein, affect absorption. Approximately 10 – 50% of the total β-Carotene consumed is absorbed in the gastrointestinal tract. The proportion of carotenoids absorbed decreases as dietary intake increases. Within the intestinal wall (mucosa), β-Carotene is partially converted into vitamin A (retinol) by the enzyme β-Carotene monoxygenase 1 (BCMO1). This mechanism is regulated by the individual’s vitamin A status. If the body has enough vitamin A, the conversion of β-Carotene decreases. Therefore, β-Carotene is a very safe source of vitamin A and high intakes will not lead to hypervitaminosis A. Excess β-Carotene is stored in the fat tissues of the body and the liver. The adult’s fat stores are often yellow from accumulated carotene while the infant’s fat stores are white.

Measurement

Plasma carotenoid concentration is determined by HPLC. It reflects the intake of carotenoids. Traditionally, vitamin A activity of β-Carotene has been expressed in International Units (IU; 1 IU = 0.60 µg of all-trans β-Carotene). However, this conversion factor does not take into account the poor bioavailability of carotenoids in humans. Thus, the FAO/WHO Expert Committee proposed that vitamin A activity be expressed as retinol equivalents (RE). 6 µg β-Carotene provide 1 µg retinol. For labelling, official national directives should be followed.

Deficiency

Although consumption of provitamin A carotenoids can prevent vitamin A deficiency, there are no known adverse clinical effects of a low carotenoid diet, provided vitamin A intake is adequate.

Groups at risk of deficiency
- Pregnant and lactating women (additional demands)
- Infants and children, adolescents
- Alcoholics (alcohol hampers the capacity of vitamin A storage)
- Chronically ill people, i.e. cystic fibrosis patients
- People with protein malnutrition and malabsorption
- Vegetarians and vegans with additional polymorphisms in the BCMO1 gene

Bioavailability of β-Carotene

Bioavailability refers to the proportion of β-Carotene that can be absorbed, transported and utilised by the body once it has been consumed. It is influenced by a number of factors:

- β-Carotene from dietary supplements is better absorbed than β-Carotene from foods
- Food processing such as chopping, mechanical homogenisation and cooking enhances bioavailability of β-Carotene
- The presence of fat in the intestine affects absorption of β-Carotene. The amount of dietary fat required to ensure carotenoid absorption seems to be low (approximately 3 – 5 g per meal)

Stability

Carotenoids can lose some of their activity in foods during storage due to the action of enzymes and exposure to light and oxygen. Dehydration of vegetables and fruits may greatly reduce the biological activity of carotenoids. On the other hand, carotenoid stability is retained in frozen foods.

Interactions

Positive interactions
- Vitamin C and E stabilizes and rescues β-Carotene

Negative interactions
- Chronic liver and kidney diseases can impair storage and transport of β-Carotene
- Alcohol abuse also hampers the capacity of β-Carotene storage
- Protein malnutrition as well as general malabsorption can influence and decrease the transport and uptake of β-Carotene within the intestine
- Reduced blood levels of lutein
Disease prevention and therapeutic use

**Immune system**
In a number of animal and human studies β-Carotene supplementation was found to enhance certain immune responses. Early studies demonstrated the ability of β-Carotene and other carotenoids to prevent infections. Some clinical trials have found that β-Carotene supplementation improves several biomarkers of immune function. It can lead to an increase in the number of white blood cells and the activity of natural killer cells. Both of these are important in combating various diseases. It may be the case that β-Carotene stimulates the immune system once it has undergone conversion to vitamin A. Another explanation could be that the antioxidant actions of β-Carotene protect cells of the immune system from damage by reducing the toxic effects of reactive oxygen species.

**Skin**
Recent evidence points to a role of β-Carotene in protecting the skin from sunlight damage. β-Carotene can be used as an oral sun protectant in combination with sunscreens for the prevention of sunburn. Its effectiveness has been shown both alone and in combination with other carotenoids or antioxidant vitamins.

**Cancer and cardiovascular diseases**
Epidemiological studies consistently indicate that as consumption of β-Carotene-rich fruits and vegetables increases, the risk of certain cancers (i.e. lung and stomach cancer) and cardiovascular diseases decreases.

**Erythropoietic protoporphyria**
In patients with erythropoietic protoporphyria – a photosensitivity disorder leading to abnormal skin reactions to sunlight – β-Carotene in doses of up to 180 mg has been shown to exert a photoprotective effect.

**Recommended Dietary Allowance (RDA)**
Until now, dietary intake of β-Carotene has been expressed as part of the RDA for vitamin A. The daily vitamin A requirements for adult men and women are 900 µg and 700 µg of preformed vitamin A (retinol) respectively (Food and Nutrition Board, FNB, 2001). Apart from its provitamin A function, data continue to accumulate supporting a role for β-Carotene as an important micronutrient in its own right. Consumption of foods rich in β-Carotene is being recommended by scientific and government organisations such as the U.S. National Cancer Institute (NCI) and the U.S. Department of Agriculture (USDA). If these dietary guidelines are followed, dietary intake of β-Carotene (about 6 mg) would be several times the average amount presently consumed in the U.S. (about 1.5 mg daily).

**Safety**
β-Carotene is a safe source of vitamin A. Due to the regulated conversion of β-Carotene into vitamin A, overconsumption does not produce hypervitaminosis A. Excessive intakes of β-Carotene may cause carotenodermia, which manifests itself in a yellowish tint of the skin, mainly in the palms of the hands and soles of the feet. The yellow colour disappears when carotenoid consumption is reduced or stopped.

High doses of β-Carotene (up to 180 mg/day) used for the treatment of erythropoietic protoporphyria have shown no adverse effects. In two studies investigating the effect of β-Carotene supplementation on the risk of developing lung cancer, an apparent increase of lung cancer in chronic heavy smokers with intakes of more than 20 mg/day over several years has been observed. The reasons for these findings are not yet clear.

The British Expert Committee on Vitamins and Minerals (EVM) recommends a Safe Upper Level for supplementation of 7 mg/day over a life-time period. Other agencies such as the European DACH Society (German Society of Nutrition, Austrian Society of Nutrition, Swiss Society of Nutrition Research) have concluded that a daily intake of up to 10 mg of β-Carotene is safe. The level of supplemental intake of β-Carotene for which epidemiological studies did not reveal any increased cancer risk is 15 mg/day (Latest evaluation by EFSA in March 2012).

**Supplements and food fortification**
β-Carotene is available in hard and soft gelatine capsules, in multi-vitamin tablets, and in antioxidant vitamin formulas and as food colour. Margarine and fruit drinks are often fortified with β-Carotene. In 1941, the U.S. Food and Drug Administration (FDA) established a standard of identity for the addition of vitamin A to margarine; since then, however, vitamin A has been partly replaced by β-Carotene, which additionally imparts an attractive yellowish colour to this product. Due to its high safety margin, β-Carotene has been recognised as more suitable for fortification purposes than vitamin A.

**Industrial production**
Isler and coworkers developed a method to synthesise β-Carotene, and it has been commercially available in crystalline form since 1954.
History

1831 Wackenroder isolates the orange-yellow pigment in carrots and coins the term ‘carotene’.

1847 Zeise provides a more detailed description of carotene.

1866 Carotene is classified as a hydrocarbon by Arnaud and coworkers.

1887 Arnaud describes the widespread presence of carotenes in plants.

1897 Willstatter and Mieg establish the molecular formula for carotene, a molecule consisting of 40 carbon and 56 hydrogen atoms.

1907 Palmer and Eckles discover the presence of carotene and xanthophylls in human blood plasma.

1914 Steenbock suggests a relationship between yellow plant pigments (β-Carotene) and vitamin A.

1919 Moore demonstrates that β-Carotene is converted into the colourless form of vitamin A in the liver.

1929 Karrer and collaborators determine the structures of β-Carotene and vitamin A.

1931 Wagner and coworkers suggest that the conversion of β-Carotene into vitamin A occurs within the intestinal mucosa.

1939 Isler and colleagues develop a method for synthesising β-Carotene.

1946 β-Carotene is found acceptable for use in foods by the Joint FAO/WHO Expert Committee on Food Additives.

1972 Specifications for β-Carotene use in foods is established by the U.S. Food Chemicals Codex.

1979 Carotene is established as ‘GRAS’, which means that the ingredient is ‘Generally Recognised As Safe’ and can be used as a dietary supplement or in food fortification.

1980/2 β-Carotene/carotenoids are recognised as important factors (independent of their provitamin A activity) in potentially reducing the risk of certain cancers.

1982 Krinsky and Deneke show the interaction between oxygen and oxyradicals using carotenoids.

1984 β-Carotene is demonstrated to be an effective antioxidant in vitro.

1988 Due to the large number of epidemiological studies that demonstrate the potential reduction of cancer incidence with increased consumption of dietary β-Carotene, the U.S. National Cancer Institute (NCI) issues dietary guidelines advising Americans to include a variety of vegetables and fruits in their daily diet.

1997 Evidence indicates that β-Carotene acts synergistically with vitamins C and E.

1999 The Women’s Health Study shows no increased risk of lung cancer for women receiving 50 mg β-Carotene on alternate days.

2004 Results from the French SU.VI.MAX study indicate that a combination of antioxidant vitamins (C, E and β-Carotene) and minerals lowers total cancer incidence and all-cause mortality in men.
Introduction

Vitamin D is the general name given to a group of fat-soluble compounds that are essential for maintaining the mineral balance in the body. The chemical structure of vitamin D was identified in the 1930s. The main forms are vitamin D2 (ergocalciferol: found in plants, yeasts and fungi) and vitamin D3 (cholecalciferol: of animal origin).

As cholecalciferol is synthesised in the skin by the action of ultraviolet light on 7-dehydrocholesterol, a cholesterol derivative, vitamin D does not fit the classical definition of a vitamin. Nevertheless, because of the numerous factors that influence its synthesis, such as latitude, season, air pollution, area of skin exposed, pigmentation and age, vitamin D is recognized as an essential dietary nutrient.

Functions

Following absorption or endogenous synthesis, the vitamin has to be converted before it can perform its biological functions. Calciferol is transformed in the liver to 25-hydroxycholecalciferol (25(OH)D, calcidiol). This is the major circulating form, which is metabolised in the kidney to the active forms as required. The most important of these is 1,25-dihydroxy-cholecalciferol (1,25(OH)2D, calcitriol) because it is responsible for most of the biological functions. The formation of 1,25(OH)2D, which is considered a hormone, is strictly controlled according to the body’s calcium needs. The main controlling factors are the existing levels of 1,25(OH)2D itself and the blood levels of parathyroid hormone, calcium and phosphorus. Therefore it plays an important role for the proper functioning of muscles, nerves and blood clotting and for normal bone formation and mineralisation.

To perform its biological functions, 1,25(OH)2D, like other hormones, binds to a specific nuclear receptor (vitamin D receptor, VDR).

Upon interaction with this receptor, 1,25(OH)2D regulates more than 250 genes in a wide variety of tissues. Vitamin D is essential for the control of normal calcium and phosphate blood concentrations. It is required for the absorption of calcium and phosphate in the small intestine and can maintain blood calcium and phosphate concentrations through bone mobilisation and increased reabsorption in the kidney.

It has been suggested that vitamin D also plays an important role in controlling cell proliferation and differentiation, immune responses and insulin secretion.

Main functions in a nutshell:

- Regulation of calcium and phosphate homeostasis
- Bone mineralization and teeth function
- Cell function, proliferation and differentiation
- Modulation of the immune system
- Neurotransmitter signaling
- Muscle contraction
- Heart beat regulation
- Blood clotting
**Vitamin D**

**Dietary sources**

Vitamin D is found only in a few foods. The richest natural sources of vitamin D are fish liver oils and salt-water fish such as sardines, herring, salmon and mackerel. Eggs, meat, milk and butter also contain small amounts. Plants are poor sources, with fruit and nuts containing no vitamin D at all. The amount of vitamin D in human milk is insufficient to cover infant needs.

**Vitamin D content of foods**

<table>
<thead>
<tr>
<th>Food</th>
<th>µg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring</td>
<td>25</td>
</tr>
<tr>
<td>Salmon</td>
<td>16</td>
</tr>
<tr>
<td>Sardines</td>
<td>11</td>
</tr>
<tr>
<td>Mackerel</td>
<td>4</td>
</tr>
<tr>
<td>Eggs</td>
<td>2.9</td>
</tr>
<tr>
<td>Butter</td>
<td>1.2</td>
</tr>
<tr>
<td>Milk (whole)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

(Source: Fachmann, Kraut)

**Absorption and body stores**

Absorption of dietary vitamin D takes place in the upper part of the small intestine with the aid of bile salts. It is incorporated into the chylomicron fraction and absorbed through the lymphatic system. Vitamin D is stored in adipose tissue. It has to be metabolised to become active.

**Measurement**

Vitamin D status is best determined by the plasma 25(OH)D concentration because this reflects dietary sources as well as vitamin D production by UV light in the skin. Usual plasma 25(OH)D values are between 25 and 130 nmol/L depending on geographic location. 1 µg vitamin D is equivalent to 40 IU (international unit). Concentrations less than 25 nmol/L are considered to be deficient.

**Stability**

Vitamin D is relatively stable in foods. Storage, processing and cooking have little effect on its activity, although in fortified milk up to 40% of the vitamin D added may be lost as a result of exposure to light.

**Interactions**

**Positive interactions**

- Vitamin D together with vitamin K, vitamin C, vitamin B6 and calcium are required for bone formation
- Women taking oral contraceptives have been found to have slightly elevated blood levels of 1,25(OH)D
- Statins – There is evidence to suggest that statins are also associated with elevated vitamin D concentrations

**Negative interactions**

- Cholestyramine (a resin used to stop reabsorption of bile salts) and laxatives based on mineral oil inhibit the absorption of vitamin D from the intestine
- Corticosteroid hormones, anticonvulsant drugs and alcohol can affect the absorption of calcium by reducing the response to vitamin D
- Animal studies also suggest that anticonvulsant drugs stimulate enzymes in the liver, resulting in an increased breakdown and excretion of the vitamin D
- Certain anti-epileptic drugs may decrease plasma 25(OH)D levels and thus may induce vitamin D insufficiency

**Deficiency**

Vitamin D deficiency leads to increased parathyroid hormone (PTH) levels, followed by a disturbance of the calcium and phosphate homeostasis. In children, unspecific symptoms such as restlessness, irritability, excessive sweating and impaired appetite may appear. Prolonged vitamin D deficiency will induce rickets that is characterised by developmental delay and skeletal abnormalities as a result of decreased calcium and phosphate availability. Rickets also result in inadequate mineralisation of tooth enamel.

Among the first signs of osteomalacia in adults due to vitamin D deficiency are bone and muscle pain that can progress to muscle weakness and tetany, as well as an increased risk of infection. Severe vitamin D deficiency will result in bone brittleness. Insufficient vitamin D status has been associated with osteoporosis (a disorder of older age in which there is loss of bone), increased risk of falls resulting in fractures and muscle weakness. Besides the skeletal effects, vitamin D deficiency has also been linked to increased risk of many chronic diseases, including autoimmune diseases, heart diseases, infection diseases and type 2 diabetes.

**Groups at risk of deficiency**

- All ages living in a geographic location higher than 40 degrees latitude during winter time
- People with naturally very dark skin
- Vegetarians, Vegans
- People with little or no sun exposure:
  - Elderly in old peoples homes
  - Individuals that avoid sun exposure for cosmetic or health reasons (e.g. sun screens)
  - Shift workers, coal miners
  - Individuals with protective dress code (e.g. religious, cultural)
  - Diseased people (e.g. skin cancer patients, long term hospitalized subjects)
- Certain medical conditions (e.g. obesity, underweight, end stage liver disease, renal disease and fat malabsorption syndromes such as cystic fibrosis, coeliac disease, inflammatory bowel disease) or medications affecting vitamin D metabolism
- Infants (if breast milk contains little vitamin D)
Disease prevention and therapeutic use

In the treatment of rickets, a daily dose of 40 µg (1,600 IU) vitamin D usually results in normal plasma concentrations of calcium and phosphorus within 10 days. The dose can be reduced gradually to 10 µg (400 IU) per day after one month of therapy. Vitamin D analogues are used in the treatment of inflammatory skin conditions such as psoriasis. Vitamin D is discussed as a prevention factor for a number of diseases. Results from epidemiological studies and evidence from animal models suggest that the risk of several autoimmune diseases (multiple sclerosis, insulin-dependent diabetes mellitus, rheumatoid arthritis) may be decreased by adequate vitamin D status. Vitamin D plays a major role in the prevention of osteoporosis because vitamin D insufficiency can be an important contributing factor in this disease.

A prospective study among 72,000 postmenopausal women over 18 years indicated that women consuming at least 15 µg/d (600 IU) vitamin D/day from food plus supplements had a 37% lower risk of hip fracture. Evidence from most clinical trials suggests that vitamin D supplementation slows down bone mineral density loss and decreases the risk of osteoporotic fracture in men and women. Various surveys and studies indicate that poor vitamin D intake or status is associated with an increased risk of colon, breast and prostate cancer. Recent studies have shown that vitamin D3 is up to 87% more potent than vitamin D2, which may explain why vitamin D3 exerts stronger effects on the prevention of fractures and falls.

Recommended Dietary Allowance (RDA)

In 1997 the Food and Nutrition Board based adequate intake levels (AI) on the assumption that no vitamin D is produced by UV light in the skin. An AI of 5 µg (200 IU)/day was recommended for infants, children and adults (ages 19 – 50 years). Based on the considerable body of science which has been published since the last review by the Institute of Medicine (IOM) in 1997, on 30th November 2010 a new report was issued by the IOM, proposing new reference values for vitamin D.

Safety

Hypervitaminosis D is a potentially serious problem as it can cause permanent kidney damage, growth retardation, calcification of soft tissues and death. Mild symptoms of intoxication are nausea, weakness, constipation and irritability. Hypercalcemia has only been associated with excessive prolonged intake of vitamin D usually well above 20,000 IU per day.

Hypervitaminosis D is not associated with overexposure to the sun because a regulating mechanism prevents overproduction of vitamin D.

The Food and Nutrition Board (FNB) has set the upper intake level for vitamin D to 100 µg/day or 4,000 IU/day (IOM, 2010). EFSA is currently evaluating a revision of the current upper intake level (50 µg/day).

Supplements and food fortification

Monopreparations of vitamin D and related compounds are available as tablets, capsules, oily solutions and injections. Vitamin D is also incorporated in combinations with vitamin A, calcium, and in multivitamins. In many countries, milk and milk products, margarine and vegetable oils fortified with vitamin D serve as a major dietary source of the vitamin.

Industrial production

Cholecalciferol is produced commercially by the action of ultraviolet light on 7-dehydrocholesterol, which is obtained from cholesterol by various methods. Ergocalciferol is produced in a similar manner from ergosterol, which is extracted from yeast. The starting material for the production of calcitriol is the cholesterol derivative pregnenolone.

Recommended daily intakes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0 – 12 months</td>
<td>400 IU (50 µg) (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 18 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Males</td>
<td>19 – 50 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Females</td>
<td>19 – 50 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Males</td>
<td>51 – 70 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Females</td>
<td>51 – 70 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Males</td>
<td>&gt; 70 years</td>
<td>800 IU (100 µg)</td>
</tr>
<tr>
<td>Females</td>
<td>&gt; 70 years</td>
<td>800 IU (100 µg)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>600 IU (75 µg)</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>600 IU (75 µg)</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2010)
** In the absence of adequate exposure to sunlight
AI Adequate Intake

Hereditary vitamin D-dependent rickets (type I and II):

These rare forms of rickets occur in spite of an adequate supply of vitamin D. They are inherited illnesses in which the formation or utilisation of 1,25(OH)2D is impaired.

Recommended daily intakes**

* Institute of Medicine (2010)
** In the absence of adequate exposure to sunlight
AI Adequate Intake

If not otherwise specified, this table presents Recommended Dietary Allowances (RDA). Allowable levels of nutrients vary depending on national regulations and the final application.
Vitamin D

History

1645 Whistler writes the first scientific description of rickets.

1865 In his textbook on clinical medicine, Trousseau recommends cod liver oil as treatment for rickets. He also recognises the importance of sunlight and identifies osteomalacia as the adult form of rickets.

1919 Mellanby proposes that rickets is due to the absence of a fat-soluble dietary factor.

1922 McCollum and coworkers establish the distinction between vitamin A and the antirachitic factor.

1925 McCollum and coworkers name the antirachitic factor vitamin D. Hess and Weinstock show that a factor with antirachitic activity is produced in the skin by ultraviolet irradiation.

1936 Windaus identifies the structure of vitamin D in cod liver oil.

1937 Schenck obtains crystallised vitamin D3 by activation of 7-dehydro-cholesterol.

1967 Bruck and colleagues accomplished the first pure chemical synthesis of vitamin D, without photochemical irradiation steps.

