Vitamins

(Important Source of Nutrition)

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Chapter 1

Vitamin

A **vitamin** is an organic compound required as a nutrient in tiny amounts by an organism. In other words, an organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animals, and biotin and vitamin D are required in the human diet only in certain circumstances. By convention, the term *vitamin* does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids (which are needed in larger amounts than vitamins), nor does it encompass the large number of other nutrients that promote health but are otherwise required less often. Thirteen vitamins are presently universally recognized.

Vitamins are classified by their biological and chemical activity, not their structure. Thus, each "vitamin" refers to a number of *vitamer* compounds that all show the biological activity associated with a particular vitamin. Such a set of chemicals is grouped under an alphabetized vitamin "generic descriptor" title, such as "vitamin A", which includes the compounds retinal, retinol, and four known carotenoids. Vitamers by definition are convertible to the active form of the vitamin in the body, and are sometimes inter-convertible to one another, as well.

Vitamins have diverse biochemical functions. Some have hormone-like functions as regulators of mineral metabolism (e.g., vitamin D), or regulators of cell and tissue growth and differentiation (e.g., some forms of vitamin A). Others function as antioxidants (e.g., vitamin E and sometimes vitamin C). The largest number of vitamins (e.g., B complex vitamins) function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism. In this role, vitamins may be tightly bound to enzymes as part of prosthetic groups: For example, biotin is part of enzymes involved in making fatty acids. Vitamins may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules. For

example, folic acid carries various forms of carbon group – methyl, formyl, and methylene – in the cell. Although these roles in assisting enzyme-substrate reactions are vitamins' best-known function, the other vitamin functions are equally important.

Until the mid-1930s, when the first commercial yeast-extract and semi-synthetic vitamin C supplement tablets were sold, vitamins were obtained solely through food intake, and changes in diet (which, for example, could occur during a particular growing season) can alter the types and amounts of vitamins ingested. Vitamins have been produced as commodity chemicals and made widely available as inexpensive semisynthetic and synthetic-source multivitamin dietary supplements, since the middle of the 20th century.

The term *vitamin* was derived from "vitamine," a combination word made up by Polish scientist Casimir Funk from *vital* and *amine,* meaning amine of life, because it was suggested in 1912 that the organic micronutrient food factors that prevent beriberi and perhaps other similar dietary-deficiency diseases might be chemical amines. This proved incorrect for the micronutrient class, and the word was shortened to vitamin.

History

The discovery dates of the vitamins and their sources

Year of discovery	Vitamin	Food source
1913	Vitamin A (Retinol)	Cod liver oil
1910	Vitamin B_1 (Thiamine)	Rice bran
1920	Vitamin C (Ascorbic acid)	Citrus, most fresh foods
1920	Vitamin D (Calciferol)	Cod liver oil
1920	Vitamin B_2 (Riboflavin)	Meat, eggs
1922	Vitamin E (Tocopherol)	Wheat germ oil, unrefined vegetable oils
1926	Vitamin B_{12} (Cobalamins)	liver, eggs, animal products
1929	Vitamin K_1 (Phylloquinone)	Leafy green vegetables
1931	Vitamin B_5 (Pantothenic acid)	Meat, whole grains, in many foods
1931	Vitamin B_7 (Biotin)	Meat, dairy products, eggs
1934	Vitamin B_6 (Pyridoxine)	Meat, dairy products
1936	Vitamin B_3 (Niacin)	Meat, eggs, grains
1941	Vitamin B_9 (Folic acid)	Leafy green vegetables

The value of eating a certain food to maintain health was recognized long before vitamins were identified. The ancient Egyptians knew that feeding liver to a patient would help cure night blindness, an illness now known to be caused by a vitamin A deficiency. The advancement of ocean voyage during the Renaissance resulted in prolonged periods without access to fresh fruits and vegetables, and made illnesses from vitamin deficiency common among ships' crews.