1968 Haussler and colleagues report the presence of an active metabolite of vitamin D in the intestinal mucosa of chicks.

1969 Haussler and Norman discover calcitriol receptors in chick intestine.

1970 Fraser and Kodicek discover that calcitriol is produced in the kidney.

1971 Norman and coworkers identify the structure of calcitriol.

1973 Fraser and associates discover the presence of an inborn error of vitamin D metabolism that produces rickets resistant to vitamin D therapy.

1978 De Luca’s group discovers a second form of vitamin D-resistant rickets (Type II).

1981 Abe and colleagues in Japan demonstrate that calcitriol is involved in the differentiation of bone-marrow cells.

1983 Provvedini and colleagues demonstrate the presence of calcitriol receptors in human leukocytes.

1984 The same group presents evidence that calcitriol has a regulatory role in immune function.

1986 Morimoto and associates suggest that calcitriol may be useful in the treatment of psoriasis.

1989 Baker and associates clone the vitamin D receptor and show that it belongs to the steroid-hormone receptor gene family.

1994 The U.S. Food and Drug Administration (FDA) approves a vitamin D-based topical treatment for psoriasis, called calcipotriol.

2003 A prospective study from Feskanich and coworkers among 72,000 postmenopausal women in the U.S. over 18 years indicates that women consuming at least 600 IU vitamin D/day from food plus supplements have a 37% lower risk of hip fracture.

2006 Researchers from the Harvard School of Public Health examine cancer incidence and vitamin D exposure in over 47,000 men in the Health Professionals Follow-Up Study. They find that a high level of vitamin D (~1500 IU daily) is associated with a 17% reduction in all cancer incidences and a 29% reduction in total cancer mortality with even stronger effects for digestive-system cancers.
Introduction

The term vitamin E covers eight fat-soluble compounds found in nature. Four of them are called tocopherols and the other four tocotrienols. They are identified by the prefixes α, β, γ and δ. α-Tocopherol is the most common and biologically the most active of these naturally occurring forms of vitamin E. Natural tocopherols occur in RRR-configuration only (RRR-α-tocopherol was formerly designated as d-α-tocopherol). The chemical synthesis of α-tocopherol results in a mixture of eight different stereoisomeric forms which is called all-rac-α-tocopherol (or dl-α-tocopherol). The biological activity of the synthetic form is lower than that of the natural form. The name tocopherol derives from the Greek words tocos, meaning childbirth, and pherein, meaning to bring forth. The name was coined to highlight its essential role in the reproduction of various animal species. The ending -ol identifies the substance as being an alcohol. The importance of vitamin E in humans was not accepted until fairly recently. Because its deficiency is not manifested by a well recognised, widespread vitamin deficiency disease such as scurvy (vitamin C deficiency) or rickets (vitamin D deficiency), science only began to recognise the significance of vitamin E at a relatively late stage.

Functions

The major biological function of vitamin E is that of a lipid soluble antioxidant preventing the propagation of free-radical reactions. Free radicals are formed in normal metabolic processes and upon exposure to exogenous toxic agents (e.g. cigarette smoke, pollutants). Vitamin E is located within the cellular membranes. It protects polyunsaturated fatty acids (PUFAs) and other components of cellular membranes from oxidation by free radicals. Apart from maintaining the integrity of the cell membranes in the human body, it also protects low density lipoprotein (LDL) from oxidation. Recently, non-antioxidant functions of α-tocopherol have been identified. α-tocopherol inhibits protein kinase C activity, which is involved in cell proliferation and differentiation. Vitamin E inhibits platelet aggregation and enhances vasodilation. Vitamin E enrichment of endothelial cells downregulates the expression of cell adhesion molecules, thereby decreasing the adhesion of blood cell components to the endothelium.

Dietary sources

Vegetable oils (olive, soya bean, palm, corn, safflower, sunflower, etc.), nuts, whole grains and wheat germ are the most important sources of vitamin E. Other sources are seeds and green leafy vegetables. The vitamin E content of vegetables, fruits, dairy products, fish and meat is relatively low. The vitamin E content in foods is often reported as α-tocopherol equivalents (α-TE). This term was established to account for the differences in biological activity of the various forms of vitamin E. 1 mg of α-tocopherol is equivalent to 1 TE. 1 IU = 1 mg all-rac-α-tocopheryl acetate and 0.74 mg RRR-α-tocopheryl acetate, 1 mg TE = 1 mg of RRR-α-tocopherol = 1.49 IU.

Vitamin E content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>mg α-TE/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ oil</td>
<td>174</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>63</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>26</td>
</tr>
<tr>
<td>Rape seed oil</td>
<td>23</td>
</tr>
<tr>
<td>Soya bean oil</td>
<td>17</td>
</tr>
<tr>
<td>Olive oil</td>
<td>12</td>
</tr>
<tr>
<td>Peanuts</td>
<td>11</td>
</tr>
<tr>
<td>Walnuts</td>
<td>6</td>
</tr>
<tr>
<td>Butter</td>
<td>2</td>
</tr>
<tr>
<td>Spinach</td>
<td>1.4</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.8</td>
</tr>
<tr>
<td>Apples</td>
<td>0.5</td>
</tr>
<tr>
<td>Milk (whole)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

(Source: Fachmann, 1990)
**Absorption and body stores**

Vitamin E is absorbed together with lipids in the small intestine, depending on adequate pancreatic function and biliary secretion. Tocopherol esters which are present in food supplements and processed food are hydrolysed before absorption. Vitamin E is incorporated into chylomicrons and transported via the lymphatic system to the liver.

α-tocopherol is the vitamin E form that predominates in blood and tissue. This is due to the action of a liver protein (α-tocopherol transfer protein) preferentially incorporating α-tocopherol into the lipoproteins which deliver it to the different tissues. Vitamin E is found in most human body tissues. The highest vitamin E contents are found in the adipose tissue, liver and muscles. The pool of vitamin E in the plasma, liver, kidneys and spleen turns over rapidly, whereas turnover of the content of adipose tissue is slow.

**Measurement**

Normal α-tocopherol concentrations in plasma measured by high performance liquid chromatography range from 12 – 45 µM (0.5 – 2 mg/100 ml). Plasma α-tocopherol concentrations of <11.6 µM, the level at which erythrocyte haemolyses occurs, indicate poor vitamin E nutritional status. Since plasma levels of α-tocopherol correlate with cholesterol levels, the α-tocopherol concentration is often indicated as α-tocopherol-cholesterol ratio. Vitamin E content is generally expressed by biological activity, using the scale of International Units (IU). According to this system, 1 mg of RRR-α-tocopherol, biologically the most active of the naturally occurring forms of vitamin E, is equivalent to 1.49 IU vitamin E. The biological activity of 1 mg of all-rac-α-tocopheryl acetate, the synthesised form of vitamin E commonly used in food enrichment, is equivalent to 1 IU. Recently, the unit of α-tocopherol equivalent was established (see: Dietary sources).

**Stability**

Light, oxygen and heat, detrimental factors encountered in long storage of foodstuffs and food processing, lower the vitamin E content of food. In some foods it may decrease by as much as 50% after only two weeks’ storage at room temperature. To a large extent, frying destroys the vitamin E in vegetable oils. Esters of α-tocopherol (α-tocopheryl acetate and α-tocopheryl succinate) are used for supplements because they are more resistant to oxidation during storage.

**Interactions**

**Positive interactions**
- The presence of other antioxidants, such as vitamin C and β-carotene, supports the antioxidative, protective action of vitamin E; the same is true of the mineral selenium
- The requirement for vitamin E is related to the amount of polyunsaturated fatty acids consumed in the diet. The higher the amount of PUFAs, the more vitamin E is required

**Negative interactions**
- When taken at the same time, iron reduces the availability of vitamin E to the body; this is especially critical in the case of anaemic newborns
- Vitamin K deficiency may be exacerbated by vitamin E, thereby affecting blood coagulation
- Various medications decrease absorption of vitamin E (e.g. cholestyramine, colestipol, isoniazid)

**Deficiency**

Because depletion of vitamin E tissue stores takes a very long time, no overt clinical deficiency symptoms have been noted in otherwise healthy adults. Symptoms of vitamin E deficiency are seen in patients with fat malabsorption syndromes or liver disease, in individuals with genetic defects affecting the α-tocopherol transfer protein and in new-born infants, particularly premature infants. Vitamin E deficiency results in neurological symptoms (neuropathy), myopathy (muscle weakness) and pigmented retinopathy. Early diagnostic signs are leakage of muscle enzymes, increased plasma levels of lipid peroxidation products and increased haemolysis of erythrocytes (red blood cells). In premature infants, vitamin E deficiency is associated with haemolytic anaemia, intraventricular haemorrhage (a condition in which blood vessels within the brain burst and bleed into the hollow chambers (ventricles) and retrolental abnormal blood vessel development in the retina of the eye in a premature infant.

**Groups at risk of deficiency**
- Vitamin E deficiency may occur as a result of genetic abnormalities in α-TTP, various fat malabsorption syndromes, protein-energy malnutrition

**Disease prevention and therapeutic use**

Research studies indicate that vitamin E has numerous health benefits. Vitamin E is thought to play a role in preventing atherosclerosis and cardiovascular diseases (heart disease and stroke) due to its effects on a number of steps in the development of atherosclerosis (e.g. inhibition of LDL oxidation, inhibition of smooth muscle cell proliferation, inhibition of platelet adhesion, aggregation and platelet release reaction). Recent studies suggest that vitamin E enhances immunity in the elderly, and that supplementation with vitamin E lowers the risk of contracting an upper respiratory tract infection, particularly the common cold. Researchers are investigating the prophylactic role of vitamin E in protecting against exogenous pollutants and lowering the risk of cancer and of cataracts. Vitamin E in combination with vitamin C may protect the body from oxidative stress caused by extreme sports (e.g. ultra marathon running). A role of vitamin E supplementation in the treatment of neurodegenerative diseases (Alzheimer’s disease, amyotrophic lateral sclerosis) is also under investigation.
Vitamin E

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin E varies according to age, sex and criteria applied in individual countries. In the USA, the RDA for adults is 15 mg RRR-α-tocopherol/day (FNB, 2000). In Europe, adult recommendations range from 4 to 15 mg α-TE/day for men and from 3 to 12 mg α-TE/day for women. The RDA for vitamin E of 15 mg cannot easily be acquired even with the best nutritional intentions. Vitamin E intake should also be adapted to that of PUFA, which influences the requirement for this vitamin. The E.C. Scientific Committee on Foods (SCF) has suggested a consumption ratio of 0.4 mg α-TE per gram of PUFA.

Recommended daily intakes *

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt; 6 months</td>
<td>4 mg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>6 – 12 months</td>
<td>5 mg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>6 mg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>7 mg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>11 mg</td>
</tr>
<tr>
<td>Males</td>
<td>14 – 50 years</td>
<td>15 mg</td>
</tr>
<tr>
<td>Females</td>
<td>&gt; 14 years</td>
<td>15 mg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>15 mg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>15 mg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** As α-tocopherol
A Adequate Intake
If not otherwise specified, this table presents Recommended Dietary Allowances (RDAs). Allowable levels of nutrients vary depending on national regulations and the final application.

Safety

Vitamin E has low toxicity. After reviewing more than 300 scientific studies, the US-based Institute of Medicine (IOM) concluded that vitamin E is safe for chronic use even at doses of up to 1000 mg per day. A recently published meta-analysis suggested that taking more than 400 IU (267 mg RRR-α-tocopherol) of vitamin E per day brought a weekly increase in the risk of all-cause mortality. However, much of the research was done in patients at high risk of a chronic disease and these findings may not be generalisable to healthy adults. Many human long-term studies with higher doses of vitamin E have not reported any adverse effects, and it has been concluded that vitamin E intakes of up to 1600 IU (1073 mg RRR-α-tocopherol) are safe for most adults. The Antioxidant Panel of the Food and Nutrition Board (FNB, 2000) has set the UL (tolerable upper intake level) for adults at 1000 mg/day of any form of supplemental α-tocopherol. In 2003 the E.C. Scientific Committee on Foods (SCF) established the UL of 300 mg α-TE for adults. Also in 2003, the UK Expert group on Vitamins and Minerals (EVM; 2003) set the UL at 540 mg α-TE for supplemental vitamin E.

Supplements and food fortification

Vitamin E is available in soft gelatine capsules, or as chewable or effervescent tablets, and is found in most multivitamin supplements. The most common fortified foods are soft drinks and cereals.

The all-rac-α-tocopherol form of vitamin E is widely used as an antioxidant in stabilising edible oils, fats and fat-containing food products. Research has shown that vitamin E in combination with vitamin C reduces the formation of nitrosamines (a proven carcinogen in animals) in bacon more effectively than vitamin C alone.

Vitamin E has been used topically as an anti-inflammatory agent, to enhance skin moisturisation and to prevent cell damage by UV light. In pharmaceutical products tocopherol is used, for example, to stabilise syrups, aromatic components, and vitamin A or provitamin A components.

α-tocopherol is used as an antioxidant in plastics, technical oils and greases, and in the purified, so-called white oils, employed in cosmetics and pharmaceuticals.

Industrial production

Vitamin E derived from natural sources is obtained by molecular distillation and, in most cases, subsequent methylation and esterification of edible vegetable oil products. Synthetic vitamin E is produced from fossil plant material by condensation of trimethylhydroquinone with isophytol.
**Vitamin E**

**History**

1911  Hart and coworkers publish the first report of a suspected ‘anti-sterility factor’ in animals.

1920  Matthill and Conklin observe reproductive anomalies in rats fed on special milk diets.

1922  Vitamin E is discovered by Evans and Scott Bishop.

1936  Evans and coworkers isolate what turns out to be α-tocopherol in its pure form from wheat germ oil.

1938  Fernholz provides the structural formula of vitamin E and Nobel laureate Karrer synthesises dl-α-tocopherol.

1945  Dam and coworkers discover peroxides in the fat tissue of animals fed on vitamin E-deficient diets. The first antioxidant theory of vitamin E activity is proposed.

1962  Tappel proposes that vitamin E acts as an *in vivo* antioxidant to protect cell lipids from free radicals.

1968  The Food and Nutrition Board of the U.S. National Research Council recognises vitamin E as an essential nutrient for humans.

1974  Fahrenholz proposes singlet oxygen quenching abilities of α-tocopherol.

1977  Human vitamin E deficiency syndromes are described.

1980  Walton and Packer propose that vitamin E may prevent the generation of potentially carcinogenic oxidative products of unsaturated fatty acids.

1980  McKay and King suggest that vitamin E functions as an antioxidant located primarily in the cell membrane.

1980s  Vitamin E is demonstrated to be the major lipid-soluble antioxidant protecting cell membranes from peroxidation. Vitamin E is shown to stabilise the superoxide and hydroxyl free radicals.

1990  Effectiveness of vitamin E in inhibiting LDL (low density lipoprotein) oxidation is shown.

1990  Kaiser and coworkers elucidate the singlet oxygen quenching capability of vitamin E.

1991  Azzi and coworkers describe an inhibitory effect of α-tocopherol on the proliferation of vascular smooth muscle cells and protein kinase C activity.

2004  Barella and coworkers demonstrate that vitamin E regulates gene expression in the liver and the testes of rats.
Vitamin K

Introduction

In 1929 Henrik Dam observed that chicks fed on fat-free diets developed haemorrhages and started bleeding. In 1935 he proposed that the antihæmorrhagic substance was a new fat-soluble vitamin, which he called vitamin K (after the first letter of the German word ‘Koagulation’). Vitamin K is indeed fat-soluble, and it occurs naturally in two forms: vitamin K1 (phylloquinone) is found in plants; vitamin K2 is the term for a group of compounds called menaquinones (MK-n, n being the number of isoprenyl units in the side chain of the molecule) which are synthesised by bacteria in the intestinal tract of humans and various animals. Vitamin K3 (menadione) is a synthetic compound that is only used in animal nutrition.

Functions

Vitamin K is essential for the synthesis of the biologically active forms of a range of proteins called vitamin K-dependent proteins. Vitamin K participates in the conversion of glutamate residues of these proteins to γ-carboxylglutamate residues by addition of a carboxyl-group (carboxylation).

In the absence of vitamin K, carboxylation of these proteins is incomplete, and they are secreted in plasma in various so called under-carboxylated forms, which are biologically inactive. Vitamin K is also essential for the functioning of several proteins involved in blood coagulation (clotting), a mechanism that prevents bleeding to death from cuts and wounds, as well as internal bleeding.

Vitamin K-dependent proteins

Prothrombin (factor II), factors VII, IX, and X, and proteins C, S and Z are proteins that are involved in the regulation of blood coagulation. They are synthesised in the liver. Protein S has also been detected in bone.

The vitamin K-dependent proteins osteocalcin and MGP (matrix Gla-protein) have been found in bone. Osteocalcin is thought to be related to bone mineralisation. Matrix Gla-protein is present in bone, cartilage and vessel walls and has recently been established as an inhibitor of calcification. The role of protein S in bone metabolism is not clear.

Recently, several other vitamin K-dependent proteins have been identified.

Dietary sources

A typical western diet provides 90% in the form of Phylloquinone (vitamin K1) and 10% of Menaquinone (MK-n, vitamin K2).

Phylloquinone
Rich food sources are green leafy vegetables such as spinach, broccoli, Brussels sprouts, cabbage and lettuce.

Menaquinone
Bacterial by-product in dairy products
High MK-7 content is found in Natto (fermented soy beans, a traditional Japanese food) (0.8-1g/100g).
Vitamin K

Absorption and body stores

Vitamin K is absorbed from the jejunum and ileum. As with other fat-soluble vitamins, absorption depends on the presence of bile and pancreatic juices and is enhanced by dietary fat. While the liver is the main storage site, vitamin K is also found in extrahepatic tissues, e.g. bone and heart. Liver stores consist of about 10% phylloquinones and 90% menaquinones. Compared with that of other fat-soluble vitamins, the total body pool of vitamin K is small and turnover of vitamin K in the liver is rapid. The body recycles vitamin K in a process called the vitamin K cycle, allowing the vitamin to function in the γ-carboxylation of proteins many times. Although the liver contains menaquinones synthesised by intestinal bacteria, the absorption of menaquinones and their contribution to the human vitamin K requirement have not yet been fully elucidated.

Measurement

Plasma vitamin K concentration is measured by high performance liquid chromatography. The normal range of plasma vitamin K in adults is 0.2–3.2 ng/ml. Levels below 0.5 ng/ml have been associated with impaired blood-clotting functions. However, measuring plasma vitamin K concentrations is of limited use as it responds to changes in dietary intake within 24 hours. As vitamin K deficiency results in impaired blood clotting, laboratory tests measure clotting time. Plasma concentration of vitamin K dependent blood-clotting factors (e.g. prothrombin, factor VII, factor IX, or factor X) are measured to assess inadequate vitamin K intake or vitamin K status.

Stability

Vitamin K compounds are moderately stable to heat and reducing agents, but are sensitive to acid, alkali, light and oxidising agents.

Interactions

**Negative interactions**

- Coumarin anticoagulants (such as warfarin), salicylates and certain antibiotics act as vitamin K antagonists
- Very high dietary or supplemental intakes of vitamin K may inhibit the anticoagulant effect of vitamin K antagonists (e.g. warfarin)
- High doses of vitamins A and E have been shown to interfere with vitamin K and precipitate deficiency states
- Absorption of vitamin K may be decreased by mineral oil, bile acid sequestrants (cholestyramine, colestipol) and orlistat (weight loss medication)

**Groups at risk of deficiency**

- Individuals with gastrointestinal disorders, fat malabsorption, liver disease, after prolonged antibiotic therapy coupled with compromised dietary intake
- Patients taking oral anticoagulant drugs which are vitamin K antagonists
- Newborn infants have a well established risk of vitamin K deficiency, which may result in fatal intracranial haemorrhage (bleeding within the skull) in the first weeks of life
- Breast-fed infants in particular have a low vitamin K status because placental transfer of vitamin K is poor and human milk contains low levels of vitamin K

Deficiency

Vitamin K deficiency is uncommon in healthy adults but occurs in individuals with gastrointestinal disorders, fat malabsorption or liver disease, or after prolonged antibiotic therapy coupled with compromised dietary intake. Impaired blood clotting is the clinical symptom of vitamin K deficiency which is demonstrated by measuring clotting time. In severe cases, bleeding occurs. Adults at risk of vitamin K deficiency also include patients taking anticoagulant drugs which are vitamin K antagonists.

Newborn infants have a well established risk of vitamin K deficiency, which may result in fatal intracranial haemorrhage (bleeding within the skull) in the first weeks of life. Breast-fed infants in particular have a low vitamin K status because placental transfer of vitamin K is poor and human milk contains low levels of vitamin K. The concentrations of plasma clotting factors are low in infants due to immaturity of the liver. Haemorrhagic disease in the new-born is a significant worldwide cause of infant morbidity and mortality. Therefore, in many countries vitamin K is routinely administered prophylactically to all newborns.

Disease prevention and therapeutic use

Phylloquinone is the preferred form of the vitamin for clinical use. It is used for intravenous and intramuscular injections as a colloidal suspension, emulsion or aqueous suspension, and as a tablet for oral use. Vitamin K1 is used in the treatment of hypoprothrombinemia (low amounts of prothrombin), secondary to factors limiting absorption or synthesis of vitamin K. During operations in which bleeding is expected to be a problem, for example, in gall-bladder surgery, vitamin K1 is administered. Anticoagulants inhibit vitamin K recycling, which can be corrected rapidly and effectively by the administration of vitamin K1. Vitamin K1 is often given to mothers before delivery and to newborn infants to protect against intracranial haemorrhage. A putative role of vitamin K in osteoporosis has been investigated since vitamin K-dependent proteins have been discovered in bone. However, further investigations are required to resolve whether vitamin K is a significant etiological component of osteoporosis.
Vitamin K

A role for vitamin K in the development of atherosclerosis is also under discussion, but studies supporting this hypothesis are limited and future research is recommended.

Recently, studies with cancer cell lines and animal studies have indicated that a combination of vitamin C and vitamin K3 has antitumor activity and inhibits the development of metastases.

Recommended Daily Allowance (RDA)

The U.S. Food and Nutrition Board of the Institute of Medicine (2001) has established an adequate intake (AI) level for adults based on reported dietary intakes of vitamin K in apparently healthy population groups. Other health authorities have come to similar conclusions.

Safety

Even when large amounts of vitamin K1 and K2 are ingested over an extended period, toxic manifestations have not been observed. Therefore, the major health authorities have not established a tolerable upper level of intake (UL) for vitamin K. Allergic reactions have been reported, however. Furthermore, administered menadione (K3) has been known to cause haemolytic anaemia, jaundice and kernicterus (a grave form of jaundice in the new-born) and is no longer used for treatment of vitamin K deficiency.

Recommended daily intakes *

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt; 6 months</td>
<td>2 µg</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>2.5 µg</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>30 µg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>55 µg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>60 µg</td>
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<tr>
<td>Children</td>
<td>14 – 18 years</td>
<td>75 µg</td>
</tr>
<tr>
<td>Males</td>
<td>&gt; 19 years</td>
<td>120 µg</td>
</tr>
<tr>
<td>Females</td>
<td>&gt; 19 years</td>
<td>90 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 18 years</td>
<td>75 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>&gt; 19 years</td>
<td>90 µg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 18 years</td>
<td>75 µg</td>
</tr>
<tr>
<td>Lactation</td>
<td>&gt; 19 years</td>
<td>90 µg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** AI: Adequate Intake

Allowable levels of nutrients vary depending on national regulations and the final application.

Supplements, food fortification and other applications

Supplements of vitamin K are available alone in tablets and capsules, and also in multivitamin preparations. Infant formula products, beverages and cookies are fortified with vitamin K. Menadione salts are generally preferred for farm animals because of their stability.

Industrial production

The procedure involves the use of monoester, menadion and an acid catalyst. Purification of the desired product to remove unreacted reagents and side products occurs either at the quinol stage or after oxidation.
**Vitamin K**

**History**

- **1929**: A series of experiments by Dam results in the discovery of vitamin K.
- **1931**: A clotting defect is observed by McFarlane and coworkers.
- **1935**: Dam proposes that the antihaemorrhagic vitamin in chicks is a new fat-soluble vitamin, which he calls vitamin K.
- **1936**: Dam and associates succeed in preparing a crude plasma prothrombin fraction, and demonstrate that its activity is decreased when it is obtained from vitamin K-deficient chick plasma.
- **1939**: Vitamin K1 is synthesised by Doisy and associates.
- **1940**: Brikhouz observes haemorrhagic conditions resulting from malabsorption syndromes or starvation, and finds that haemorrhagic disease of the newborn responds to vitamin K.
- **1943**: Dam receives half of the Nobel prize for his discovery of vitamin K, the blood coagulation factor.
- **1943**: Doisy receives half of the Nobel prize for his discovery of the chemical nature of vitamin K.
- **1974**: The vitamin K-dependent step in prothrombin synthesis is demonstrated by Stenflo and associates and Nelsestuen and colleagues.
- **1975**: Esmon discovers a vitamin K-dependent protein carboxylation in the liver.
Vitamin C

Introduction

Vitamin C is water-soluble, and probably the most famous of all the vitamins. Even before its discovery in 1932, physicians recognised that there must be a compound in citrus fruits preventing scurvy, a disease that killed as many as 2 million sailors between 1500 and 1800. Later researchers discovered that man, other primates and the guinea pig depend on external sources to cover their vitamin C requirements. Most other animals are able to synthesise vitamin C from glucose and galactose in their body.

Functions

The most prominent role of vitamin C is its immune stimulating effect, which is important for the defence against infections such as common colds. It also acts as an inhibitor of histamine, a compound that is released during allergic reactions. As a powerful antioxidant it can neutralise harmful free radicals and aids in neutralising pollutants and toxins. Thus it is able to prevent the formation of potentially carcinogenic nitrosamines in the stomach (due to consumption of nitrite-containing foods, such as smoked meat). The reduction of oxidative stress has an impact on cardiovascular disease (CVD). Individuals experiencing oxidative stress have ascorbic acid blood levels lower than healthy individuals. Importantly, vitamin C is also able to regenerate other antioxidants such as vitamin E.

As an enzyme co-factor vitamin C is required for the synthesis of collagen, the intercellular ‘cement’ substance which gives structure to muscles, vascular tissues, bones, tendons and ligaments. Due to these functions vitamin C, especially in combination with zinc, is important for the healing of wounds. Vitamin C contributes to the health of teeth and gums, preventing haemorrhaging and bleeding. It also improves the absorption of iron from the diet, and is needed for the metabolism of bile acids, which may have implications for blood cholesterol levels and gallstones. In addition, vitamin C plays an important role in the synthesis of several peptide hormones and neurotransmitters and carnitine. Finally, vitamin C is also a crucial factor in the eye’s ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration (AMD) and vision-loss in combination with other antioxidant vitamins and zinc.

Main functions in a nutshell:

- Antioxidant
- Immune stimulation
- Anti-allergic
- Collagen synthesis ‘Cement’ for connective tissues
- Wound healing
- Teeth and gum health
- Regeneration of vitamin E
- Aids iron absorption
- Eye health

Vitamin C content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose hip</td>
<td>2000</td>
</tr>
<tr>
<td>Acerolas</td>
<td>1600</td>
</tr>
<tr>
<td>Blackcurrants</td>
<td>200</td>
</tr>
<tr>
<td>Peppers</td>
<td>138</td>
</tr>
<tr>
<td>Broccoli</td>
<td>115</td>
</tr>
<tr>
<td>Fennel</td>
<td>95</td>
</tr>
<tr>
<td>Kiwis</td>
<td>71</td>
</tr>
<tr>
<td>Strawberries</td>
<td>64</td>
</tr>
<tr>
<td>Oranges</td>
<td>49</td>
</tr>
</tbody>
</table>

(Souci, Fachmann, Kraut)
Dietary sources

Vitamin C is widely distributed in fruits and vegetables. Citrus fruits, blackcurrants, peppers, green vegetables (e.g. broccoli, Brussels sprouts), and fruits like strawberries, guava, mango and kiwi are particularly rich sources. On a quantity basis, the intake of potatoes, cabbage, spinach and tomatoes is also of importance. Depending on the season, one medium-sized glass of freshly pressed orange juice (i.e. 100 g) yields from 15 to 35 mg vitamin C.

Absorption and body stores

Intestinal absorption of vitamin C depends on the amount of dietary intake, decreasing with increasing intake levels. At an intake of 30 to 180 milligrams, about 70% to 90% is absorbed; about 50% of a single dose of 1 to 15 grams is absorbed; and only 16% of a single dose of 12 grams is absorbed. Up to 500 milligrams are absorbed via a sodium-dependent active transport process, while at higher doses simple diffusion occurs.

The storage capacity of water-soluble vitamins is generally low compared to that of fat-soluble ones. Humans have an average tissue store of vitamin C of 20 mg/kg body weight. The highest concentration is found in the pituitary gland (400 mg/kg); other tissues of high concentration are the adrenal glands, eye lenses, white blood cells (esp. lymphocytes), brain, liver, and white blood cells (leukocytes).

Measurement

Vitamin C can be measured in the blood plasma and other body tissues by various techniques. Also dipstick tests for estimation of vitamin C levels in the urine are available. Less satisfying, however, is the evaluation of the analytical data concerning the true reflection of the body status. Threshold values are difficult to define and the subject of controversial discussion. Typical blood plasma levels are in the range of 20 to 100 µmol/L.

Stability

Vitamin C is sensitive to heat, light and oxygen. In food it can be partly or completely destroyed by long storage or overcooking. Refrigeration can substantially diminish vitamin C loss in food.

Influence of storage and preparation on vitamin C loss in foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Storage / preparation</th>
<th>Vitamin C loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>1 month</td>
<td>50%</td>
</tr>
<tr>
<td>Fruits</td>
<td>1 month</td>
<td>20%</td>
</tr>
<tr>
<td>Apples</td>
<td>6 – 9 months</td>
<td>100%</td>
</tr>
<tr>
<td>Milk</td>
<td>UHT</td>
<td>25%</td>
</tr>
<tr>
<td>Fruits</td>
<td>Sterilisation</td>
<td>50%</td>
</tr>
<tr>
<td>Fruits</td>
<td>Air drying</td>
<td>50 – 70%</td>
</tr>
<tr>
<td>Leafy vegetables</td>
<td>Canning</td>
<td>48%</td>
</tr>
</tbody>
</table>

Interactions

Positive interactions

The presence of other antioxidants, such as vitamin E and β-Carotene, supports the protective antioxidant action of vitamin C. Other vitamins, such as those of the B-complex (particularly B6, B12, folic acid and pantothenic acid) and some pharmacologically active substances, as well as the naturally occurring compounds known as bioflavonoids, may have a sparing effect on vitamin C.

Negative interactions

Due to toxic compounds in smoke, the vitamin C requirement for smokers and passive smokers is about 35 mg/day higher than for non-smokers. Also several pharmacologically active compounds, among them some antidepressants, diuretics, birth control pills and aspirin (acetylsalicylic acid), deplete the tissues of vitamin C. This is also true of certain habits, for example alcohol consumption and (passive) smoking.

Modified from Oberbeil, Fit durch Vitamine, Die neuen Wunderwaffen, Südwest Verlag GmbH & Co. KG, München 1995
Deficiency

Early symptoms of vitamin C deficiency are very general and could also indicate other diseases. They include fatigue, lassitude, loss of appetite, drowsiness and insomnia, feeling rundown, irritability, low resistance to infections and petechiae (minor capillary bleeding). Severe vitamin C deficiency leads to scurvy, characterised by weakening of collagenous structures, resulting in widespread capillary bleeding. Infantile scurvy causes bone malformations. Bleeding gums and loosening of the teeth are usually the earliest signs of clinical deficiency. Haemorrhages under the skin cause extreme tenderness of extremities and pain during movement. If left untreated, gangrene and death may ensue. Nowadays this is rare in developed countries and can be prevented by a daily intake of about 10 – 15 mg of vitamin C. However, for optimal physiological functioning much higher amounts are required.

Groups at risk of deficiency

- Smokers and passive smokers – increased oxidative stress and metabolic turnover of Vitamin C
- Low income groups – Individuals with low vitamin / inadequate nutrient intake e.g. elderly, chronic dieters
- People suffering from illness, infectious and inflammatory diseases, allergies, arteriosclerosis, high blood pressure, others (e.g. cancer, stroke, tinnitus)
- Mentally and physically stressed people
- Pregnant and and breast feeding women

Disease prevention and therapeutic use

Dozens of prospective studies suggest that vitamin C plays a role in preventing a variety of diseases. As this nutrient is important for a variety of diseases, only a selection of them are presented here in detail.

Cardiovascular diseases (CVD)
(heart disease and stroke)

The data for the CVD protective benefits of vitamin C are inconsistent. While some studies have failed to find significant reductions in the risk of coronary heart disease (CHD), numerous prospective cohort studies have found inverse associations between dietary vitamin C intake or vitamin C plasma levels and CVD risk. Vitamin C may protect coronary arteries by reducing the build-up of plaque, as this helps to prevent the oxidation of LDL cholesterol (the ‘bad’ cholesterol), especially in combination with vitamin E. Some data has shown that vitamin C may also boost blood levels of HDL cholesterol (the ‘good’ cholesterol), which is also considered positive for the prevention of heart diseases. The risk of stroke may be reduced by an adequate intake of vitamin C through fruits, vegetables and supplements. However, due to the inconsistency of the data and its lack of specificity to vitamin C, the interpretation of these results is difficult.

Cancer

The role of vitamin C in cancer prevention has been studied extensively, and until now no beneficial effect has been shown for breast, prostate, or lung cancer. However, a number of studies have associated higher intakes of vitamin C with decreased incidence of cancers of the upper digestive tract, cervix, ovary, bladder, and colon. Studies find significant cancer risk reduction after vitamin C supplementation has been used in cases of severe colds. This may be due to the antihistaminic action of very large doses of vitamin C.

Wound healing

During a postoperative period, or during healing of superficial wounds, supplemental vitamin C contributes to the prevention of infections and promotes skin repair.

Blood pressure

Several studies have shown a blood pressure lowering effect of vitamin C supplementation at about 500 mg per day due to improved dilation of blood vessels.
Vitamin C

Supplements, food fortification and other applications

Vitamin C is offered in conventional tablets, effervescent and chewable tablets, time-release tablets, syrups, powders, granules, capsules, drops and ampoules, either alone or in multivitamin-mineral preparations. Buffered vitamin C forms are less acidic, which can be an advantage in terms of preventing gastric irritation. Vitamin C can also be used in the form of injections. A number of fruit juices, fruit flavour drinks and breakfast cereals are enriched with vitamin C. On average in Europe, vitamin C supplements provide up to 8.3% of total vitamin C intake.

Safety

As much as 6 – 10 g vitamin C per day (more than 100 times the RDA) has been ingested regularly by many people with no evidence of side effects. Although a number of possible problems with very large doses of vitamin C have been suggested, none of these adverse health effects have been confirmed, and there is no reliable scientific evidence that large amounts of vitamin C (up to 10 g/day in adults) are toxic. In the year 2000 the U.S. Food and Nutrition Board recommended a tolerable upper intake level (UL) for vitamin C of 2 g (2,000 mg, adults) daily in order to prevent most adults from experiencing osmotic diarrhoea and gastrointestinal disturbances.

Industrial production

The synthesis of ascorbic acid was achieved by Reichstein in 1933, and this was followed by industrial production five years later by Hoffman La Roche Ltd., the vitamin division of which is now DSM Nutritional Products Ltd. Today synthetic vitamin C, identical to that occurring in nature, is produced from glucose on an industrial scale by chemical and biotechnological synthesis.
History

400BC Hippocrates describes the symptoms of scurvy.

1747 British naval physician James Lind prescribes oranges and lemons as a cure for scurvy.

1907 Scurvy is experimentally produced in guinea pigs by Hoist and Frohlich.

1917 A bioassay is developed by Chick and Hume to determine the anti-scorbutic properties of foods.

1930 Szent-Györgyi demonstrates that the hexuronic acid he first isolated from the adrenal glands of pigs in 1928 is identical to vitamin C, which he extracts in large quantities from sweet peppers.

1932 In independent efforts, Haworth and King establish the chemical structure of vitamin C.

1932 The relationship between vitamin C and anti-scorbutic factor is discovered by Szent-Györgyi and at the same time by King and Waugh.

1933 In Basel, Reichstein synthesises ascorbic acid identical to natural vitamin C. This is the first step towards the vitamin’s industrial production in 1936.

1937 Haworth and Szent-Györgyi receive a Nobel prize for their research on vitamin C.

1940 In a self experiment, Crandon proves the mandatory contribution of vitamin C in wound healing.

1975-9 Experimental studies in vitro illustrate the antioxidant and singlet-oxygen quenching properties of vitamin C.

1979 Packer and coworkers observe the free radical interaction of vitamin E and vitamin C.

1982 Niki demonstrates the regeneration of vitamin E by vitamin C in model reactions.

1988 The National Cancer Institute (USA) recognises the inverse relationship between vitamin C intake and various forms of cancer, and issues guidelines to increase vitamin C in the diet.

1998/9 Three studies show that supplementation with vitamin C can dramatically lower lead levels.

2003 A systematic review of thirty studies addressing the effect of supplemented vitamin C on the duration of colds reveals that there is a consistent benefit, with a reduction in duration of 8% to 14%.

2004 Levine calls for a re-evaluation of vitamin C as cancer therapy, especially intravenous vitamin C.

2006 A 5 year Japanese study shows that the risk of contracting three or more colds in the five-year period was decreased by 66% by daily intake of a 500-mg vitamin C supplement.
**Introduction**

Thiamine is a water-soluble B-complex vitamin. It was the first B vitamin to be identified and one of the first organic compounds to be recognised as a vitamin in the 1930s. In fact it was through the discovery and naming of thiamine that the word 'vitamin', from the Latin 'vita' = life and 'amine' = nitrogen-containing compound, was coined. The notion that the absence of a substance in food could cause a disease (in this case beriberi) was a revolutionary one. Man and other primates rely on their food intake to cover their thiamine requirements.

**Functions**

The main functions of thiamine are connected to its role as a coenzyme in the form of thiamine pyrophosphate (TPP). Coenzymes are ‘helper molecules’ which activate enzymes, the proteins that control the thousands of biochemical processes occurring in the body. TPP acts as a ‘helper molecule’ in about 25 enzymatic reactions and plays an essential role in the production of energy from food in carbohydrate metabolism as well as in the links between carbohydrate, protein and fat metabolism. Furthermore, TPP is a coenzyme for the metabolism of branched-chain keto acids that are derived from branched-chain amino acids. Its triphosphate form (TTP) in particular plays a role in the conduction of nerve impulses, in the metabolism of the neurotransmitters acetylcholin, adrenaline, and serotonin and in aerobic metabolism.

**Dietary sources**

Thiamine is found in most foods, but mostly in small amounts. The best source of thiamine is dried brewer’s yeast. Other good sources include meat (especially pork and ham products), some species of fish (eel, tuna), whole grain cereals and bread, nuts, pulses, dried legumes and potatoes. Concerning cereal grains, the thiamine-rich bran is removed during the milling of wheat to produce white flour, and during the polishing of brown rice to produce white rice. As a consequence, enriched and fortified grain-products are common today.

**Absorption and body stores**

Gastrointestinal absorption of nutritional thiamine occurs in the lumen of the small intestine (mainly the jejunum) by means of a sodium and energy dependent active transport mechanism. For thiamine levels higher than 2 µmol/L, passive diffusion plays an additional role. Thiamine occurs in the human body as free thiamine and its phosphorylated forms (see Chemistry).

**Thiamine content of foods**

<table>
<thead>
<tr>
<th>Food</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>12</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>2</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>1.5</td>
</tr>
<tr>
<td>Brazil nuts</td>
<td>1</td>
</tr>
<tr>
<td>Pork</td>
<td>0.9</td>
</tr>
<tr>
<td>Beans</td>
<td>0.8</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>0.59</td>
</tr>
<tr>
<td>Beef</td>
<td>0.23</td>
</tr>
</tbody>
</table>

(Source, Fachmann, Kraut)

**Main functions in a nutshell:**

- Co-enzyme in energy metabolism
- Co-enzyme for pentose metabolism as a basis for nucleic acids
- Nerve impulse conduction and muscle action

![Thiamine crystals in polarized light](image)

**Synonyms**

Vitamin B1, antineuritic factor, nerve vitamin

**Chemistry**

Pyrimidine and thiazole moiety linked by a methylene bridge – phosphorylated forms: thiamine monophosphate (TMP), thiamine diphosphate (TDP), thiamine triphosphate (TTP).

Molecular formula of thiamine-chloride

![Thiamine crystals in polarized light](image)

![Molecular formula of thiamine-chloride](image)
Because thiamine has a high turnover rate (10 – 20 days) and is not appreciably stored in the body (approx. 1 mg/day is used up in tissues), a daily supply is required. The limited stores may be depleted within two weeks or less on a thiamine-free diet, with clinical signs of deficiency beginning shortly after. Regular intake of thiamine is therefore critical. The heart, kidney, liver and brain have the highest concentrations, followed by the leukocytes and red blood cells. Excess thiamine and its acid metabolites are excreted principally in the urine.