In 1749, the Scottish surgeon James Lind discovered that citrus foods helped prevent scurvy, a particularly deadly disease in which collagen is not properly formed, causing

poor wound healing, bleeding of the gums, severe pain, and death. In 1753, Lind published his *Treatise on the Scurvy*, which recommended using lemons and limes to avoid scurvy, which was adopted by the British Royal Navy. This led to the nickname Limey for sailors of that organization. Lind's discovery, however, was not widely accepted by individuals in the Royal Navy's Arctic expeditions in the 19th century, where it was widely believed that scurvy could be prevented by practicing good hygiene, regular exercise, and maintaining the morale of the crew while on board, rather than by a diet of fresh food. As a result, Arctic expeditions continued to be plagued by scurvy and other deficiency diseases. In the early 20th century, when Robert Falcon Scott made his two expeditions to the Antarctic, the prevailing medical theory was that scurvy was caused by "tainted" canned food.

During the late 18th and early 19th centuries, the use of deprivation studies allowed scientists to isolate and identify a number of vitamins. Lipid from fish oil was used to cure rickets in rats, and the fat-soluble nutrient was called "antirachitic A". Thus, the first "vitamin" bioactivity ever isolated, which cured rickets, was initially called "vitamin A"; however, the bioactivity of this compound is now called vitamin D. In 1881, Russian surgeon Nikolai Lunin studied the effects of scurvy while at the University of Tartu in present-day Estonia. He fed mice an artificial mixture of all the separate constituents of milk known at that time, namely the proteins, fats, carbohydrates, and salts. The mice that received only the individual constituents died, while the mice fed by milk itself developed normally. He made a conclusion that "a natural food such as milk must therefore contain, besides these known principal ingredients, small quantities of unknown substances essential to life." However, his conclusions were rejected by other researchers when they were unable to reproduce his results. One difference was that he had used table sugar (sucrose), while other researchers had used milk sugar (lactose) that still contained small amounts of vitamin B.

The Ancient Egyptians knew that feeding a patient liver (back, right) would help cure night blindness.

In east Asia, where polished white rice was the common staple food of the middle class, beriberi resulting from lack of vitamin B_1 was endemic. In 1884, Takaki Kanehiro, a British trained medical doctor of the Imperial Japanese Navy, observed that beriberi was endemic among low-ranking crew who often ate nothing but rice, but not among officers who consumed a Western-style diet. With the support of the Japanese navy, he experimented using crews of two battleships; one crew was fed only white rice, while the other was fed a diet of meat, fish, barley, rice, and beans. The group that ate only white rice documented 161 crew members with beriberi and 25 deaths, while the latter group had only 14 cases of beriberi and no deaths. This convinced Takaki and the Japanese Navy that diet was the cause of beriberi, but mistakenly believed that sufficient amounts of protein prevented it. That diseases could result from some dietary deficiencies was further investigated by Christiaan Eijkman, who in 1897 discovered that feeding unpolished rice instead of the polished variety to chickens helped to prevent beriberi in the chickens. The following year, Frederick Hopkins postulated that some foods contained "accessory factors" — in addition to proteins, carbohydrates, fats, et cetera — that are necessary for the functions of the human body. Hopkins and Eijkman were awarded the Nobel Prize for Physiology or Medicine in 1929 for their discovery of several vitamins.

In 1910, the first vitamin complex was isolated by Japanese scientist Umetaro Suzuki, who succeeded in extracting a water-soluble complex of micronutrients from rice bran and named it aberic acid (later *Orizanin*). He published this discovery in a Japanese

scientific journal. When the article was translated into German, the translation failed to state that it was a newly discovered nutrient, a claim made in the original Japanese article, and hence his discovery failed to gain publicity. In 1912 Polish biochemist Casimir Funk isolated the same complex of micronutrients and proposed the complex be named "vitamine" (a portmanteau of "vital amine"). The name soon became synonymous with Hopkins' "accessory factors", and, by the time it was shown that not all vitamins are amines, the word was already ubiquitous. In 1920, Jack Cecil Drummond proposed that the final "e" be dropped to deemphasize the "amine" reference, after researchers began to suspect that not all "vitamines" (in particular, vitamin A) has an amine component.