**Measurement**

The standard way to assess thiamine status used to be to determine erythrocyte transketolase (α-ETK) activity both with and without stimulation of this enzyme by the addition of TDP cofactor. Technical difficulties led to an increasing use of direct determination of TDP in whole blood, e.g. by HPLC (High Performance Liquid Chromatography), in order to assess thiamine status. The HPLC assay is more robust and easier to perform. Thiamine status determined by this method is considered to be in good correlation with results from transketolase activation assays. Usually, whole blood concentrations are found to be between 66.5 and 200 nmol/L.

**Stability**

Thiamine is unstable when exposed to heat, alkali, oxygen and radiation. Water solubility is also a factor in the loss of thiamine from foods. About 25% of the thiamine in food is lost during the normal cooking process. Considerable amounts may be lost in thaw drip from frozen meats or in the water used to cook meats and vegetables. To preserve thiamine, foods should be cooked in a covered pan for the shortest time possible and should not be soaked in water or heated for too long. Juices and cooking water should be re-used in stews and sauces.

**Positive interactions**

- Magnesium: necessary for the conversion of thiamine to its active form
- Vitamins E and C prevent its oxidation to an inactive form
- The catalytic mechanism of pyruvate dehydrogenase and other enzymes requires the interplay of several vitamin-derived and other cofactors

**Negative interactions**

- Smoking, sulfonamide and estrogen may raise requirements
- Alcohol reduces thiamine absorption and blocks phosphorylation of thiamine to its cofactor form (TDP)
- Drugs that cause nausea and lack of appetite, or which increase intestinal function or urinary excretion, decrease the availability of thiamine
- Digoxin, indomethacin, anticonvulsants, antacids and some diuretics may lead to the risk of deficiency
- Coffee and tea may act as antagonists
- Thiamine is degraded by thiaminases (present in raw fish and shellfish)

**Deficiency**

Marginal thiamine deficiency may manifest itself in such vague symptoms as fatigue, insomnia, irritability and lack of concentration, anorexia, abdominal discomfort, constipation and loss of appetite. When there is not enough thiamine, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism has severe consequences. The two principal thiamine deficiency diseases are ‘beriberi’ and ‘Wernicke-Korsakoff syndrome’.

Beriberi manifests itself primarily in disorders of the nervous and cardiovascular systems. This serious disease is still common in parts of south-east Asia, where polished rice is a staple food and thiamine enrichment programs are not fully in place. Many other countries fortify rice and other cereal grains to replace the nutrients lost in processing.

**The disease exists in three forms:**

- **Dry beriberi** – a polyneuropathy with severe muscle wasting
- **Wet beriberi** – which in addition to neurologic symptoms is characterised by cardiovascular manifestations, edema and ultimately heart failure
- **Infantile beriberi** – which occurs in breast-fed infants whose nursing mothers are deficient in thiamine. Symptoms of vomiting, convulsions, abdominal distention and anorexia appear quite suddenly and may be followed by death from heart failure

The ‘Wernicke-Korsakoff syndrome’ (cerebral beriberi) is the thiamine deficiency disease seen most often in the Western world. It is frequently associated with chronic alcoholism in conjunction with limited food consumption. Symptoms include confusion, paralysis of eye motor nerves, abnormal oscillation of the eyes, psychosis, confabulation, and impaired retentive memory and cognitive function. The syndrome is also seen occasionally in people who fast, have chronic vomiting (hook worm) or have gross malnutrition due to e.g. AIDS or stomach cancer. If treatment of amnestic symptoms is delayed, the memory may be...
Thiamine

The development of thiamine deficiency can be caused by:
- Alcoholic disease
- Inadequate storage and preparation of food
- Increased demand due to pregnancy and lactation, heavy physical exertion, fever and stress, or adolescent growth
- Inadequate nutrition
  - high carbohydrate intake (e.g. milled or polished rice)
  - regular heavy consumption of tea and coffee (Tannin = antithiamine)
  - foods such as raw fish or betel nuts (thiaminases)
- Certain diseases (dysentery, diarrhea, cancer, nausea/vomiting, liver diseases, infections, malaria, AIDS, hyperthyroidism)
- Certain drugs (birth-control pills, neuroleptica, some cancer drugs)
- Long-term parenteral nutrition (e.g. highly concentrated dextrose infusions)

Groups at risk of deficiency
- People on diuretic medication (water tablets) or digoxin (a drug used in heart failure)
- People recovering from heart failure
- Those suffering from or recovering from infections
- People with stomach disease and those with cancer, liver or thyroid disease
- Chronic alcoholics

Disease prevention and therapeutic use

Thiamine is specific in the prevention and treatment of beriberi and other manifestations of thiamine deficiency (e.g. Wernicke-Korsakoff, peripheral neuritis). The dosage range is from 100 mg daily in mild deficiency states to 200 – 300 mg in severe cases. Thiamine administration is often beneficial in neuritis accompanied by excessive alcohol consumption or pregnancy. With alcoholic and diabetic polyneuropathies, the therapeutic dose is most often in the range of 10 – 100 mg/daily. When alcoholism has led to delirium tremens, large doses of thiamine, together with other vitamins should be given by slow injection. Large doses of thiamine (100 – 600 mg daily) have been advocated in the treatment of such diverse conditions as lumbago, sciatica, trigeminal neuritis, facial paralysis and optic neuritis. However, the response to such treatment has been variable.

Recommended Dietary Allowance (RDA)

Because thiamine facilitates energy utilisation, estimated requirements are calculated on the basis of energy intake, which can be very much dependent on activity levels. For adults, the RDA is 0.5 mg per 1000 kcal, which amounts to a range of 1.0 – 1.1 mg per day for women and 1.2 mg for men, based on an average caloric intake. An additional 0.5 mg per day are recommended during pregnancy and lactation.

Safety

Thiamine has been found to be well tolerated in healthy people, even at very high oral doses (up to 200 mg/day). Due to its very broad safety margin for oral administration and long history of safe use, none of the official regulatory authorities has defined a safe upper limit for this vitamin. The only reaction found in humans is of the hypersensitivity type. In the vast majority of cases these have occurred after injection of thiamine in patients with a history of allergic reactions. For parenteral administration, the doses that produced these reactions varied from 5 to 100 mg, though most of them occurred at the higher end of this range.

Recommended daily intakes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt; 6 months</td>
<td>0.2 mg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>0.3 mg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>0.9 mg</td>
</tr>
<tr>
<td>Males</td>
<td>&gt; 14 years</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Females</td>
<td>14 – 18 years</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Females</td>
<td>19 years</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>1.4 mg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>1.4 mg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
AI Adequate Intake
If not otherwise specified, this table presents Recommended Dietary Allowances (RDAs). Allowable levels of nutrients vary depending on national regulations and the final application.

Supplements and food fortification

Thiamine is mostly formulated in combination with other B-vitamins (B-complex) or included in multivitamin supplements. Fortification of white flour, cereals, pasta, beverages and rice began in the United States during the second World War (1939 – 1945), with other countries quickly following suit. Fortification of staple foods has virtually eradicated the B-vitamin deficiency diseases in developed nations.

Industrial production

Chemical synthesis of thiamine is a complicated process, involving some 15 – 17 different steps. Although commercial production of thiamine was first accomplished in 1937, the production did not develop on a broad scale until the 1950s, when demand rose sharply because of food fortification.
History

7th c. First classical description of beriberi in a ‘General Treatise on the Etiology and Symptoms of Diseases’ (author: Ch’ao-Yuan-fang Wu Ching).

1882 Takaki, surgeon general, dramatically decreases the incidence of beriberi in the Japanese navy by improving sailors’ diets.

1897 Dutch medical officers Eijkman and Grijns show that the symptoms of beriberi can be reproduced in chickens fed on polished rice, and that these symptoms can be prevented or cured by feeding them rice bran.

1912 Funk isolates the antiberiberi factor from rice bran extracts and calls it a ‘vitamine’ – an amine essential for life. The name finds ready acceptance and helps to focus attention on the new concept of deficiency diseases.

1915 McCollum and Davis propose water-soluble thiamine as antiberiberi factor.

1926 Jansen and Donath isolate antiberiberi factor from rice bran.

1927 The British Medical Research Council proposes thiamine as anti-beriberi factor.

1927 The British Medical Research Council proposes thiamine as anti-beriberi factor.

1936 Williams, who first began experimenting with thiamine and beriberi in Manila around 1910, identifies and publishes the chemical formula and names it thiamine.

1937 The first commercial production of thiamine is accomplished.

1943 Williams and coworkers, and Foltz and colleagues carry out dietary studies that document widespread thiamine deficiency in the United States.

1943 Standards of identity for enriched flour are created by the U.S. Food and Nutrition Board, requiring that thiamine, niacin, riboflavin and iron be added to white flour.
Riboflavin

Introduction
Riboflavin is one of the most widely distributed water-soluble vitamins. The above synonyms, lactoflavin and ovoeflavin, as well as the terms hepatoflavin, verdoflavin and uroflavin, indicate the source from which the vitamin was originally isolated, i.e. milk, eggs, liver, plants and urine. The term ‘flavin’ originates from the Latin word ‘flavus’ referring to the yellow colour of this vitamin. Riboflavin is also part of the vitamin B complex. Riboflavin is in the form of free riboflavin or as its coenzymatic forms flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). In the body the majority of riboflavin is in the form of free riboflavin and FAD, with only a small amount in the FMN form.

Functions
Flavin coenzymes are essential for energy production via the respiratory chain, as they act as catalysts in the transfer of electrons in numerous reduction-oxidation reactions (redox reactions). Flavin coenzymes participate in many metabolic reactions of carbohydrates, fats and proteins. Riboflavin coenzymes are also essential for the conversion of pyridoxine (vitamin B6) and folic acid into their coenzyme forms and for the transformation of tryptophan to niacin. Riboflavin also promotes normal growth and assists in the synthesis of steroids, red blood cells, and glycogen. Furthermore, it helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system, and is involved in the production of adrenaline by the adrenal glands. Riboflavin is also important for the antioxidant status within cell systems, both by itself and as part of the glutathione reductase and xanthine oxidase system. This defence system may also help defend against bacterial infections and tumour cells.

Dietary sources
Riboflavin is present as an essential constituent of all living cells, and is therefore widely distributed. However, there are very few rich sources in food. Yeast and offal have the highest concentrations, but they do not have much relevance in today’s human nutrition. The most important and common dietary sources are milk and milk products, lean meat, eggs and green leafy vegetables. Cereal grains, although poor sources of riboflavin, are important for those who rely on cereals as their main dietary component. Fortified cereals and bakery products supply large amounts. Animal sources of riboflavin are more readily absorbed than vegetable sources. In milk from cows, sheep and goats, at least 90% of the riboflavin is in the free form; in most other sources, it occurs bound to proteins.

Riboflavin content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>mg/100g</th>
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<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>3.7</td>
</tr>
<tr>
<td>Pork liver</td>
<td>3.2</td>
</tr>
<tr>
<td>Chicken breast</td>
<td>0.9</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>0.7</td>
</tr>
<tr>
<td>Camembert/Parmesan</td>
<td>0.6</td>
</tr>
<tr>
<td>White mushrooms</td>
<td>0.4</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.3</td>
</tr>
<tr>
<td>Spinach</td>
<td>0.23</td>
</tr>
<tr>
<td>Milk/Yoghurt</td>
<td>0.18</td>
</tr>
</tbody>
</table>

(Souci, Fachmann, Kraut)

Main functions in a nutshell:
- Reduction-oxidation reactions
- Energy production
- Antioxidant functions
- Conversion of pyridoxine (vitamin B6) and folic acid into their active coenzyme forms
- Growth and reproduction
- Growth of skin, hair, and nails
Absorption and body stores

Most dietary riboflavin is bound to a food protein such as FMN and FAD. These are released in the stomach by acidification and absorbed in the upper part of the small intestine by an active, rapid, saturable transport mechanism. The rate of absorption is proportional to intake and increases when riboflavin is ingested along with other foods. Approximately 15% is absorbed if taken alone versus 60% absorption when taken with food. Passive diffusion plays only a minor role at the physiological doses ingested in the diet. In the mucosal cells of the intestine, riboflavin is again converted to the coenzyme form (FMN). In the portal system it is bound to plasma albumin or to other proteins, mainly immunoglobulins, and transported to the liver, where it is converted to the other coenzyme form, FAD, and bound to specific proteins as flavoproteins.

Riboflavin, mainly as FAD, is distributed in all tissues, but concentrations are low and little is stored. The liver and retinal tissues are the main storage places, albeit riboflavin is not stored to any significant extent in the body. Riboflavin is excreted mainly in the urine where it contributes to the yellow colour. Small amounts are also excreted in sweat and bile. During lactation, about 10% of absorbed riboflavin passes into the milk.

Measurement

Body status can be determined by direct and indirect methods. Direct methods include the determination of FAD and FMN in whole blood by HPLC (High Performance Liquid Chromatography). Usually, whole blood concentrations (FAD) of 175 – 475 nmol/L are measured. Another possibility for riboflavin status assessment is the monitoring of urinary excretion. Values < 27 µg/g creatinine point to deficiency, 27 – 79 µg/g creatinine are considered marginal, and values > 80 µg/g creatinine are considered normal. Urinary excretion rises sharply after tissue saturation is reached. Indirect methods include determining the activity of the FAD-dependent enzyme erythrocyte glutathione reductase (EGR). This biochemical method gives a valid indication of riboflavin status. During riboflavin deficiency EGR is no longer saturated with FAD, so enzyme activity increases when FAD is added in vitro. The difference in activity in erythrocytes with and without added FAD is called the activity coefficient (EGRAC). An EGRAC > 1.30 is indicative of biochemical riboflavin deficiency.

Stability

Riboflavin is degraded by light and up to 50% may be lost if foods are left out in sunlight or any UV light. Because of this light sensitivity, riboflavin will rapidly disappear from milk kept in glass bottles exposed to the sun or bright daylight (85% within 2 hours). Riboflavin is stable when heated and so is not easily destroyed in the ordinary processes of cooking, but it will leach into cooking water. The pasteurisation process causes milk to lose about 20% of its riboflavin content. Alkalis such as baking soda also destroy riboflavin. Sterilisation of foods by irradiation or treatment with ethylene oxide may also cause destruction of riboflavin.

Interactions

Positive interactions

• Thryoxine and triiodothyronine stimulate the FMN and FAD in mammalian systems
• Anticholinergic drugs increase the absorption of riboflavin by allowing it to stay longer at absorption sites

Negative interactions

Impact on metabolism, absorption, utilization and storage of riboflavin e.g. by:
• Ouabain (treatment of congestive heart failure)
• Theophylline (muscle relaxant, diuretic, central nervous stimulant)
• Penicillin (displaces riboflavin from its binding protein, thus inhibiting transport to the central nervous system)
• Chlorpromazin (anti-psychotic drug), barbiturates and possibly tricyclic antidepressants prevent the incorporation of riboflavin into FAD
• Riboflavin impairs the antibiotic activity of streptomycin, erythromycin, tyrothricin, carbomycin and tetracyclines
• Caffeine, zinc, copper and iron may chelate with riboflavin and affect its absorption
Riboflavin

**Deficiency**

Overt clinical symptoms of riboflavin deficiency are rarely seen in developed countries. However, the sub-clinical stage of deficiency, characterised by a change in biochemical indices, is common. Riboflavin deficiency rarely occurs in isolation but usually in combination with deficiencies of other B-complex vitamins, because flavoproteins are also involved in the metabolism of other B-complex vitamins. The absorption of iron, zinc and calcium is impaired in riboflavin deficiency.

Clinically, riboflavin-deficiency affects many organs and tissues. Most prominent are the effects on the skin, mucosa and eyes:

- Glossitis (magenta tongue, geographical tongue)
- Cheilosis, angular stomatitis (fissures at the corners of the mouth)
- Sore throat
- Burning of the lips, mouth, and tongue
- Inflamed mucous membranes
- Pruritus (itching)
- Seborrheic dermatitis (moist scaly skin inflammation)
- Corneal vascularisation associated with sensitivity to bright light, impaired vision, itching and a feeling of grittiness in the eyes

In severe long-term deficiency, damage to nerve tissue can cause depression and hysteria. Other symptoms are normocytic and normochromic anaemia, and peripheral neuropathy of the extremities (tingling, coldness and pain). Low intracellular levels of flavin coenzymes could effect mitochondrial function, oxidative stress and blood vessel dilation, which have been associated with pre-eclampsia during pregnancy.

**Groups at risk of deficiency**

- Individuals with inadequate food intake e.g. elderly, vegans, chronic dieters
- Pregnant and lactating women (additional demands)
- Infants and school children
- Adolescents, particularly girls
- Chronic alcoholics
- People with chronic disorders (e.g. tuberculosis, diabetes) and intestinal malabsorption (e.g. morbus crohn, lactose intolerance) and trauma, including burns and surgery
- Medication users (oral-contraceptives, antibiotics, tranquilisers)
- Athletes
- Newborns after phototherapy for newborn hyperbilirubinemia
**Disease prevention and therapeutic use**

**Eye-related diseases**
Oxidative damage of lens proteins by light may lead to the development of age-related cataracts. Riboflavin deficiency leads to decreased glutathione reductase activity, which can result in cataracts. Therefore, riboflavin is used in combination with other antioxidants, like vitamin C and carotenoids, in disease prevention for age-related cataracts. Riboflavin has been used to treat corneal ulcers, photophobia and noninfective conjunctivitis in patients without any typical signs of deficiency, with beneficial results. Most cases of riboflavin deficiency respond to daily oral doses of 5 – 10 mg.

**Migraines**
People suffering from migraine headaches have a modified mitochondrial oxygen metabolism. Because riboflavin plays an important role in energy production, supplemental riboflavin has been investigated as a treatment for migraine. The effect of riboflavin supplementation at 400 mg/day for 3 months was a decrease in gravity and frequency of migraine attacks.

**Prevention of deficiencies in high-risk patients**
Patients suffering from achlorhydria, vomiting, diarrhoea, hepatic disease, or other disorders preventing absorption or utilisation, should be treated parenterally. Deficiency symptoms begin to improve in 1 – 3 days, but complete resolution may take weeks.

**Elevated blood pressure**
A placebo controlled double-blind randomized controlled trial in CVD patients recently reported that riboflavin intervention at the dietary level of 1.6mg/d resulted in a reduction of systolic blood pressure by 13mmHg and diastolic blood pressure by almost 8 mmHg, specifically in those individuals with the MTHFR 677 TT genotype. The frequency of the TT genotype is 10% worldwide, ranging from 4% to 18% in the United States, 20% in Northern China, and to as high as 32% in Mexico.

**Recommended Dietary Allowance (RDA)**
Dietary recommendations for riboflavin exist in many countries, where mean values for adult males vary between 1.2 and 2.2 mg daily. The recommendations of the Food and Nutrition Board of the U.S. National Research Council are based on feeding studies conducted in the 1940s, which showed that a riboflavin intake of 0.55 mg or less per day results in clinical signs of deficiency after about 90 days. These data have led to the assumption that an intake of 0.6 mg per 1000 kcal should supply the needs for essentially all healthy people.

**Safety**
Riboflavin is nontoxic. No cases of toxicity from ingestion of riboflavin have been reported. No toxic or adverse reactions to riboflavin in humans have been identified. A harmless yellow coloration of urine occurs at high doses. The limited capacity of the gastrointestinal tract to absorb this vitamin makes any significant risk unlikely, and because riboflavin is water-soluble, excess amounts are simply excreted.

**Supplements and food fortification**
Riboflavin is available as oral preparations, alone or most commonly in multivitamin and vitamin B-complex preparations, and as an injectable solution. Crystalline riboflavin (E101) is poorly soluble in water, so riboflavin-5’-phosphate (E 106), a more expensive but more soluble form of riboflavin, has been developed for use in liquid formulations. Riboflavin is one of the vitamins often added to flour and bakery products and beverages to compensate for losses due to processing. It is also used to enrich milk, breakfast cereals and dietetic products. Because of its bright yellow colour, riboflavin is sometimes added to other drugs or infusion solutions as a marker.

**Industrial production**
Riboflavin can be produced by chemical synthesis or by fermentation processes. Chemical processes are usually refinements of the procedures developed by Kuhn and by Karrer in 1934 using oxylene, D-ribose and alloxan as starting materials. Various bacteria and fungi are commercially employed to synthesise riboflavin, using cheap natural materials and industrial wastes as a growth medium.