In 1931, Albert Szent-Györgyi and a fellow researcher Joseph Svirbely suspected that "hexuronic acid" was actually vitamin C, and gave a sample to Charles Glen King, who proved its anti-scorbutic activity in his long-established guinea pig scorbutic assay. In 1937, Szent-Györgyi was awarded the Nobel Prize in Physiology or Medicine for his discovery. In 1943, Edward Adelbert Doisy and Henrik Dam were awarded the Nobel Prize in Physiology or Medicine for their discovery of vitamin K and its chemical structure. In 1967, George Wald was awarded the Nobel Prize (along with Ragnar Granit and Haldan Keffer Hartline) for his discovery that vitamin A could participate directly in a physiological process.

In humans

Vitamins are classified as either water-soluble or fat-soluble. In humans there are 13 vitamins: 4 fat-soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C). Water-soluble vitamins dissolve easily in water and, in general, are readily excreted from the body, to the degree that urinary output is a strong predictor of vitamin consumption. Because they are not readily stored, consistent daily intake is important. Many types of water-soluble vitamins are synthesized by bacteria. Fat-soluble vitamins are absorbed through the intestinal tract with the help of lipids (fats). Because they are more likely to accumulate in the body, they are more likely to lead to hypervitaminosis than are water-soluble vitamins. Fat-soluble vitamin regulation is of particular significance in cystic fibrosis.

List of vitamins

Each vitamin is typically used in multiple reactions, and, therefore, most have multiple functions.

Vitamin generic descriptor name	Vitamer chemical name(s) (list not complete)	Solubility	Recommended dietary allowances (male, age 19–70)	Deficiency disease	Upper Intake Level (UL/day)	Overdose disease
Vitamin A	Retinol, retinal, and four carotenoids including beta carotene	Fat	**900 µg**	Night-blindness, Hyperkeratosis, and Keratomalacia	3,000 µg	Hypervitaminosis A
Vitamin B_1	Thiamine	Water	**1.2 mg**	Beriberi, Wernicke-Korsakoff syndrome	N/D	Drowsiness or muscle relaxation with large doses.
Vitamin B_2	Riboflavin	Water	**1.3 mg**	Ariboflavinosis	N/D	
Vitamin B_3	Niacin, niacinamide	Water	**16.0 mg**	Pellagra	35.0 mg	Liver damage (doses > 2g/day) and other problems
Vitamin B_5	Pantothenic acid	Water	5.0 mg	Paresthesia	N/D	Diarrhea; possibly nausea and heartburn.
Vitamin	Pyridoxine, pyridoxamine,	Water	**1.3–1.7 mg**	Anemia peripheral	100 mg	Impairment of

B_6	pyridoxal			neuropathy.		proprioception, nerve damage (doses > 100 mg/day)
Vitamin B_7	Biotin	Water	30.0 µg	Dermatitis, enteritis	N/D	
Vitamin B_9	Folic acid, folinic acid	Water	**400 µg**	Megaloblast and Deficiency during pregnancy is associated with birth defects, such as neural tube defects	1,000 µg	May mask symptoms of vitamin B_{12} deficiency; other effects.
Vitamin B_{12}	Cyanocobalamin, hydroxycobalamin, methylcobalamin	Water	**2.4 µg**	Megaloblastic anemia	N/D	Acne-like rash [causality is not conclusively established].
Vitamin C	Ascorbic acid	Water	**90.0 mg**	Scurvy	2,000 mg	Vitamin C megadosage
Vitamin D	Ergocalciferol, cholecalciferol	Fat	5.0 µg–10 µg	Rickets and Osteomalacia	50 µg	Hypervitaminosis D

Vitamin E	Tocopherols, tocotrienols	Fat	**15.0 mg**	Deficiency is very rare; mild hemolytic anemia in newborn infants.	1,000 mg	Increased congestive heart failure seen in one large randomized study.
Vitamin K	phylloquinone, menaquinones	Fat	120 μg	Bleeding diathesis	N/D	Increases coagulation in patients taking warfarin.

In nutrition and diseases

Vitamins are essential for the normal growth and development of a multicellular organism. Using the genetic blueprint inherited from its parents, a fetus begins to develop, at the moment of conception, from the nutrients it absorbs. It requires certain vitamins and minerals to be present at certain times. These nutrients facilitate the chemical reactions that produce among other things, skin, bone, and muscle. If there is serious deficiency in one or more of these nutrients, a child may develop a deficiency disease. Even minor deficiencies may cause permanent damage.

For the most part, vitamins are obtained with food, but a few are obtained by other means. For example, microorganisms in the intestine — commonly known as "gut flora" — produce vitamin K and biotin, while one form of vitamin D is synthesized in the skin with the help of the natural ultraviolet wavelength of sunlight. Humans can produce some vitamins from precursors they consume. Examples include vitamin A, produced from beta carotene, and niacin, from the amino acid tryptophan.

Once growth and development are completed, vitamins remain essential nutrients for the healthy maintenance of the cells, tissues, and organs that make up a multicellular organism; they also enable a multicellular life form to efficiently use chemical energy provided by food it eats, and to help process the proteins, carbohydrates, and fats required for respiration.

Deficiencies

It was suggested that, when plants and animals began to transfer from the sea to rivers and land about 500 million years ago, environmental deficiency of marine mineral

antioxidants was a challenge to the evolution of terrestrial life. Terrestrial plants slowly optimized the production of "new" endogenous antioxidants such as ascorbic acid (Vitamin C), polyphenols, flavonoids, tocopherols, etc. Since this age, dietary vitamin deficiencies appeared in terrestrial animals. Humans must consume vitamins periodically but with differing schedules, to avoid deficiency. Human bodily stores for different vitamins vary widely; vitamins A, D, and B_{12} are stored in significant amounts in the human body, mainly in the liver, and an adult human's diet may be deficient in vitamins A and D for many months and B_{12} in some cases for years, before developing a deficiency condition. However, vitamin B_3 (niacin and niacinamide) is not stored in the human body in significant amounts, so stores may last only a couple of weeks. For vitamin C, the first symptoms of scurvy in experimental studies of complete vitamin C deprivation in humans have varied widely, from a month to more than six months, depending on previous dietary history that determined body stores.

Deficiencies of vitamins are classified as either primary or secondary. A **primary deficiency** occurs when an organism does not get enough of the vitamin in its food. A **secondary deficiency** may be due to an underlying disorder that prevents or limits the absorption or use of the vitamin, due to a "lifestyle factor", such as smoking, excessive alcohol consumption, or the use of medications that interfere with the absorption or use of the vitamin. People who eat a varied diet are unlikely to develop a severe primary vitamin deficiency. In contrast, restrictive diets have the potential to cause prolonged vitamin deficits, which may result in often painful and potentially deadly diseases.

Well-known human vitamin deficiencies involve thiamine (beriberi), niacin (pellagra), vitamin C (scurvy), and vitamin D (rickets). In much of the developed world, such deficiencies are rare; this is due to (1) an adequate supply of food and (2) the addition of vitamins and minerals to common foods, often called fortification. In addition to these classical vitamin deficiency diseases, some evidence has also suggested links between vitamin deficiency and a number of different disorders.

Side-effects and overdose

In large doses, some vitamins have documented side-effects that tend to be more severe with a larger dosage. The likelihood of consuming too much of any vitamin from food is remote, but overdosing (vitamin poisoning) from vitamin supplementation does occur. At high enough dosages, some vitamins cause side-effects such as nausea, diarrhea, and vomiting. When side-effects emerge, recovery is often accomplished by reducing the dosage. The doses of vitamins different individual can tolerate varies widely, and appear to be related to age and state of health.