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**Recommended daily intakes**

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<thead>
<tr>
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<th>Life stage</th>
<th>Dose/day</th>
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<tbody>
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<td>&lt; 6 months</td>
<td>0.3 mg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>0.4 mg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>0.9 mg</td>
</tr>
<tr>
<td>Males</td>
<td>14 years</td>
<td>1.3 mg</td>
</tr>
<tr>
<td>Females</td>
<td>14 – 18 years</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Females</td>
<td>19 years</td>
<td>1.1 mg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>1.4 mg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>1.6 mg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
AI Adequate Intake

(If not otherwise specified, this table presents Recommended Dietary Allowances (RDAs). Allowable levels of nutrients vary depending on national regulations and the final application.)
**History**

- **1879** Blyth isolates lactochrome – a water-soluble, yellow fluorescent material – from whey.
- **1932** Warburg and Christian extract a yellow enzyme from brewer’s yeast and suggest that it plays an important part in cell respiration.
- **1933** Kuhn and coworkers obtain a crystalline yellow pigment with growth-promoting properties from egg white and whey, which they identify as vitamin B2.
- **1934** Kuhn and associates in Heidelberg, and Karrer and colleagues in Zurich synthesise pure riboflavin.
- **1937** The Council on Pharmacy and Chemistry of the American Medical Association names the vitamin ‘riboflavin’.
- **1937** Theorell determines the structure of flavin mononucleotide, FMN.
- **1938** Warburg and Christian isolate and characterise flavin adenine dinucleotide (FAD) and demonstrate its involvement as a coenzyme.
- **1941** Sebrell and coworkers demonstrate clinical signs of riboflavin deficiency in human feeding experiments.
- **1968** Glatzle and associates propose the use of the erythrocyte glutathione reductase test as a measurement of riboflavin status.
Vitamin B6

Introduction
Pyridoxine is a water-soluble vitamin. Man and other primates depend on external sources to cover their vitamin B6 requirements. Vitamin B6 was discovered in the 1930s almost as a by-product of the studies on pellagra, a deficiency disease caused by the absence of the vitamin niacin. Negligible amounts of vitamin B6 can be synthesised by intestinal bacteria. There are three different natural forms (vitamers) of vitamin B6, namely pyridoxine, pyridoxamine, and pyridoxal, all of which are normally present in foods. The 3 forms of vitamin B6 are also present as phosphorylated derivatives. For human metabolism the active derivative of the vitamin, pyridoxal 5-phosphate (PLP), is of major importance as the metabolically active coenzyme form.

Functions
PLP serves as a coenzyme of more than 60 enzymes that catalyse essential chemical reactions in the human body. It plays an important role in protein, carbohydrate and lipid metabolism. It is involved in the production of serotonin from the amino acid tryptophan in the brain and other neurotransmitters, and so it has a role in the regulation of mental processes and mood. Furthermore, it is involved in the conversion of tryptophan to the vitamin niacin, the formation of haemoglobin and the growth of red blood cells, the production of prostaglandines and hydrochloric acid in the gastrointestinal tract, the sodium-potassium balance, and in histamine metabolism. As part of the vitamin B-complex it may also be involved in the downregulation of the homocysteine blood level. Vitamin B6 also plays a role in the improvement of the immune system.

Dietary sources
Vitamin B6 is widely distributed in foods, mainly in bound forms. Pyridoxine is found especially in plants, whereas pyridoxal and pyridoxamine are principally found in animal tissue, mainly in the form of PLP.

Rich food sources are chicken and the liver of beef, pork and veal, fish (salmon, tuna, sardines, halibut, herring), nuts (walnuts, peanuts), brewer’s yeast, and wheat germ. Generally, vegetables and fruits are rather poor sources of vitamin B6, although there are products in these food classes which contain considerable amounts of pyridoxine, such as lentils, courgettes, avocado and bananas.

Main functions in a nutshell:

- Neurotransmitter synthesis
- Red blood cell formation
- Niacin formation
- Degradation of homocysteine to cysteine
- Inhibition of steroid hormone signalling
- Support of immune defence

Vitamin B6 content of foods

<table>
<thead>
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<th>Food</th>
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</tr>
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<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>4.4</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.98</td>
</tr>
<tr>
<td>Walnuts</td>
<td>0.87</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>0.72</td>
</tr>
<tr>
<td>Pork liver</td>
<td>0.59</td>
</tr>
<tr>
<td>Lentils</td>
<td>0.57</td>
</tr>
<tr>
<td>Avocado</td>
<td>0.53</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.5</td>
</tr>
<tr>
<td>Courgettes</td>
<td>0.46</td>
</tr>
<tr>
<td>Bananas</td>
<td>0.36</td>
</tr>
</tbody>
</table>

(Souci, Fachmann, Kraut)
Absorption and body stores

All three forms of vitamin B6 (pyridoxine, pyridoxal and pyridoxamine) are readily absorbed in the small intestine by an energy dependent process. All three are converted to pyridoxal phosphate in the liver, a process which requires zinc and riboflavin. The bioavailability of plant-based vitamin B6 varies considerably, ranging from 0% to 80%. Some plants contain pyridoxine glycosides that cannot be hydrolysed by intestinal enzymes. Although these glycosides may be absorbed, they do not contribute to vitamin activity. The storage capacity of water-soluble vitamins is generally low compared to that of fat-soluble ones. Small quantities of pyridoxine are widely distributed in body tissue, mainly as PLP in the liver and muscle. PLP is tightly bound to the proteins albumin and haemoglobin in plasma and red blood cells. Because the half-life of pyridoxine is about 25 days and it is not significantly bound to plasma proteins, the limited stores may be depleted within two to six weeks on a pyridoxin-free diet, a daily supply is required. Excess pyridoxine is primarily excreted in the urine as 4-pyridoxic acid (4-PA) and to a limited extent in faeces.

Measurement

There are several direct and indirect methods that can be used for assessing a person’s vitamin B6 status. Direct methods include determination of PLP in plasma, and determination of urinary excretion of 4-pyridoxic acid (4-PA). The method of choice for quantification of both compounds is high performance liquid chromatography. Whole blood concentrations usually are 35 – 110 nmol/L PLP. Concentrations of PLP have been found to correlate well with the vitamin B6 deficiency determined by indirect methods. Indirect methods measure the stimulated activity of pyridoxine dependent enzymes in erythrocytes by addition of PLP. This mainly determines the erythrocyte alanine aminotransferase activation coefficient (EAST-AC) or the erythrocyte aspartate aminotransferase activation coefficient. The coefficient of activity with stimulation to activity without stimulation indicates the vitamin B6 status. For EAST-AC, values > 1.8 are considered to show deficiency, 1.7 – 1.8 to be marginal, and < 1.7 to be adequate. For large-scale population surveys there is another method of assessing a vitamin B6 deficiency state: the tryptophan load test. Vitamin B6 participates in the conversion of tryptophan to the vitamin niacin. A vitamin B6 deficiency blocks this process, producing more xanthurenic acid. If the administration of tryptophan leads to an increased excretion of xanthurenic acid, a vitamin B6 deficiency can be diagnosed.

Stability

Pyridoxine is relatively stable to heat, but pyridoxal and pyridoxamine are not. Pasteurisation therefore causes milk to lose up to 20% of its vitamin B6 content. Vitamin B6 is decomposed by oxidation and ultraviolet light, and by an alkaline environment. Because of this light sensitivity, vitamin B6 will disappear (50% within a few hours) from milk kept in glass bottles exposed to the sun or bright daylight. Alkalis, such as baking soda, also destroy pyridoxine. Freezing of vegetables causes a reduction of up to 25%, while milling of cereals leads to wastes as high as 90%. Cooking losses of processed foods may range from a few percent to nearly half the vitamin B6 originally present. Cooking and storage losses are greater with animal products.
Interactions

Positive interactions
Certain vitamins of the B-complex (niacin, riboflavin, biotin) may act synergistically with vitamin B6 derivatives.

Vitamin B6 additionally requires zinc and magnesium to fulfill its physiological functions.

Negative interactions
There are more than 40 drugs that interfere with vitamin B6 metabolism, potentially causing low status e.g.
- Phenytoin (an antiepileptic drug)
- Theophylline (a drug for respiratory diseases)
- Phenobarbitone (a barbiturate mainly used for its antiepileptic properties)
- Desoxypyridoxine (a tuberculostatic drug)
- Hydralazine (an antihypertensive)
- Cycloserine (an antibiotic)
- Vitamin B6 reduces the therapeutic effect of levodopa by accelerating its metabolism
- Levodopa also reduces vitamin B6 status as the drug forms a Schiff base complex with PLP

Deficiency

A deficiency of vitamin B6 alone is uncommon, because it usually occurs in combination with a deficit in other B-complex vitamins, especially with riboflavin deficiency, because riboflavin is needed for the formation of the coenzyme PLP.

A dietary deficiency state showing definable clinical deficiency symptoms is rare, although recent diet surveys revealed that a significant part of the following population groups have B6 intakes below the RDA.

Groups at risk for deficiency
- The elderly (higher dietary requirements, increased B6 catabolism and decreased protein binding capacity with advancing age)
- Pregnant and lactating women (additional demands)
- Women in general, especially those taking oral contraceptives
- Patients on drugs interacting with B-vitamin metabolism
- Underweight people or people who eat poorly, (e.g. people with eating disorders)
- Chronic alcoholics (heavy drinking may severely impair the ability of the liver to synthesize PLP)

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<td>Females</td>
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</tr>
<tr>
<td>Males</td>
<td>&gt; 51 years</td>
<td>1.7 mg</td>
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<tr>
<td>Females</td>
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<td>1.7 mg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>1.9 mg</td>
</tr>
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<td>Lactation</td>
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**Disease prevention and therapeutic use**

**Sideroblastic anemias and pyridoxine-dependent abnormalities of metabolism**

Pyridoxine is an approved treatment for sideroblastic anemias and pyridoxine-dependent abnormalities of metabolism. In such cases, therapeutic doses of approximately 40-200 mg vitamin B6 per day are indicated. Vitamin B6 deficiency is also associated with hypochromic microcytic anaemia.

**PMS (premenstrual syndrome)**

Some studies suggest that vitamin B6 doses of up to 100 mg/day may be of value for relieving the symptoms of premenstrual syndrome. However, final conclusions are still limited and more research is needed.

**Hyperemesis gravidarum**

Pyridoxine is often administered in doses of up to 40 mg/day in the treatment of nausea and vomiting during pregnancy (hyperemesis gravidarum). However, as ‘morning sickness’ improves even without treatment it is difficult to prove the therapeutic benefit.

**Depression**

Pyridoxine is also used to assist in the relief of depression (especially in women taking oral contraceptives). However, clinical trials have not yet provided evidence for its efficacy.

**Carpal tunnel syndrome**

Pyridoxine has also been claimed to alleviate the symptoms of carpal tunnel syndrome. Some studies report benefits while others do not.

**Hyperhomocystinaemia / cardiovascular disease**

Elevated homocysteine levels in the blood are considered a risk factor for atherosclerotic disease. Several studies have shown that vitamin B6, vitamin B12 and folic acid can lower critical homocysteine levels. However, randomised controlled trials have shown that homocysteine lowering through supplementation with B-vitamins, including vitamin B6, was not effective for secondary prevention of cardiovascular disease.

**Immune function**

The elderly are a group that often suffers from impaired immune function. Adequate B6 intake is thus important, and it has been shown that the amount of vitamin B6 required to improve the immune system is higher (2.4 mg/day for men; 1.9 mg/day for women) than the current RDA.

**Asthma**

Asthma patients taking vitamin B6 supplements may have fewer and less severe attacks of wheezing, coughing and breathing difficulties.

**Diabetes**

Research has also suggested that certain patients with diabetes mellitus or gestational diabetes experience an improvement in glucose tolerance when given vitamin B6 supplements.

**Kidney stones**

Glyoxylate can be oxidised to oxalic acid that may lead to calcium oxalate kidney stones. Pyridoxal phosphate is a cofactor for the degradation of glyoxylate to glycine. There is some evidence that high doses of vitamin B6 (> 150 mg/day) may be useful for normalising the oxalic acid metabolism to reduce the formation of kidney stones. However, the relationship between B6 and kidney stones must be studied further before any definite conclusions can be drawn.

**Glutamate sensivity**

People who are sensitive to glutamate, which is often used for the preparation of Asian dishes, can react with headache, tachycardia (accelerated heart rate), and nausea. 50 to 100 mg of pyridoxine can be of therapeutic value.

**Autism**

High dose therapy with pyridoxine improves the status of autistics in about 30% of cases.

**Recommended Dietary Allowance (RDA)**

The recommended daily intake of vitamin B6 varies according to age, sex, risk group (see ‘Groups at risk’) and criteria applied. The vitamin B6 requirement is increased when high-protein diets are consumed, since protein metabolism can only function properly with the assistance of vitamin B6 derivatives. Pregnant and lactating women need an additional 0.7 mg to compensate for increased demands made by the foetus or baby.

**Safety**

Vitamin B6 in all its forms is well tolerated, but large excesses are toxic. Daily oral doses of pyridoxine of up to 50 times the RDA (ca. 100 mg) for periods of 3 – 4 years have been administered without adverse effects. Daily doses of 500 mg and more may cause sensory neuropathy after several years of ingestion, whereas the intake of amounts in excess of 1 gram daily may lead to reversible sensory neuropathy within a few months. Sensory neuropathy has been selected as a critical end-point on which to base a tolerable upper intake level (UL) of 100 mg/day (IOM) for adults, although supplements somewhat higher than this may be safe for most individuals. Fortunately these side-effects are largely reversible upon cessation of vitamin B6 intake. EFSA (2006) set a Tolerable Upper Intake Level of 25mg/day. Today, prolonged intake of doses exceeding 500 mg a day is considered to carry the risk of adverse side-effects.

**Supplements and food fortification**

The most commonly available form of vitamin B6 is pyridoxine hydrochloride, which is used in food fortification, nutritional supplements and therapeutic products such as capsules, tablets and ampoules. Vitamins, mostly of the B-complex, are widely used in the enrichment of cereals. Dietetic foods such as infant formulas and slimming diets are often fortified with vitamins, including pyridoxine.
History

1926  Goldberger and coworkers feed rats a diet deficient in what is considered to be the pellagra-preventive factor; these animals develop skin lesions.

1934  György first identifies the factor as vitamin B6 or adermin, a substance capable of curing a characteristic skin disease in rats (dermatitis acrodynia). The factor is then called the rat anti-acro-dynia factor, deficiency of which causes so-called 'rat-pellagra'.

1935  Birch and György succeed in differentiating riboflavin and vitamin B6 from the specific pellagra preventive factor (P-P) of Goldberger and his associates.

1938  Lepkovsky is the first to report the isolation of pure crystalline vitamin B6. Independently, but slightly later, several other groups of researchers also report the isolation of crystallised vitamin B6 from rice polishings (Keresztesy and Stevens; György; Kuhn and Wendt; Ichiba and Michi).

1939  Harris and Farkas determine the structure of pyridoxine and succeed in synthesising the vitamin. György proposes the name pyridoxine.

1945  Snell demonstrates that two other natural forms of the vitamin exist, namely pyridoxal and pyridoxamine.

1957  Snyderman determines the levels of vitamin B6 required by humans.
Vitamin B12

Introduction

Vitamin B12 is the largest and most complex of all the vitamins. The name vitamin B12 is generic for a specific group of cobalt-containing corrinoids with biological activity in humans. Interestingly, it is the only known metabolite to contain cobalt, which gives this water-soluble vitamin its red colour. This group of corrinoids is also known as cobalamins. The main cobalamins in humans and animals are hydroxycobalamin, adenosylcobalamin and methylcobalamin, the last two being the biological active forms. Cyanocobalamin is a form of vitamin B12 that is widely used in fortified foods and supplements owing to its availability and stability. It is transformed into biologically active forms in the body. In 1934, three researchers won the Nobel prize in medicine for discovering the lifesaving properties of vitamin B12. They found that eating large amounts of raw liver, which contains high amounts of vitamin B12, could save the life of previously incurable patients with pernicious anaemia. This finding saves 10,000 lives a year in the U.S. alone. Vitamin B12 was isolated from liver extract in 1948 and its structure was elucidated 7 years later.

Functions

Vitamin B12 is necessary for the formation of blood cells, nerve sheaths and various proteins. It is therefore, essential for the prevention of pernicious anaemia and neurological disturbances. It is also involved in fat and carbohydrate metabolism and is essential for growth. In humans, vitamin B12 functions primarily as a cofactor in intermediary metabolism. Two enzymes are dependent on vitamin B12:

1) Methionine synthase which converts homocysteine to methionine

2) Methylmalonyl CoA mutase which converts methylmalonyl CoA to succinyl CoA

In its methylcobalamin form vitamin B12 is the direct cofactor for methionine synthase, the enzyme that recycles homocysteine back to methionine. In addition, methionine synthase and vitamin B12 are involved in the production of the active forms of folate and low vitamin B12 may disrupt folate metabolism. Methylmalonyl CoA mutase converts 1-methylmalonyl CoA to succinyl CoA (an important reaction in lipid and carbohydrate metabolism). Adenosylcobalamin is also the coenzyme in ribonucleotide reduction (which provides building blocks for DNA synthesis).

Main functions in a nutshell:

• Coenzyme-function in intermediary metabolism, especially in cells of the nervous tissue, bone marrow and gastrointestinal tract

• Essential growth factor

• Formation of blood cells and myelin sheaths

• Regeneration of folate

• Involved in the production of melatonin (controls the release of many hormones in the body and is involved with the sleep/wake cycle)
Vitamin B12 is produced exclusively by microbial synthesis in the digestive tract of animals. Therefore, animal protein products are the source of vitamin B12 in the human diet, in particular organ meats (liver, kidney). Other good sources are fish, eggs and dairy products. In foods, hydroxo- , methyl- and 5'-deoxyadenosyl-cobalamins are the main cobalamins present. Bacteria in the intestine synthesize vitamin B12, but under normal circumstances not in areas where absorption occurs. Some foods are also fortified with vitamin B12.

### Vitamin B12 content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef liver</td>
<td>65</td>
</tr>
<tr>
<td>Crab</td>
<td>27</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>8</td>
</tr>
<tr>
<td>Steak</td>
<td>5</td>
</tr>
<tr>
<td>Coalfish</td>
<td>3.5</td>
</tr>
<tr>
<td>Cheese (Camembert)</td>
<td>3</td>
</tr>
<tr>
<td>Eggs</td>
<td>1 – 3</td>
</tr>
</tbody>
</table>

Source: Fachmann, Kraut

Absorption and body stores

Vitamin B12 from food sources is bound to proteins and is only released by an adequate concentration of hydrochloric acid in the stomach. Free vitamin B12 is then immediately bound to glycoproteins originating from the stomach and salivary glands. This glycoprotein complex protects vitamin B12 from chemical denaturation. Gastrointestinal absorption of vitamin B12 occurs in the small intestine by an active process requiring the presence of intrinsic factor, another glycoprotein, which the gastric parietal cells secrete after being stimulated by food. The absorption of physiological doses of vitamin B12 is limited to approximately 2μg/dose. The vitamin B12 intrinsic factor complex is then absorbed through phagocytosis by specific ileal receptors. Once absorbed, the vitamin is transferred to a plasma-transport protein which delivers the vitamin to target cells. A lack of intrinsic factor prevents vitamin B12 absorption. If this is untreated, potentially irreversible neurological damage and life-threatening anaemia develops (see Deficiency).

### Measurement

Measurement of vitamin B12 in plasma is routinely used to determine deficiency, but may not be a reliable indication in all cases. In pregnancy, for example, tissue levels are normal but serum levels are low. Vitamin B12 can be measured by chemical, microbiological or immunoassay isotope dilution methods. Microbiological assays, which are widely used for blood and tissue samples, are sensitive but non-specific.

Serum cobalamin concentration is often determined by automated immunoassays using intrinsic factor as a binding agent. These assays have mainly replaced microbiological methods.

Data in the literature about vitamin B12 concentration in serum varies. However, values < 110 – 150 pmol/L are considered to reflect deficiency, whereas values > 150 – 200 pmol/L represent an adequate status. Major vitamin B12-dependent metabolic processes include the formation of methionine from homocysteine, and the formation of succinyl coenzyme A from methylmalonyl coenzyme A. Thus, apart from directly determining vitamin B12 concentration in the blood, elevated concentrations of both methylmalonic acid (MMA) and homocysteine may indicate a vitamin B12 deficiency. Vitamin B12 concentrations can also be measured using the novel biomarker holoTC. HoloTC is a direct measure of the active fraction of the vitamin and is proposed to be a more sensitive and reliable indicator of status.