In 2008, overdose exposure to all formulations of vitamins and multivitamin-mineral formulations was reported by 68,911 individuals to the American Association of Poison Control Centers (nearly 80% of these exposures were in children under the age of 6), leading to 8 "major" life-threatening outcomes and 0 deaths.

Supplements

Dietary supplements, often containing vitamins, are used to ensure that adequate amounts of nutrients are obtained on a daily basis, if optimal amounts of the nutrients cannot be obtained through a varied diet. Scientific evidence supporting the benefits of some vitamin supplements is well established for certain health conditions, but others need further study. In some cases, vitamin supplements may have unwanted effects, especially if taken before surgery, with other dietary supplements or medicines, or if the person taking them has certain health conditions. Dietary supplements may also contain levels of vitamins many times higher, and in different forms, than one may ingest through food.

There have been mixed studies on the importance and safety of dietary supplementation. A meta-analysis published in 2006 suggested that Vitamin A and E supplements not only provide no tangible health benefits for generally healthy individuals but may actually increase mortality, although two large studies included in the analysis involved smokers, for which it was already known that beta-carotene supplements can be harmful. Another study published in May 2009 found that antioxidants such as vitamins C and E may actually curb some benefits of exercise. While others findings suggest that evidence of Vitamin E toxicity is limited to specific form taken in excess.

Governmental regulation of vitamin supplements

Most countries place dietary supplements in a special category under the general umbrella of *foods*, not drugs. This necessitates that the manufacturer, and not the government, be responsible for ensuring that its dietary supplement products are safe before they are marketed. Regulation of supplements varies widely by country. In the United States, a dietary supplement is defined under the Dietary Supplement Health and Education Act of 1994. In addition, the Food and Drug Administration uses the Adverse Event Reporting System to monitor adverse events that occur with supplements. In the European Union, the Food Supplements Directive requires that only those supplements that have been proven safe can be sold without a prescription

Names in current and previous nomenclatures

Nomenclature of reclassified vitamins

Previous name	Chemical name	Reason for name change
Vitamin B_4	Adenine	DNA metabolite; synthesized in body
Vitamin B_8	Adenylic acid	DNA metabolite; synthesized in body
Vitamin F	Essential fatty acids	Needed in large quantities (does not fit the definition of a vitamin).
Vitamin G	Riboflavin	Reclassified as Vitamin B_2
Vitamin H	Biotin	Reclassified as Vitamin B_7
Vitamin J	Catechol, Flavin	Catechol nonessential; flavin reclassified as B_2
Vitamin L_1	Anthranilic acid	Non essential

Vitamin L_2	Adenylthiomethylpentose	RNA metabolite; synthesized in body
Vitamin M	Folic acid	Reclassified as Vitamin B_9
Vitamin O	Carnitine	Synthesized in body
Vitamin P	Flavonoids	No longer classified as a vitamin
Vitamin PP	Niacin	Reclassified as Vitamin B_3
Vitamin S	Salicylic acid	Proposed inclusion of salicylate as an essential micronutrient
Vitamin U	S-Methylmethionine	Protein metabolite; synthesized in body

The reason that the set of vitamins skips directly from E to K is that the vitamins corresponding to letters F-J were either reclassified over time, discarded as false leads, or renamed because of their relationship to vitamin B, which became a complex of vitamins.

The German-speaking scientists who isolated and described vitamin K (in addition to naming it as such) did so because the vitamin is intimately involved in the *Koagulation* of blood following wounding. At the time, most (but not all) of the letters from F through to J were already designated, so the use of the letter K was considered quite reasonable. The table on the right lists chemicals that had previously been classified as vitamins, as well as the earlier names of vitamins that later became part of the B-complex.

Anti-vitamins

Anti-vitamins are chemical compounds that inhibit the absorption or actions of vitamins. For example, avidin is a protein in egg whites that inhibits the absorption of biotin. Pyrithiamine is similar to thiamine vitamin B1 and inhibits the enzymes that use thiamine.

Chapter 2

Vitamin A

Vitamin A is a vitamin that is needed by the retina of the eye in the form of a specific metabolite, the light-absorbing molecule retinal, that is absolutely necessary for both low-light (scotopic vision) and color vision. Vitamin A also functions in a very different role, as an irreversibly oxidized form of retinol known as retinoic acid, which is an important hormone-like growth factor for epithelial and other cells.

In foods of animal origin, the major form of vitamin A is an ester, primarily retinyl palmitate, which is converted to the retinol (chemically an alcohol) in the small intestine. The retinol form functions as a storage form of the vitamin, and can be converted to and from its visually active aldehyde form, retinal. The associated acid (retinoic acid), a metabolite that can be irreversibly synthesized from vitamin A, has only partial vitamin A activity, and does not function in the retina for the visual cycle.

All forms of vitamin A have a beta-ionone ring to which an isoprenoid chain is attached, called a *retinyl group*. Both structural features are essential for vitamin activity. The orange pigment of carrots – beta-carotene – can be represented as two connected retinyl groups, which are used in the body to contribute to vitamin A levels. Alpha-carotene and gamma-carotene also have a single retinyl group, which give them some vitamin activity. None of the other carotenes have vitamin activity. The carotenoid beta-cryptoxanthin possesses an ionone group and has vitamin activity in humans.

Vitamin A can be found in two principal forms in foods:

- retinol, the form of vitamin A absorbed when eating animal food sources, is a yellow, fat-soluble substance. Since the pure alcohol form is unstable, the vitamin is found in tissues in a form of retinyl ester. It is also commercially produced and administered as esters such as retinyl acetate or palmitate.

- The carotenes alpha-carotene, beta-carotene, gamma-carotene; and the xanthophyll beta-cryptoxanthin (all of which contain beta-ionone rings), but no other carotenoids, function as vitamin A in herbivores and omnivore animals, which possess the enzyme required to convert these compounds to retinal. In general, carnivores are poor converters of ionine-containing carotenoids, and pure carnivores such as cats and ferrets lack beta-carotene 15,15'-monooxygenase and cannot convert any carotenoids to retinal (resulting in *none* of the carotenoids being forms of vitamin A for these species).

History

The discovery of vitamin A may have stemmed from research dating back to 1906, indicating that factors other than carbohydrates, proteins, and fats were necessary to keep cattle healthy. By 1917 one of these substances was independently discovered by Elmer McCollum at the University of Wisconsin–Madison, and Lafayette Mendel and Thomas Burr Osborne at Yale University. Since "water-soluble factor B" (vitamin B) had recently been discovered, the researchers chose the name "fat-soluble factor A" (vitamin A). In 1919, Steenbock (University of Wisconsin) proposed a relationship between yellow plant pigments (beta-carotene) and vitamin A. Vitamin A was first synthesized in 1947 by two Dutch chemists, David Adriaan van Dorp and Jozef Ferdinand Arens.

Equivalencies of retinoids and carotenoids (IU)

As some carotenoids can be converted into vitamin A, attempts have been made to determine how much of them in the diet is equivalent to a particular amount of retinol, so that comparisons can be made of the benefit of different foods. The situation can be confusing because the accepted equivalences have changed. For many years, a system of equivalencies in which an international unit (IU) was equal to 0.3 μg of retinol, 0.6 μg of β-carotene, or 1.2 μg of other provitamin-A carotenoids was used. Later, a unit called retinol equivalent (RE) was introduced. Prior to 2001, one RE corresponded to 1 μg retinol, 2 μg β-carotene dissolved in oil (it is only partly dissolved in most supplement pills, due to very poor solubility in any medium), 6 μg β-carotene in normal food (because it is not absorbed as well as when in oils), and 12 μg of either α-carotene, γ-carotene, or β-cryptoxanthin in food.