### Stability

Vitamin B12 is stable to heat, but slowly loses its activity when exposed to light, oxygen and acid or alkali-containing environments. Loss of activity during cooking is due to the water solubility of vitamin B12 (loss through meat juices or leaching into water) rather than to its destruction.
Vitamin B12

Interactions

Negative interactions
Absorption of cobalamins is impaired by alcohol and vitamin B6 deficiency. Furthermore, a number of drugs reduce the absorption of vitamin B12, and supplementation with the affected nutrient may be necessary:

- Antibiotics (e.g. chloramphenicol)
- Anti-diabetics (e.g. metformin and phenformin)
- Anti-epileptic drugs
- Anti-gout medication (Colchicine)
- Stomach medication (H2 receptor antagonists, Proton pump inhibitors)
- Nitrous oxide (anesthetic)
- Oral contraceptives
- Tuberculostatics (Para-aminosalicylic acid)

Groups at risk for deficiency
- Vegetarians
- Elderly
- Alcoholics
- People with
  - pernicious anaemia (autoimmune disease, chiefly affects persons post middle age)
  - food-bound vitamin B12 malabsorption (in patients on long term treatment with certain drugs, elderly patients with gastric atrophy, patients with atrophic gastritis)
  - after gastrectomy
  - after ingestion of corrosive agents with destruction of gastric mucosa
  - lesions of the small bowel; bacterial overgrowth; patients with small intestinal defects; inborn errors of cobalamin metabolism etc.
  - pancreatic insufficiency
  - AIDS

Recommended daily intakes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt; 6 months</td>
<td>0.4 µg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>0.5 µg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>0.9 µg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>1.2 µg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>1.8 µg</td>
</tr>
<tr>
<td>Adults</td>
<td>&gt; 14 years</td>
<td>2.4 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>2.6 µg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>2.8 µg</td>
</tr>
</tbody>
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* Institute of Medicine (2001)
AI Adequate Intake
If not otherwise specified, this table presents Recommended Dietary Allowances (RDAs). Allowable levels of nutrients vary depending on national regulations and the final application.

Deficiency

Vitamin B12 deficiency affects 10 – 15% of individuals over the age of 60.

Deficiency of vitamin B12 leads to defective DNA synthesis in cells, which affects the growth and repair of all cells. Tissues most affected are those with the greatest rate of cell turnover, e.g. those of the haematopoietic system. This can lead to megaloblastic anaemia (characterised by large and immature red blood cells) and neuropathy, with numerous symptoms including: glossitis, weakness, loss of appetite, loss of taste and smell, impotence, irritability, memory impairment, mild depression, hallucination, breathlessness (dyspnea) on exertion, tingling and numbness (paraesthesia). Vitamin B12 deficiency can also lead to hyperhomocysteinaemia, a possible risk factor for occlusive vascular disease. Low vitamin B12 has been associated with a variety of chronic diseases of ageing such as dementia and cognitive impairment, cardiovascular disease and osteoporosis.

The symptoms of vitamin B12 deficiency are similar to those of folic acid deficiency, the major difference being only that vitamin B12 deficiency is associated with spinal cord degeneration. If folic acid is used to treat vitamin B12 deficiency, anaemia may be alleviated but the risk of damage to the nervous system remains. Nervous dysfunction associated with vitamin B12 can be irreversible and potentially life threatening if left untreated. It is therefore essential to diagnose the deficiency accurately before starting therapy.

Cause of deficiency is usually as a result of vitamin B12 malabsorption, or lack of intrinsic factor. Without intrinsic factor, absorption is not possible and a severe and persistent deficiency develops that cannot be prevented by the usual dietary intakes of vitamin B12.

Recommended daily intakes*

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Pernicious anaemia:
Pernicious anaemia is the classical symptom of B12 deficiency, but it is actually the end-stage of an autoimmune inflammation of the stomach, resulting in destruction of stomach cells by the body’s own antibodies. Anaemia is a condition in which red blood cells do not provide adequate oxygen to body tissues. Pernicious anaemia is a type of megaloblastic anaemia.

Gastric atrophy:
Gastric atrophy is a chronic inflammation of the stomach resulting in decreased stomach acid production. Because this is necessary for the release of vitamin B12 from the proteins in food, vitamin B12 absorption is reduced.
Disease prevention and therapeutic use

**Pernicious anaemia**
Pernicious anaemia patients are traditionally treated with intramuscular injections; however they can be effectively treated with large oral doses of the vitamin but require lifetime vitamin B12 therapy. When used alone, oral doses of at least 150 µg/day are necessary, although single weekly oral doses of 1000 µg have proved satisfactory in some cases. Combinations of vitamin B12 and intrinsic factor may be given, but as a variable number of patients become refractory to intrinsic factor after prolonged treatment, parenteral therapy with cyanocobalamin or hydroxocobalamin is preferred.

**Hyperhomocysteinaemia**
Homocysteine appears to be a nerve and vessel toxin, promoting mortality and cardiovascular disease (CVD) as well as stroke, Alzheimer’s disease, birth defects, recurrent pregnancy loss, and eye disorders. Keeping homocysteine at levels associated with lower rates of disease requires adequate B12, folic acid and B6 intake.

**Cancer**
Vitamin B12 deficiency may lead to an elevated rate of DNA damage and altered methylation of DNA. These are obvious risk factors for cancer. In a recent study, chromosome breakage was minimised in young adults by supplementation with 700 µg of folic acid and 7 µg of vitamin B12 daily in cereal for two months.

Recommended Dietary Allowance (RDA)
The US Institute of Medicine (IOM) recommends that anyone over 50 years should consume most of their vitamin B12 from fortified foods or supplements. An increase to 2.6 µg/day is recommended during pregnancy and to 2.8 µg/day for lactation to cover the additional requirements of the foetus/infant. The Committee on Nutrition of the American Academy of Paediatrics recommends a daily vitamin B12 intake of 0.15 µg/100 kcal energy intake for infants and preadolescent children. Other authorities have suggested intakes of 0.4 – 0.5 µg (0 – 1 year of age), 0.9 – 1.8 µg (1 – 10 years of age) and 2.4 µg (> 10 years). The ‘average’ western diet probably supplies 3 – 15 µg/day, but can range from 1 – 100 µg/day.

Safety
Large intakes of vitamin B12 from food or supplements have caused no toxicity in healthy people. No adverse effects have been reported from single oral doses as high as 100 mg and chronic administration of 1 mg (500 times the RDA) weekly for up to 5 years. Moreover, there have been no reports of carcinogenic or mutagenic properties, and studies to date indicate no teratogenic potential. The main food safety authorities have not set a tolerable upper intake level (UL) for vitamin B12 because of its low toxicity.

Supplements and food fortification
The principal form of vitamin B12 used in supplements is cyanocobalamin. It is available in the form of injections and as a nasal gel for the treatment of pernicious anaemia. Cyanocobalamin is also available in tablet and oral liquid form for vitamin B-complex, multivitamin and vitamin B12 supplements. Vitamin B12 is widely used to enrich cereal products and certain beverages. Dietetic foods such as slimming foods and infant formulas are often fortified with vitamins, including vitamin B12. Fortification with vitamin B12 is especially important for products aimed at people with a low dietary B12 intake, such as vegans.

Industrial production
Vitamin B12 is produced commercially from bacterial fermentation, usually as cyanocobalamin.
Vitamin B12

History

1824 The first case of pernicious anaemia and its possible relation to disorders of the digestive system is described by Combe.

1855 Combe and Addison identify clinical symptoms of pernicious anaemia.

1925 Whipple and Robscheit-Robbins discover the benefit of liver in the regeneration of blood in anaemic dogs.

1926 Minot and Murphy report that a diet of large quantities of raw liver to patients with pernicious anaemia restores the normal level of red blood cells. Liver concentrates are developed and studies on the presumed active principle(s) ('antipernicious anaemia factor') are initiated.

1929 Castle postulates that two factors are involved in the control of pernicious anaemia: an 'extrinsic factor' in food and an 'intrinsic factor' in normal gastric secretion. Simultaneous administration of these factors causes red blood cell formation which alleviates pernicious anaemia.

1934 Whipple, Minot and Murphy are awarded the Nobel prize for medicine for their work in the treatment of pernicious anaemia.

1948 Rickes and associates and Smith and Parker, working separately, isolate a crystalline red pigment which they name vitamin B12.

1948 West shows that injections of vitamin B12 dramatically benefit patients with pernicious anaemia.

1949 Pierce and coworkers isolate two crystalline forms of vitamin B12 equally effective in combating pernicious anaemia. One form is found to contain cyanide (cyanocobalamin) while the other is not (hydroxocobalamin).

1955 Hodgkin and coworkers establish the molecular structure of cyanocobalamin and its coenzyme forms using X-ray crystallography.

1955 Eschenmoser and colleagues in Switzerland and Woodward and coworkers in the USA synthesise vitamin B12 from cultures of certain bacteria/fungi.

1973 Total chemical synthesis of vitamin B12 by Woodward and coworkers.
Niacin
Nicotinic Acid and Nicotinamide

Introduction
The term niacin refers to both nicotinic acid and its amide derivative, nicotinamide (niacinamide). Both are used to form the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Niacin is a member of the water-soluble B-vitamin complex. The amino acid tryptophan can be converted to nicotinic acid in humans, nicotinic acid was isolated as early as 1867. In 1937 it was demonstrated that this substance cures the disease pellagra. The name niacin is derived from nicotinic acid and vitamin.

Functions
The coenzymes NAD and NADP are required for many biological reduction-oxidation (redox) reactions. About 200 enzymes require NAD or NADP. NAD is mainly involved in reactions that generate energy in tissues by the biochemical degradation of carbohydrates, fats and proteins. NAD is also required as a substrate for non-redox reactions. It is the source of adenosine diphosphate (ADP)-ribose, which is transferred to proteins by different enzymes. These enzymes and their products seem to be involved in DNA replication, DNA repair, cell differentiation and cellular signal transduction. NADP functions in reductive biosyntheses such as the synthesis of fatty acids and cholesterol.

Dietary sources
Nicotinamide and nicotinic acid occur widely in nature. Nicotinic acid is more prevalent in plants, whereas in animals nicotinamide predominates. Yeast, liver, poultry, lean meats, nuts and legumes contribute most of the niacin obtained from food. Milk and green leafy vegetables contribute lesser amounts. In cereal products (corn, wheat), nicotinic acid is bound to certain components of the cereal and is thus not bioavailable. Specific food processing, such as the treatment of corn with lime water involved in the traditional preparation of tortillas in Mexico and Central America, increases the bioavailability of nicotinic acid in these products. Tryptophan contributes as much as two thirds of the niacin activity required by adults in typical diets. Important food sources of tryptophan are meat, milk and eggs.

Absorption and body stores
Both acid and amide forms of the vitamin are readily absorbed from the stomach and the small intestine. At low concentrations the two forms are absorbed by a sodium-dependent facilitated diffusion, and at higher concentrations by passive diffusion. Niacin is present in the diet mainly as NAD and NADP, and nicotinamide is released from the coenzyme forms by enzymes in the intestine. The main storage organ, the liver, may contain a significant amount of the vitamin, which is stored as NAD. The niacin coenzymes NAD and NADP are synthesised in all tissues from nicotinic acid or nicotinamide.
Measurement

Determination of the urinary excretion of two niacin metabolites, N-methyl-nicotinamide and N-methyl-2-pyridone-5-carboxamide has been used to assess niacin status. Excretion of $5.8 \pm 3.6$ mg N-methyl-nicotinamide/24hrs and $20.0 \pm 12.9$ mg N-methyl-2-pyridone-5-carboxamide/24hrs are considered normal. A ratio of the two metabolites is also used for status assessment. An adequate niacin status is considered when the ratio of N-methyl-2-pyridone-5-carboxamide to N-methyl-nicotinamide is between 1.3 and 4.0. Recent studies suggest that the measurement of NAD and NADP concentrations and their ratio in red blood cells may be sensitive and reliable indicators for the determination of niacin status. A ratio of erythrocyte NAD to NADP $< 1.0$ may identify subjects at risk of developing niacin deficiency. Plasma tryptophan concentration is also used for assessment of niacin status.

Stability

Both nicotinamide and nicotinic acid are stable when exposed to heat, light, air and alkali. Little loss occurs during the cooking and storage of foods.

Interactions

Negative interactions

Copper deficiency can inhibit the conversion of tryptophan to niacin. The drug penicillamine has been demonstrated to inhibit the tryptophan-to-niacin pathway in humans; this may be due in part to the copper-chelating effect of penicillamine. The pathway from tryptophan to niacin is sensitive to a variety of nutritional alterations. Inadequate iron, riboflavin, or vitamin B6 status reduces the synthesis of niacin from tryptophan.

Long-term treatment of tuberculosis with isoniazid may cause niacin deficiency because isoniazid is a niacin antagonist. Other drugs which interact with niacin metabolism may also lead to niacin deficiency, e.g. tranquillisers (diazepam) and anticonvulsants (phenytoin, phenobarbitol).

Deficiency

Symptoms of a marginal niacin deficiency include: insomnia, loss of appetite, weight and strength loss, soreness of the tongue and mouth, indigestion, abdominal pain, burning sensations in various parts of the body, vertigo, headaches, numbness, nervousness, poor concentration, apprehension, confusion and forgetfulness.

Severe niacin deficiency leads to pellagra, a disease characterised by dermatitis, diarrhoea and dementia. In the skin, a pigmented rash develops symmetrically in areas exposed to sunlight (the term pellagra comes from the Italian phrase for raw skin). Symptoms affecting the digestive system include a bright red tongue, stomatitis, vomiting, and diarrhoea. Headaches, fatigue, depression, apathy, and loss of memory are neurological symptoms of pellagra. If untreated, pellagra is fatal. Since the synthesis of NAD from tryptophan requires an adequate supply of riboflavin and vitamin B6, insufficiencies of these vitamins may also contribute to niacin deficiency, resulting in pellagra.

Pellagra is rarely seen in industrialised countries, except for its occurrence in people with chronic alcoholism. In other parts of the world where maize and jowar (barley) are the major staples, pellagra persists. It also occurs in India and parts of China and Africa.

Symptoms of a marginal niacin deficiency include: insomnia, loss of appetite, weight and strength loss, soreness of the tongue and mouth, indigestion, abdominal pain, burning sensations in various parts of the body, vertigo, headaches, numbness, nervousness, poor concentration, apprehension, confusion and forgetfulness.

Groups at risk for deficiency

- Patients with Hartnup’s disease, a genetic disorder, may develop pellagra because of defective tryptophan absorption
- Patients with carcinoid syndrome may develop pellagra because dietary tryptophan is preferentially used for serotonin and this increased turnover restricts the amounts of tryptophan which can be used for niacin synthesis
- Alcoholics
- Long-term intake of certain drugs (see interactions)

Disease prevention and therapeutic use

Niacin is specific in the treatment of glossitis, dermatitis and the mental symptoms seen in pellagra. High doses of nicotinic acid ($1.5 – 4$ g/day) can reduce total and low-density lipoprotein cholesterol and triacylglycerols and increase high-density lipoprotein cholesterol in patients at risk of cardiovascular disease. There is a flush reaction to high doses of nicotinic acid, which is seen primarily with a rising blood level and may wear off once a plateau level has been reached. Nicotinic acid has also been used in doses of $100$ mg as a vasodilator in patients suffering from diseases causing vasoconstriction. Type 1 diabetes mellitus results from the autoimmune destruction of insulin-secreting β-cells in the pancreas. There is evidence that nicotinamide may delay or prevent the development of diabetes. Clinical trials are in progress to investigate this effect of nicotinamide.

Recent studies suggest that an infection with human immunodeficiency virus (HIV) increases the risk of niacin deficiency. Higher intakes of niacin were associated with decreased progression rate to AIDS in an observational study of HIV-positive men.
DNA damage is an important risk factor for cancer. NAD is consumed as a substrate in ADP-ribose transfer reactions to proteins which play a role in DNA repair. This has aroused interest in the relationship between niacin and cancer. A large case-control study found increased consumption of niacin, along with antioxidant nutrients, to be associated with decreased incidence of cancers of the mouth, throat and oesophagus.

Recommended Dietary Allowance (RDA)

The actual daily requirement of niacin depends on the quantity of tryptophan in the diet and the efficiency of the tryptophan to niacin conversion. The conversion factor is 60 mg of tryptophan to 1 mg of niacin, which is referred to as 1 niacin equivalent (NE). This conversion factor is used for calculating both dietary contributions from tryptophan and recommended allowances of niacin. In the USA, the RDA for adults is 16 mg NEs for men and 14 mg NEs for women. The RDA for niacin is based on energy intake because niacin is involved in the metabolism of energy producing nutrients. RDA is estimated as 6.6 mg NE per 1000 kcal. Other regulatory authorities have established similar RDAs.

Recommended daily intakes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt; 6 months</td>
<td>2 mg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>4 mg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>6 mg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>8 mg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>12 mg</td>
</tr>
<tr>
<td>Males</td>
<td>&gt; 14 years</td>
<td>16 mg</td>
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<td>Females</td>
<td>&gt; 14 years</td>
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<tr>
<td>Pregnancy</td>
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<td>18 mg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>17 mg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** As niacin equivalents (NE): 1 mg niacin = 60 mg of tryptophan; 0-6 months = preformed niacin (not NE).
AIAdequate intake

If not otherwise specified, this table presents Recommended Dietary Allowances (RDAs). Allowable levels of nutrients vary depending on national regulations and the final application.

Safety

There is no evidence that niacin from foods causes adverse effects. Pharmacological doses of nicotinic acid, but not nicotinamide, exceeding 300 mg per day have been associated with a variety of side effects including nausea, diarrhoea and transient flushing of the skin. Doses exceeding 2.5 g per day have been associated with hepatotoxicity, glucose intolerance, hyperglycaemia, elevated blood uric acid levels, heartburn, nausea, headaches. Severe jaundice may occur, even with doses as low as 750 mg per day, and may eventually lead to irreversible liver damage. Doses of 1.5 to 5 g/day of nicotinic acid have been associated with blurred vision and other eye problems. Tablets with a buffer and time release capsules are available to reduce flushing and gastrointestinal irritation for persons with a sensitivity to nicotinic acid. These should be used with caution, however, because time-release niacin tablets used at high levels are linked to liver damage. The Food and Nutrition Board (1998) set the tolerable upper intake level (UL) for niacin (nicotinic acid plus nicotinamide) at 35 mg/day. The EU Scientific Committee on Food (2002) developed different upper levels for nicotinic acid and nicotinamide: the UL for nicotinic acid has been set at 10 mg/day, for nicotinamide at 900 mg/day.

Supplements and food fortification

Single supplements of nicotinic acid are available in tablets, capsules and syrups. Multivitamin and B-complex vitamin infusions, tablets and capsules also contain nicotinamide. Niacin is used to fortify grain including corn and bran breakfast cereals and wheat flour (whole meal, white and brown). U.S. standards of identity and state standards require enrichment of bread, flour, farina, macaroni, spaghetti and noodle products, corn meal, corn grits and rice.

Industrial production

Although other routes are known, most nicotinic acid is produced by oxidation of 5-ethyl-2-methylpyridine. Nicotinamide is produced via 3-methylpyridine. This compound is derived from two carbon sources, acetaldehyde and formaldehyde, or from acrolein plus ammonia. 3-Methylpyridine is first oxidised to 3-cyanopyridine, which in a second stage converts to nicotinamide by hydrolysis.
Niacin

History

1755 The disease pellagra is first described by Thiery who calls the disease ‘mal de la rosa’.

1867 Huber provides the first description of nicotinic acid.

1873 Weidel describes the elemental analysis and crystalline structure of the salts and other derivatives of nicotinic acid in some detail.

1894 First preparation of nicotinamide by Engler.

1913 Funk isolates nicotinic acid from yeast.

1915 Goldberger demonstrates that pellagra is a dietary deficiency disease.

1928 Goldberger and Wheeler use the experimental model of black tongue disease in dogs as the human disease pellagra.

1937 Spies cures human pellagra using nicotinamide.

1945 Krehl discovers that the essential amino acid tryptophan is transformed into niacin by mammalian tissues.

1955 The concept of niacin equivalents is proposed by Horwitt.

1955 Altschul and associates report that high doses of nicotinic acid reduce serum cholesterol in man.

1961 Turner and Hughes demonstrate that the main absorbed form of niacin is the amide.

1978 Shepperd and colleagues report that high doses of nicotinic acid lower both serum cholesterol and triglycerides.

1980 Bredehorst and colleagues show that niacin status affects the extent of ADP-ribosylation of proteins.
Pantothenic acid

Introduction

Pantothenic acid was discovered in 1933 and belongs to the group of water-soluble B vitamins. Its name originates from the Greek word ‘pantos’, meaning ‘everywhere’, as it can be found throughout all living cells.

Functions

Pantothenic acid, as a constituent of coenzyme A (a coenzyme of acetylation) and acyl carrier protein (ACP, an enzyme involved in the synthesis of fatty acids), plays a key role in the metabolism of carbohydrates, proteins and fats, and is therefore essential for the maintenance and repair of all cells and tissues. Coenzyme A (CoA) is involved in a broad range of acetyl- and acyl-transfer steps and reactions of the oxidative metabolism and catabolism (tricarboxylic acid [TCA] cycle) hence in reactions that supply energy. For example in the process of fat burning (β-oxidation), pantothenic acid works in concert with coenzyme Q10 and L-carnitine. The acyl carrier protein however is required for biosynthetic (anabolic) reactions such as fatty acid synthesis.

CoA is also engaged in the synthesis of neurotransmitters (e.g. acetylcholine), porphyrin (a component of haemoglobin, the oxygen-carrying red blood cell pigment) and antibodies, and in the metabolism of drugs (e.g. sulphonamides) and in alcohol detoxification.

Dietary sources

The active vitamin is present in virtually all plant, animal and microbial cells. Thus pantothenic acid is widely distributed in foods, mostly incorporated into coenzyme A. Its richest sources are yeast and organ meats (liver, kidney, heart, brain), but eggs, milk, vegetables, nuts and whole-grain cereals are more common sources.

Pantothenic acid is only biosynthesised by plants, bacteria, eubacteria and archaea. The extent and significance of the enteral synthesis by intestinal microorganisms is unknown.

Main functions in a nutshell:

- Metabolism of carbohydrates, proteins and fats
- Energy supply from nutrients
- Biosynthesis of essential lipids, steroids, hormones, neurotransmitters, and porphyrin
- Metabolism of xenobiotics

Calcium pantothenate crystals in polarized light

Synonyms

Vitamin B5, pantothenate, pantothenol, D-panthenol, anti-dermatosis vitamin, chick antipelagra factor

Chemistry

Pantothenic acid is composed of β-alanine and 2,4-dihydroxy-3,3-dimethylbutyric acid (pantoic acid), acid amide-linked. Pantetheine consists of pantothenic acid linked to a β-mercaptoethylamine group.

Molecular formula of pantothenic acid

Pantothenic acid content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veal liver</td>
<td>7.9</td>
</tr>
<tr>
<td>Brewer’s yeast</td>
<td>7.2</td>
</tr>
<tr>
<td>Peanuts</td>
<td>2.1</td>
</tr>
<tr>
<td>White mushrooms</td>
<td>2.1</td>
</tr>
<tr>
<td>Egg</td>
<td>1.6</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>0.94</td>
</tr>
<tr>
<td>Herring</td>
<td>0.94</td>
</tr>
<tr>
<td>Milk</td>
<td>0.35</td>
</tr>
<tr>
<td>Fruits / Vegetables</td>
<td>0.2 – 0.6</td>
</tr>
</tbody>
</table>

(Source: Fachmann, Kaut)
Pantothenic acid

Absorption and body stores

Most of the pantothenic acid in food exists in the form of coenzyme A or acyl carrier protein (ACP), which are converted into pantetheine by a series of enzyme reactions (hydrolases) in the small intestine. Pantetheine can be directly absorbed or is further metabolised to pantothenic acid. The absorption happens by passive diffusion and by a saturable sodium-dependent active transport system, which is also shared by biotin. Pantothenic acid is transported to the tissues via the blood circulation, primarily incorporated into erythrocytes or bound to plasma proteins and, there it is embedded into coenzyme A and ACP again. The cellular pantothenic acid uptake is similar to the intestinal absorption.

Intracellular concentrations are regulated by the pantothenic acid kinase. If nutritional supplement formulations such as calcium pantothenate are ingested, they must also first be converted by intestinal enzymes before being taken up by the small intestine. About half of the pantothenic acid in the diet is actually absorbed. Topically applied D-panthenol (the alcoholic form of pantothenic acid that can, for example, be found in many cosmetic products) is also absorbed by passive diffusion through the skin and transformed to pantothenic acid by enzymatic oxidation.

The highest concentrations in the body are found in the liver, adrenal glands, kidneys, brain, heart and testes. Total pantothenic acid levels in whole blood range from 1.6 to 2.7 mM in healthy adults; most of it exists as CoA in the red blood cells. Urinary excretion in the form of pantothenate is generally correlated with dietary intake, but variation is large (0.9 – 1.5 µmol/L).

Measurement

Due to the fact that dietary deficiency is practically unknown, little research has been conducted to assess pantothenate status in man. Nutritional status can be deduced from amounts of pantothenate excreted in urine. Less than 1 mg daily is considered abnormally low. A more thorough approach is the determination of pantothenate in serum, or preferably whole blood, by microbiological methods using Lactobacillus plantarum. Although these assays are highly sensitive and specific, they are slow and tedious to perform, as whole blood needs enzyme pre-treatment because the bacterium strain does not respond to CoA. New methods, such as HPLC/MS (High Performance Liquid Chromatography / Mass Spectrometry) and immunological methods (radioimmunossay, ELISA) have also been applied. Another method suggested for assessing nutritional status is the sulphanylamide acetylation test, which directly measures the activity of coenzyme A in the blood. Whole blood levels typically range from 0.9 – 1.5 µmol/L.

Stability

Pantothenic acid is stable under neutral conditions, but is readily destroyed by heat in alkaline or acid solutions. Up to 50% may be lost during cooking (due to leaching) and up to 80% as a result of food processing and refining (canning, freezing, milling etc.). Pasteurisation of milk only causes minor losses.

Interactions

Positive interactions

Various studies have indicated that vitamin B12 may aid in the conversion of free pantothenic acid into coenzyme A. In the absence of B12, CoA production is decreased and fat metabolism impaired. In animal experiments, ascorbic acid (vitamin C) was shown to lessen the severity of symptoms due to pantothenic acid deficiency; vitamin A, vitamin B6, folic acid and biotin are also necessary for proper utilisation of pantothenic acid. Coenzyme Q10 and L-carnitine enable together with pantothenic acid the β-oxidation of fatty acids in the mitochondria.

Negative interactions

Ethanol causes a decrease in the amount of pantothenic acid in tissues, with a resulting increase in serum levels. It has therefore been suggested that pantothenic acid utilisation is impaired in alcoholics. Birth control pills containing estrogens and progestin may increase the requirement for pantothenic acid. The most common antagonist of pantothenic acid used experimentally to accelerate the appearance of deficiency symptoms is omega-methyl pantothenic acid. L-pantothenic acid has also been shown to have an antagonistic effect in animal studies. Methyl bromide, a fumigant used to control vermin in places where food is stored, destroys the pantothenic acid in foods exposed to it.
Since pantothenic acid occurs to some extent in all foods, it is generally assumed that dietary deficiency of this vitamin is extremely rare. However, pantothenic acid deficiency in humans is not well documented and probably does not occur in isolation but in conjunction with deficiencies of other B vitamins. Clinical manifestations that can be clearly ascribed to dietary deficiency of pantothenic acid have not been identified, although it has been implicated in ‘burning feet’ syndrome, a condition observed among malnourished prisoners of war in the 1940s. Deficiency symptoms have been produced experimentally by administering the antagonist omega-methyl pantothenic acid in addition to a pantothenic acid-deficient diet. They include fatigue, headaches, insomnia, nausea, abdominal cramps, vomiting and flatulence. The subjects complained of tingling sensations in the arms and legs, muscle cramps and impaired coordination. There was cardiovascular instability and impaired responses to insulin, histamine and ACTH (a stress hormone). Nearly all symptoms are reversed when pantothenic acid is ingested again. The symptoms are the result of low coenzyme A levels, impaired acetylcholine synthesis and altered carbohydrate and lipid metabolism. Homopantothenate is a pantothenic acid antagonist that has been used in Japan to enhance mental function, especially in Alzheimer’s disease. A rare side effect was an abnormal brain function resulting from the failure of the liver to eliminate toxins (hepatic encephalopathy). This condition was reversed by pantothenic acid supplementation, suggesting it was due to pantothenic acid deficiency caused by the antagonist. In experiments with mice it has been shown that a deficiency of pantothenic acid leads to skin irritation and greying of the fur, which were reversed by giving pantothenic acid. Panthenol has since been added to shampoo, although it has never been successful in restoring hair colour in humans.

**Groups at risk of deficiency**
- Alcoholics
- People with insufficient food intake
- People with impaired absorption

**Recommended daily intakes***

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0 – 6 months</td>
<td>1.7 mg</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>1.8 mg</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>2 mg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>3 mg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>4 mg</td>
</tr>
<tr>
<td>Adults</td>
<td>19 – 70 years</td>
<td>5 mg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>6 mg</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>7 mg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** AI, Adequate Intake

Allowable levels of nutrients vary depending on national regulations and the final application.

**Deficiency**

**Disease prevention and therapeutic use**

Although isolated deficiency states are rarely observed, various investigators have noted changes in pantothenic acid levels in various diseases, and pharmacological amounts of the vitamin are used in the treatment of numerous conditions. In most cases, however, the claimed therapeutic responses have not been confirmed by controlled studies in humans. For the treatment of deficiency due to impaired absorption, intravenous or intramuscular injections of 500 mg several times a week are recommended. Postoperative ileus (paralysis of the intestine) requires doses of up to 1000 mg every six hours. Panthenol is applied topically to skin and mucosa to speed up healing of wounds, (diabetic) ulcers and inflammation, such as cuts and grazes, burns, sun-burn, nappy rash, bed sores, laryngitis and bronchitis (the basis for this application is an *in vitro* study with human dermal fibroblasts from 1999). In combination, pantothenic acid and ascorbic acid significantly enhance post surgical therapy and wound healing. The healing process of conjunctiva and the cornea after reconstructive surgery of the epithelium has also been accelerated. Pantothenic acid has been tried, with varying results, to treat various liver conditions, arthritis, obesity, acne and constipation in the elderly; to prevent urinary retention after surgery or childbirth; and (together with biotin) to prevent baldness. It has also been reported to have a protective effect against radiation sickness. Pantethine is used to normalise lipid profiles, as it lowers elevated triglycerides and LDL cholesterol while raising levels of the beneficial HDL cholesterol. Pantethine actually consists of two molecules of pantetheine joined by two molecules of sulphur (a disulphide bridge). It is especially effective at lowering elevated blood lipids in patients with diabetes without hindering blood sugar control.
Recommended Dietary Allowance (RDA)

It is widely agreed that there is insufficient information available on which to base a recommended daily allowance (RDA) for pantothenic acid. Most countries that make recommendations therefore give an estimate of safe and adequate levels for daily intake. These dietary reference intakes (DRIs) are based on estimated dietary intakes in healthy population groups and range, depending on the health authority concerned, from 2 to 14 mg for adults.

Safety

Pantothenic acid is essentially considered to be nontoxic, and no cases of hypervitaminosis have ever been reported. As much as 10 g daily in humans produces only minor gastrointestinal disturbance (diarrhoea). Pantothenate derivatives are not mutagenic in bacterial tests, however high doses (≤ 10 – 15 g) can cause transient nausea and a lack of fatigue in humans. Due to the lack of reports of adverse effects the main regulatory authorities have not defined a tolerable upper level of intake (UL) for pantothenic acid.

Supplements, food fortification and other applications

Pure pantothenic acid is a viscous hygroscopic oil that is chemically not very stable. Supplements therefore usually contain the calcium salt, or alcohol, panthenol. Both are highly water-soluble and are rapidly converted to the free acid in the body. Calcium pantothenate is often included in multivitamin preparations; panthenol is the more common form used in mono-preparations, which are available in a wide variety of pharmaceutical forms (e.g. solutions for injection and local application, aerosols, tablets, ointments and creams). Pantethine, the dimer of pantetheine (the cysteamine amide analogue of pantothenic acid), is used as a cholesterol and triglyceride-lowering drug in Europe and Japan and is available in the U.S. as a dietary supplement.

Pantothenate is added to a variety of foods, the most important of which are breakfast cereals and beverages, dietetic and baby foods. D-panthenol is often used in cosmetic products. In skin care products, it helps to keep the skin moist and supple, stimulates cell growth and tissue repair, and inhibits inflammation and reddening. As a moisturiser and conditioner in hair care products, it protects against and repairs damage due to chemical and mechanical procedures (brushing, combing, shampooing, perming, colouring etc.), and imparts sheen and lustre.

Industrial production

Pantothenic acid is primarily chemically synthesised by condensation of D-pantolactone with β-alanine. Addition of a calcium salt produces colourless crystals of calcium pantothenate (100% purity).

Furthermore pantothenic acid can be purified from specific Escherichia Coli fermentation broths (biotechnological process). And brewers yeast is considered a low purity natural source.

Panthenol is produced as a clear, almost colourless, viscous hygroscopic liquid.
History

1931  Williams and Truesdail separate an acid fraction from 'bios', the growth factor for yeast discovered in 1901 by Wildiers.

1933  Williams and coworkers show this fraction to be a single acid substance essential for the growth of yeast. Because they find it in a wide range of biological materials, they suggest calling it ‘pantothenic acid’.

1938  Williams and associates establish the structure of pantothenic acid.

1939  Jukes and colleagues show the similarity between pantothenic acid and the chick antidermatitis factor.

1940  Total synthesis of the vitamin is achieved independently by Williams and Major, Stiller and associates, Reichstein and Grüssner, and Kuhn and Wieland.

1947  Lipmann and his associates identify pantothenic acid as one of the components of the coenzyme they had discovered in liver two years earlier.

1953  The full structure of coenzyme A is elucidated by Baddiley and colleagues. Lipmann receives the Nobel Prize, together with Krebs, for his work on coenzyme A and its role in metabolism.

1954  Bean and Hodges report that pantothenic acid is essential in human nutrition. Subsequently, they and their colleagues conduct several further studies to produce deficiency symptoms in healthy humans using the antagonist omega-methyl pantothenic acid.

1965  Pugh and Wakil identify the acyl carrier protein as an additional active form of pantothenic acid.

1976  Fry and her associates measure the metabolic response of humans to deprivation of pantothenic acid without involvement of an antagonist.
Vitamin basics: the facts about vitamins in nutrition

Folic acid

Introduction

Folate is a generic term for a water-soluble group of B vitamins including folic acid and naturally occurring folates. Folic acid is a synthetic folate compound used in vitamin supplements and fortified food because of its increased stability. The name comes from folium, which is the Latin word for leaves, because folates were first isolated from spinach in 1941. In 1962 Herbert consumed a folate-deficient diet for several months and recorded his development of deficiency symptoms. His findings set the criteria for the diagnosis of folate deficiency.

Functions

Tetrahydrofolate, which is the active form of folic acid in the body, acts as a coenzyme in numerous essential metabolic reactions. Folate coenzymes act as acceptors and donors of one-carbon units in these reactions. Folate coenzymes play an important role in the metabolism of several amino acids, the constituents of proteins. The synthesis of the amino acid methionine from homocysteine requires a folate coenzyme and, in addition, vitamin B12. Folate is also involved in the synthesis of nucleic acids (DNA and RNA) – the molecules that carry genetic information in cells – and also in the formation of blood cells. Folates are therefore essential for normal cell division, proper growth and are required for the prevention of anaemia, and also for normal foetal development.

Dietary sources

Folates are found in a wide variety of foods, but in relatively low density. Its richest sources are liver, dark green leafy vegetables, beans, wheat germ and yeast. Other natural sources are egg yolk, milk and dairy products, beets, orange juice and whole wheat bread. Fortified foods (e.g. breakfast cereals) are among the best dietary sources of folate because they provide the vitamin as folic acid, a highly bioavailable vitamin form. Folates synthesised by intestinal bacteria do not contribute significantly to folate nutrition in humans because bacterial folate synthesis is usually restricted to the large intestine (colon), whereas absorption occurs mainly in the upper part of the small intestine (jejunum).

Absorption and body stores

Most natural dietary folates exist as polyglutamates, which have to be converted to the monoglutamate form in the gut before absorption. The monoglutamate form is absorbed in the proximal small intestine by an active carrier-mediated transport mechanism, and also by passive diffusion. Ingested folic acid is enzymatically reduced and methylated in the mucosa cells. The predominant form of folate in the plasma is 5-methyltetrahydrofolate (5-MTHF). Folates are widely distributed in tissues, most of them as polyglutamate derivatives. The main storage organ is the liver, which contains about half of the body’s stores.

Bioavailability

Absorption of folic acid is almost 100% when consumed under fasting conditions. When folic acid is consumed with a portion of food, bioavailability is estimated from experimental data to be 85% compared with free folic acid. The bioavailability of food folates is variable and incomplete, and has been estimated to be no more than 50% that of folic acid.

Main functions in a nutshell:
- Normal cell division
- Proper growth and optimal functioning of the bone marrow

Folic acid content of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>µg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef liver</td>
<td>592</td>
</tr>
<tr>
<td>Peanuts</td>
<td>169</td>
</tr>
<tr>
<td>Spinach</td>
<td>145</td>
</tr>
<tr>
<td>Broccoli</td>
<td>114</td>
</tr>
<tr>
<td>Asparagus</td>
<td>108</td>
</tr>
<tr>
<td>Eggs</td>
<td>67</td>
</tr>
<tr>
<td>Strawberries</td>
<td>43</td>
</tr>
<tr>
<td>Orange juice (freshly squeezed)</td>
<td>41</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>22</td>
</tr>
<tr>
<td>Milk (whole)</td>
<td>6.7</td>
</tr>
</tbody>
</table>

(Souci, Fachmann, Kraut)
**Measurement**

Different methods are used for the measurement of folates in foods and human tissue such as blood. They can be measured by microbiological assay using *Lactobacillus casei* as test organism; this approach is considered to be the gold standard method for folate measurement but tends to be used in research rather than clinical settings. Radioassays based on competitive protein binding are simpler to perform and are not affected by antibiotics, which give false low values in microbiological assays. High-performance liquid chromatography (HPLC) methods have also been established for the analysis of folates in human tissue and in foods. Folate status is assessed by measuring serum and red blood cell folate levels of methyltetrahydrofolate, which is the predominant folate. Serum folate level is considered a sensitive indicator of recent folate intake. Serum concentrations < 7 nmol/L (3 ng/ml) are suggested to indicate negative folate balance. Levels in the red blood cells are considered to be a better indicator of long-term status and to be representative of tissue folate stores. Levels < 305 nmol/L (140 ng/ml) indicate inadequate folate status. Levels < 155 nmol/L (70 ng/ml) are considered to be the gold standard method for folate measurement.

**Stability**

Most naturally-occurring forms of folate in food are unstable. Fresh leafy vegetables stored at room temperature may lose up to 70% of their folate activity within three days. Considerable losses also occur during cooking through leaching into cooking water (up to 95%) and through heating. Folic acid (found in supplements and fortified foods) is more stable compared with natural folates.

**Interactions**

**Positive interactions**

Proper folate utilisation depends on an adequate supply of other vitamins of the B-group such as vitamin B12 and B6, which are involved in the chemical reactions needed for folate metabolism. Vitamin C may also provide the reducing conditions needed to preserve folates in the diet, and a diet deficient in folates is also likely to be deficient in vitamin C.

**Negative interactions**

Several chemotherapeutic agents (e.g. methotrexate, trimethoprim, pyrimethamine) inhibit the enzyme dihydrofolate reductase, which is necessary for the metabolism of folates. When nonsteroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen) are taken in very large therapeutic doses, for example in the treatment of severe arthritis, they may interfere with folate metabolism. Many drugs may interfere with the absorption, utilisation and storage of folates. These include alcohol, cholestyramine and cholestipol (drugs used to lower blood cholesterol), anti-epileptic agents such as phenytoin and diphenyl-hydantoin, and sulfasalazine, which is used in the treatment of ulcerative colitis. Drugs that reduce acidity in the intestine, such as antacids and modern anti-ulcer drugs, have also been reported to interfere with the absorption of folic acid. Early studies of oral contraceptives containing high levels of oestrogen suggested an adverse effect on folate status, but this has not been supported by more recent studies on low dose oral contraceptives.

**Deficiency**

Folate deficiency can result from inadequate intake, defective absorption, abnormal metabolism or increased requirements. Diagnosis of a subclinical deficiency relies on demonstrating reduced red cell folate concentration or on other biochemical evidence such as increased homocysteine concentration, since haematological manifestations are often absent. Early symptoms of folate deficiency are non-specific and may include tiredness, irritability and loss of appetite. Severe folate deficiency leads to megaloblastic anaemia, a condition in which the bone marrow produces giant, immature red blood cells. At an advanced stage of anaemia symptoms of weakness, fatigue, shortness of breath, irritability, headache, and palpitations appear. If left untreated, megaloblastic anaemia may be fatal. Gastrointestinal symptoms also result from severe folate deficiency. Deficiency during pregnancy may result in premature birth, infant low birth weight and foetal growth retardation. In children, growth may be retarded and puberty delayed.