Newer research has shown that the absorption of provitamin-A carotenoids is only half as much as previously thought. As a result, in 2001 the US Institute of Medicine recommended a new unit, the retinol activity equivalent (RAE). Each μg RAE corresponds to 1 μg retinol, 2 μg of β-carotene in oil, 12 μg of "dietary" beta-carotene, or 24 μg of the three other dietary provitamin-A carotenoids.

Substance and its chemical environment	**Micrograms of retinol equivalent per microgram of the substance**
retinol	1
beta-carotene, dissolved in oil	1/2

beta-carotene, common dietary	1/12
alpha-carotene, common dietary	1/24
gamma-carotene, common dietary	1/24
beta-cryptoxanthin, common dietary	1/24

Because the conversion of retinol from provitamin carotenoids by the human body is actively regulated by the amount of retinol available to the body, the conversions apply strictly only for vitamin A-deficient humans. The absorption of provitamins depends greatly on the amount of lipids ingested with the provitamin; lipids increase the uptake of the provitamin.

The conclusion that can be drawn from the newer research is that fruits and vegetables are not as useful for obtaining vitamin A as was thought; in other words, the IUs that these foods were reported to contain were worth much less than the same number of IUs of fat-dissolved oils and (to some extent) supplements. This is important for vegetarians, as Night blindness is prevalent in countries where little meat or vitamin A-fortified foods are available.

A sample vegan diet for one day that provides sufficient vitamin A has been published by the Food and Nutrition Board (page 120). On the other hand, reference values for retinol or its equivalents, provided by the National Academy of Sciences, have decreased. The RDA (for men) of 1968 was 5000 IU (1500 μg retinol). In 1974, the RDA was set to 1000 RE (1000 μg retinol), whereas now the Dietary Reference Intake is 900 RAE (900 μg or 3000 IU retinol). This is equivalent to 1800 μg of β-carotene supplement (3000 IU) or 10800 μg of β-carotene in food (18000 IU).

Recommended daily intake

Vitamin A
Dietary Reference Intake:

	RDA	**Upper limit**
Life stage group	**Adequate intakes (AI*) μg/day**	**μg/day**
Infants		
0–6 months	400*	600
7–12 months	500*	600
Children		
1–3 years	300	600
4–8 years	400	900
Males		

	600	1700
9–13 years	900	2800
14–18 years	900	3000
19 – >70 years		
Females		
9–13 years	600	1700
14–18 years	700	2800
19 – >70 years	700	3000
Pregnancy		
<19 years	750	2800
19 – >50 years	770	3000
Lactation		
<19 years	1200	2800
19 – >50 years	1300	3000

(Note that the limit refers to synthetic and natural retinol ester forms of vitamin A. Carotene forms from dietary sources are not toxic.)

According to the Institute of Medicine of the National Academies, "RDAs are set to meet the needs of almost all (97 to 98%) individuals in a group. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data prevents being able to specify with confidence the percentage of individuals covered by this intake." To reduce the possible risk of bone fracture and osteoporosis in postmenopausal women, an upper limit intake of 1500 μg RE/d has been recommended.

Sources

Vitamin A is found naturally in many foods:

- liver (beef, pork, chicken, turkey, fish) (6500 µg 722%), including cod liver oil
- carrot (835 µg 93%)
- broccoli leaf (800 µg 89%) – According to USDA database broccoli florets have much less.
- sweet potato (709 µg 79%)
- butter (684 µg 76%)
- kale (681 µg 76%)
- spinach (469 µg 52%)
- pumpkin (400 µg 41%)
- collard greens (333 µg 37%)
- Cheddar cheese (265 µg 29%)
- cantaloupe melon (169 µg 19%)
- egg (140 µg 16%)
- apricot (96 µg 11%)
- papaya (55 µg 6%)
- mango (38 µg 4%)
- pea (38 µg 4%)
- broccoli (31 µg 3%)
- milk (28 µg 3%)

Note: data taken from USDA database bracketed values are retinol activity equivalences (RAEs) and percentage of the adult male RDA, per 100 grams of the foodstuff (average).