Folate deficiency is very common in many parts of the world and is part of the general problem of undernutrition. In developed countries, nutritional folate deficiency may be encountered above all in economically underprivileged groups. Reduced folate intake is also often seen in people on special diets (e.g. weight-reducing diets). Disorders of the stomach (e.g. atrophic gastritis) and small intestine (e.g. celiac disease, sprue, Crohn’s disease) may lead to folate deficiency as a result of malabsorption. In conditions with a high rate of cell turnover (e.g. cancer, certain anaemias and skin disorders), folate requirements are increased. This is also the case during pregnancy and lactation, due to rapid tissue growth during pregnancy and to losses through the milk during lactation. People undergoing drug treatment, e.g. for epilepsy, cancer or an infection, are at high risk of developing a folate deficiency, as are patients with renal failure who require regular haemodialysis. Acute folate deficiencies have been reported to occur within a relatively short time in patients undergoing intensive care, especially those on total parenteral nutrition.
Folic acid

**Disease prevention and therapeutic use**

In situations where there is a risk of folate deficiency, oral folic acid supplementation is recommended, usually in a multivitamin preparation containing 400 µg of folic acid. In acute cases of megaloblastic anaemia, treatment often has to be started before a diagnosis of the cause (vitamin B12 or folate deficiency) has been made. To avoid complications that may arise by treating a B12 deficiency with folic acid in such circumstances (see below), both folic acid and vitamin B12 need to be administered until a specific diagnosis is available.

It has been demonstrated that peri-conceptional (before and during the first 28 days after conception) supplementation of women with folic acid can decrease the risk of neural tube defects (malformations of the brain and spinal cord, causing anencephaly or spina bifida). Therefore, a daily intake of 400 µg folic acid in addition to a healthy diet 8 weeks prior to and during the first 12 weeks after conception is recommended globally to women of reproductive age.

There is evidence that adequate folate status may also prevent the incidence of other birth defects, including cleft lip and palate, certain heart defects and limb malformations. Results from intervention studies have shown that a multivitamin supplement containing folic acid is more effective in decreasing the risk of neural tube defects and other birth defects than folic acid alone.

Numerous studies have shown that even moderately elevated levels of homocysteine in the blood increase the risk of atherosclerosis. Folic acid has been shown to decrease homocysteine levels. Several randomised placebo-controlled trials are presently being conducted to establish whether folic acid supplementation reduces the risk of cardiovascular diseases possibly by lowering homocysteine blood levels.

A number of different observational studies have found poor folate status to be associated with increased cancer risk. There is also evidence that improving folate plays a role in preventing colorectal cancer. The results of two large epidemiological investigations suggest that increased folate intake may reduce breast cancer risk associated with regular alcohol consumption. Low folate levels have also been recently associated with Alzheimer’s disease, dementia and increased risk of depression.

**Recommended Dietary Allowance (RDA)**

In the USA the recommendations of the Food and Nutrition Board (1998) are expressed as dietary folate equivalents (DFEs) to account for the different bioavailability of folates and folic acid. This organisation recommends a daily intake of 400 µg of DFE for adult females and males. To cover increased needs during pregnancy and lactation, it recommends 600 µg/day and 500 µg/day respectively. In Europe, the RDA varies between 200 – 400 µg/day for adults in different countries.

**Safety**

Oral folic acid is not considered to be toxic to humans. It has been claimed that high doses of folic acid may counteract the effect of antiepileptic medication and so increase the frequency of seizures in susceptible patients. Folic acid at very high doses can also potentially mask vitamin B12 deficiency and thereby delay its diagnosis. It should therefore not be used indiscriminately in patients with anaemia because of the risk of damage to the nervous system due to B12 deficiency. The U.S. Food and Nutrition Board (FNB) (1998) set the tolerable upper intake level (UL) of folic acid from fortified foods or supplements at 1 mg/day for adults. The EU Scientific Committee on Food (2000) also established an UL of 1 mg for folic acid.

Folic acid is available as oral preparations, alone or in combination with other vitamins or minerals (e.g. iron), and as an aqueous solution for injection. As the acid is only poorly soluble in water, folate salts are used to prepare liquid dosage forms. Folinic acid (also known as leucovorin or citrovorum factor) is a derivative of folic acid administered by intramuscular injection to circumvent the action of dihydrofolate reductase inhibitors, such as methotrexate. It is not otherwise indicated for the prevention or treatment of folic acid deficiency.

Folic acid is added to a variety of foods, the most important of which are flour, breakfast cereals, certain beverages, salt and baby foods.

To reduce the risk of neural tube defects, cereal grains are fortified with folic acid in some countries. In the USA and Canada all enriched cereal grains (e.g. enriched bread, pasta, flour, breakfast cereals, and rice) are required to be fortified with folic acid. In Hungary and Chile, wheat flour is fortified with folic acid.

**Industrial production**

Folic acid is manufactured on a large scale by chemical synthesis. Various processes are known. Most synthesised folic acid is used in animal feed.

**Recommended daily intakes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants &lt; 6 months</td>
<td>65 µg (AI)</td>
<td></td>
</tr>
<tr>
<td>Infants 7 – 12 months</td>
<td>80 µg (AI)</td>
<td></td>
</tr>
<tr>
<td>Children 1 – 3 years</td>
<td>150 µg</td>
<td></td>
</tr>
<tr>
<td>Children 4 – 8 years</td>
<td>200 µg</td>
<td></td>
</tr>
<tr>
<td>Children 9 – 13 years</td>
<td>300 µg</td>
<td></td>
</tr>
<tr>
<td>Adults ≥ 14 years</td>
<td>400 µg</td>
<td></td>
</tr>
<tr>
<td>Pregnant 14 – 50 years</td>
<td>600 µg</td>
<td></td>
</tr>
<tr>
<td>Lactation 14 – 50 years</td>
<td>500 µg</td>
<td></td>
</tr>
</tbody>
</table>

*Institute of Medicine (2000).
** As dietary folate equivalents (DFEs).
AI Adequate Intake.
Allowable levels of nutrients vary depending on national regulations and the final application.
History

1931 Wills in India observes the effect of liver and yeast extracts in treating tropical macrocytic anaemia and concludes that this disorder must be due to a dietary deficiency. She recognises that yeast contains a curative agent equal in potency to that of liver.

1938 Day and coworkers find an antianæmia factor for monkeys in yeast and designate it ‘vitamin M.’ Around the same time, Stokstad and Manning discover a growth factor for chicks, which they call ‘Factor U’.

1939 Hogan and Parrott identify an antianæmia factor for chicks in liver extracts, which they name ‘vitamin BC’.

1940 Discovery of growth factors for Lactobacillus casei and Streptococcus lactis. Snell and Peterson coin the term ‘norite-eluate factor’.

1941 Mitchell and colleagues suggest the name ‘folic acid’ (folium, Latin for leaf) for the factor responsible for growth stimulation of Streptococcus lactis, which they isolate from spinach and suspect of having vitamin-like properties for animals.

1945 Angier and coworkers report the synthesis of a compound identical to the L. casei factor isolated from liver. They later describe the chemical structures of the basic and related compounds.

1945 Spies demonstrates that folic acid cures megaloblastic anaemia during pregnancy.

1962 Herbert consums a folate-deficient diet for several months and records his development of deficiency symptoms. His findings set the criteria for the diagnosis of folate deficiency. In the same year, Herbert estimates the folic acid requirements for adults, which still serve as a basis for many RDAs.

1991 Wald establishes that folic acid supplementation reduces risk of neural tube defects by 70% among women who have already given birth to a child with such birth defects.

1992 Butterworth finds that higher than normal serum levels of folic acid are associated with decreased risk of cervical cancer in women infected with human papillomavirus. Also, Czeizel demonstrates that first-time occurrence of neural tube defects may be largely eliminated with a multivitamin containing folic acid taken in the periconceptional period.

1993 The U.S. Public Health Service recommends that all women of childbearing age consume 0.4 mg (400 µg) of folate daily in order to reduce the risk of foetal malformations such as spina bifida and other neural tube defects.

1998 Fortification of all enriched cereal grains (e.g. enriched bread, pasta, flour, rice and breakfast cereals) with folic acid becomes mandatory in the USA and in Canada. In Hungary, wheat flour is fortified with folic acid.

2000 Mandatory fortification of wheat flour with folic acid is established in Chile.
Introduction

Biotin is a colorless, water-soluble member of the B-complex group of vitamins. Although biotin was discovered in 1901 as a special growth factor for yeast, it took nearly forty years of research to establish biotin as a vitamin for humans. Due to its beneficial effects for hair, skin and nails, biotin is also known as the ‘beauty vitamin’. There are eight different forms of biotin, but only one of them – D-biotin – occurs naturally and has full vitamin activity. Biotin can only be synthesised by bacteria, moulds, yeasts and plants.

Functions

Biotin plays a key role in the metabolism of lipids, proteins and carbohydrates. The enzyme holocarboxylase synthetase (HCS) is required to covalently attach biotin to its target enzymes. These act as carboxylases and are inactive in the absence of the biotin prosthetic group:

• Acetyl-CoA carboxylase (involved in the synthesis of fatty acids from acetate)
• Pyruvate carboxylase (involved in gluconeogenesis, i.e. the generation of glucose from lactate, glycerol, and amino acids)
• β-methylcrotonyl-CoA carboxylase (necessary for the metabolism of leucin, an essential amino acid)
• Propionyl-CoA carboxylase (involved in energy metabolism, necessary for the catabolism of some amino acids and odd-chain fatty acids)

Furthermore, biotin may have a role in DNA replication and transcription arising from its interaction with nuclear histone proteins. It owes its reputation as the ‘beauty vitamin’ to the fact that it activates protein/amino acid metabolism in the hair roots and fingernail cells.

Dietary sources

Biotin is widely distributed in most foods but at very low concentrations compared to other water-soluble vitamins. It is found in free and protein-bound forms in foods. Its richest sources are yeast, liver and kidney and swiss chard. Egg yolk, soy beans, nuts and cereals are also good sources. 100 g of liver contains approximately 100 µg biotin, whereas most other meats, vegetables and fruits only contain approximately 1 µg biotin /100 g. Biotin bioavailability has been shown to vary considerably in animal experiments (5% – 62%).

Biotin-producing micro-organisms exist in the large intestine, but the extent and significance of this enteral synthesis in the overall biotin turnover is difficult to calculate and thus remains a subject of controversy.

Synonyms

Vitamin H (‘Haar und Haut’, German words for ‘hair and skin’), vitamin B7 and co-enzyme R.

Chemistry

Biotin has a bicyclic ring structure. One ring contains a ureido group and the other contains a heterocyclic sulphur atom and a valeric acid side-group. (Hexahydro-2-oxo-thieno [3,4]-dimidazole-4-pentanoic acid). Biologically active analogues: biocytin (ε-N-biotinyl-L-lysine), oxybiotin (S substituted with O).

Molecular formula of biotin

Biotin content of foods

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<th>Food</th>
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<tr>
<td>Asparagus</td>
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Source: Fachmann, Kraut
Absorption and body stores

In most foodstuffs biotin is bound to proteins from which it is released in the intestine by protein hydrolysis and a specific enzyme, biotinidase. Biotin is then absorbed unchanged in the upper part of the small intestine by an electron-neutral sodium (Na⁺) gradient dependent carrier-mediated process and also by slow passive diffusion when at therapeutic doses. The carrier is regulated by the availability of biotin, with up-regulation of the number of transporter molecules when biotin is deficient. The colon is also able to absorb biotin via an analogue transport mechanism. Once absorbed, biotin is distributed to all tissues. The presence of a specific biotin carrier protein in plasma is not yet conclusive. The liver and kidney are the main storage places. Biotin metabolites are not active as vitamins and are excreted in the urine. High amounts of biotin, synthesised by colonic bacteria, appear in the faeces.

Measurement

The body status of biotin can be determined by measuring the activity and/or activation of biotin-dependent enzymes – predominately carboxylases – by added biotin. More convenient methods are direct determination of biotin in plasma or serum by microbiological methods or avidin binding assays, or determination of biotin excretion and 3-hydroxyisovaleric acid in urine. Measurement of biotin in plasma is not a reliable indicator of nutritional status, because reported concentrations for biotin in the blood vary widely. Thus, a low plasma-biotin concentration is not a sensitive indicator of inadequate intake. Usual serum concentrations are 100 – 400 pmol/L.

Stability

Biotin is relatively stable when heated and so is not easily destroyed in the ordinary processes of cooking but it will leach into cooking water. Processing of food, e.g. canning, causes a moderate reduction in biotin content.

Interactions

Negative interactions

Raw egg whites contain avidin, a glycoprotein that strongly binds with biotin and prevents its absorption. Thus, the ingestion of large quantities of raw egg white over a long period can result in a biotin deficiency. It has also been reported that antibiotics which damage the intestinal flora (thus decreasing bacterial synthesis) can reduce biotin concentrations. Interactions with certain anticonvulsant drugs and alcohol have also been reported, as they may inhibit intestinal carrier-mediated transport of biotin. Pantothenic acid ingested in large amounts competes with biotin for intestinal and cellular uptake because they both use the same transporter.

Deficiency

Human biotin deficiency is extremely rare. This is probably due to the fact that biotin is synthesised by beneficial bacteria in the human intestinal tract. Potential deficiency symptoms include anorexia, nausea, vomiting, glossitis, depression, dry scaly dermatitis, conjunctivitis and ataxia. Long-lasting, severe, biotin deficiency can result in loss of hair colour and hair loss (alopecia). Signs of biotin deficiency in humans have been demonstrated in volunteers consuming a biotin-deficient diet together with large amounts of raw egg white. After 3 – 4 weeks they developed a fine dry scaly desquamating dermatitis, frequently around the eyes, nose, and mouth. After ten weeks on the diet, they were fatigued, depressed and sleepy, with nausea and loss of appetite. Muscular pains, hyperesthesia and paresthesia occurred, without reflex changes or other objective signs of neuropathy.

Groups at risk of deficiency

• Patients maintained on total parenteral nutrition
• Dialysis patients
• Individuals receiving some forms of long-term anticonvulsant therapy
• Individuals with biotinidase deficiency or holocarboxylase synthetase (HCS) deficiency (genetic defects)
• Patients with malabsorption, including short-bowel syndrome
• People who eat large amounts of raw egg white

Volunteers also developed anaemia and hypercholesterolaemia. Liver biopsies in sudden infant death syndrome babies reveal low biotin concentrations. Most of the affected infants were bottle-fed.
Disease prevention and therapeutic use

There is no direct evidence that marginal biotin deficiency causes birth defects in humans but an adequate biotin intake/supplementation during pregnancy is advisable. Biotin is used clinically to treat the biotin-responsive inborn errors of metabolism, holocarboxylase synthetase deficiency and biotinidase deficiency. Large doses of biotin may be given to babies with a condition called infantile seborrhea or to patients with genetic abnormalities in biotin metabolism. A large number of reports have shown a beneficial effect of biotin in infant seborrheic dermatitis, Leiner’s disease (a generalised form of seborrheic dermatitis) and also plamo plantar pustulosis. Biotin supplements are sometimes given to help reduce blood sugar in diabetes patients. People with type 2 diabetes often have low concentrations of biotin. Some patients with diabetes may have an abnormality in the biotin-dependent enzyme pyruvate carboxylase, which can lead to dysfunction of the nervous system.

The main benefit of biotin as a dietary supplement is in strengthening hair and nails. Biotin supplements may improve thin or splitting toe-and fingernails and improve hair health. Uncombable hair syndrome in children also improves with biotin supplementation, as do certain skin disorders, such as ‘cradle cap’. Biotin has also been used to combat premature graying of hair, though it is likely to be useful only for those with a low biotin status. In orthomolecular medicine biotin is used to treat hair loss, but scientific evidence is not conclusive.

Recommended Dietary Allowance (RDA)

In 1998 the Food and Nutrition Board of the Institute of Medicine felt the existing scientific evidence was insufficient to calculate an EAR, and thus an RDA, for biotin. Instead an Adequate Intake (AI) level has been defined. The AI for biotin assumes that current average intakes of biotin (35 µg to 60 µg/day) are meeting the dietary requirement. An estimation of the safe and adequate daily dietary intake for biotin was made for the first time in 1980 by the Food and Nutrition Board of the United States National Research Council. The present recommendations in the USA are 20 – 30 µg daily for adults and children over 9 years, and 5 – 12 µg daily for infants and younger children. France and South Africa recommend a daily intake of up to 300 µg, and Singapore up to 400 µg biotin. Others, including Germany, assume that diet and intestinal synthesis provide sufficient amounts.

Recommended daily intakes

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<th>Life stage</th>
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<td>Infants</td>
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<td>5 µg (AI)</td>
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<tr>
<td>Infants</td>
<td>7 – 12 months</td>
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<tr>
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<td>Children</td>
<td>4 – 8 years</td>
<td>12 µg (AI)</td>
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<td>Children</td>
<td>9 – 13 years</td>
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<tr>
<td>Children</td>
<td>14 – 18 years</td>
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<tr>
<td>Adults</td>
<td>&gt; 19 years</td>
<td>30 µg (AI)</td>
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<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>30 µg (AI)</td>
</tr>
<tr>
<td>Lactation</td>
<td>14 – 50 years</td>
<td>35 µg (AI)</td>
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</table>

* Institute of Medicine (2001)
AI, Adequate Intake

Safety

No known toxicity has been associated with biotin. Biotin has been administered in doses as high as 40 mg per day without objectionable effects. As a result, no major regulatory authorities have established a tolerable upper level of intake (UL) for biotin.

Supplements and other applications

Biotin, usually either in the form of crystalline D-biotin or brewer’s yeast, is added to many dietary supplements, infant milk formulas and baby foods, as well as various dietetic products. As a supplement, biotin is often included in combinations of the B vitamins. Mono-preparations of biotin are available in some countries as oral and parenteral formulations. Therapeutic doses of biotin for patients with a biotin deficiency range between 5 and 20 mg daily. Seborrheic dermatitis and Leiner’s disease in infants respond to daily doses of 5 mg. Patients with biotinidase deficiency require life-long biotin therapy in milligram doses (5 – 10 mg/day). Patients with HCS deficiency require supplementation of 40 – 100 mg/day. If biotin therapy is introduced in infancy, the prognosis for both these genetic defects are good. A daily supplement of 60 µg biotin for adults and 20 µg for children has been recommended to maintain normal plasma concentrations in patients on total parenteral nutrition.

Baker’s yeast (Saccharomycyes cerevisiae) is dependent on biotin for growth. Biotin is therefore added as a growth stimulant to the nutrient medium used in yeast fermentation. Many of the microorganisms used in modern biotechnology are also biotin-dependent and thus, biotin is added to the growth medium in such cases.

In cosmetics, biotin is used as an ingredient for hair care products.

Industrial production

Commercial synthesis of biotin is based on a method developed by Goldberg and Sternbach in 1949 using fumaric acid as starting material. This technique produces a pure D-biotin which is identical to the natural product.
History

1901  Wildiers discovers that yeast requires a special growth factor which he names ‘bios’. Over the next 30 years, bios proves to be a mixture of essential factors, one of which – bios IIB – is biotin.

1916  Bateman observes the detrimental effect of feeding high doses of raw egg white to animals.

1927  Boas confirms the findings of dermatosis and hair loss in rats fed with raw egg white. She shows that this egg white injury can be cured by a ‘protective factor X’ found in the liver.

1931  György also discovers this factor in the liver and calls it vitamin H (from Haut, the German word for skin).

1933  Allison and coworkers isolate a respiratory coenzyme – coenzyme R – that is essential for the growth of Rhizobium, a nitrogen-fixing bacterium found in leguminous plants.

1935  Kögl and Tönnis extract a crystalline growth factor from dried egg yolk and suggest the name ‘biotin’.

1940  György and his associates conclude that biotin, vitamin H and coenzyme R are identical. They also succeed in isolating biotin from the liver.

1942  Kögl and his group in Europe and du Vigneaud and his associates in the USA establish the structure of biotin.

1942  Sydenstricker and colleagues demonstrate the need for biotin in the human diet.

1943  Total synthesis of biotin by Harris and colleagues in the USA.

1949  Goldberg and Sterbach develop a technique for the industrial production of biotin.

1956  Traub confirms the structure of biotin by X-ray analysis.

1959  Lynen’s group describes the biological function of biotin and paves the way for further studies on the carboxylase enzymes.

1971  First description of an inborn error of biotin-dependent carboxylase metabolism by Gompertz and associates.

1981  Burri and her colleagues show that the early infantile form of multiple carboxylase deficiency is due to a mutation affecting holocarboxylase synthetase activity.

1983  Burri and coworkers suggest that late-onset multiple carboxylase deficiency results from a deficiency in biotinidase activity.
References


The Institute of Medicine, www.iom.edu

The Linus Pauling Institute, Micronutrient Information Center.
http://lpi.oregonstate.edu/


Tolerable upper intake levels for vitamins and minerals, efsa, 2006.”


‘The Nobel Prize and the Discovery of Vitamins’. Nobelprize.org, 4 Apr 2012


Oberbeil, Fit durch Vitamine, Die neuen Wunderwaffen Südwest Verlag GmbH & Co. KG, München 1993


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**Vitamin K**

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