Conversion of carotene to retinol varies from person to person and bioavailability of carotene in food varies.

Metabolic functions

Vitamin A plays a role in a variety of functions throughout the body, such as:

- Vision
- Gene transcription
- Immune function
- Embryonic development and reproduction
- Bone metabolism
- Haematopoiesis
- Skin and cellular health
- Antioxidant activity

Vision

The role of vitamin A in the visual cycle is specifically related to the retinal form. Within the eye, 11-*cis*-retinal is bound to rhodopsin (rods) and iodopsin (cones) at conserved lysine residues. As light enters the eye, the 11-*cis*-retinal is isomerized to the all-"trans" form. The all-"trans" retinal dissociates from the opsin in a series of steps called photo-bleaching. This isomerization induces a nervous signal along the optic nerve to the visual center of the brain. After separating from opsin, the all-"trans"-retinal is recycled and converted back to the 11-"cis"-retinal form by a series of enzymatic reactions. In addition, some of the all-"trans" retinal may be converted to all-"trans" retinol form and then transported with an interphotoreceptor retinol-binding protein (IRBP) to the pigment epithelial cells. Further esterification into all-"trans" retinyl esters allow for storage of all-trans-retinol within the pigment epithelial cells to be reused when needed. The final stage is conversion of 11-*cis*-retinal will rebind to opsin to reform rhodopsin in the retina. Rhodopsin is needed to in low light (contrast) as well as for night vision. It is for this reason that a deficiency in vitamin A will inhibit the reformation of rhodopsin and lead to one of the first symptoms, night blindness.

Gene transcription

Vitamin A, in the retinoic acid form, plays an important role in gene transcription. Once retinol has been taken up by a cell, it can be oxidized to retinal (retinaldehyde) by retinol dehydrogenases and then retinaldehyde can be oxidized to retinoic acid by retinaldehyde dehydrogenases. The conversion of retinaldehyde to retinoic acid is an irreversible step, meaning that the production of retinoic acid is tightly regulated, due to its activity as a ligand for nuclear receptors. Retinoic acid can bind to two different nuclear receptors to initiate (or inhibit) gene transcription: the retinoic acid receptors (RARs) or the retinoid

"X" receptors (RXRs). RAR and RXR must dimerize before they can bind to the DNA. RAR will form a heterodimer with RXR (RAR-RXR), but it does not readily form a homodimer (RAR-RAR). RXR, on the other hand, readily forms a homodimer (RXR-RXR) and will form heterodimers with many other nuclear receptors as well, including the thyroid hormone receptor (RXR-TR), the Vitamin D_3 receptor (RXR-VDR), the peroxisome proliferator-activated receptor (RXR-PPAR) and the liver "X" receptor (RXR-LXR). The RAR-RXR heterodimer recognizes retinoic acid response elements (RAREs) on the DNA whereas the RXR-RXR homodimer recognizes retinoid "X" response elements (RXREs) on the DNA. The other RXR heterodimers will bind to various other response elements on the DNA. Once the retinoic acid binds to the receptors and dimerization has occurred, the receptors undergo a conformational change that causes co-repressors to dissociate from the receptors. Coactivators can then bind to the receptor complex, which may help to loosen the chromatin structure from the histones or may interact with the transcriptional machinery. The receptors can then bind to the response elements on the DNA and upregulate (or downregulate) the expression of target genes, such as cellular retinol-binding protein (CRBP) as well as the genes that encode for the receptors themselves.

Dermatology

Vitamin A, and more specifically, retinoic acid, appears to maintain normal skin health by switching on genes and differentiating keratinocytes (immature skin cells) into mature epidermal cells. Exact mechanisms behind pharmacological retinoid therapy agents in the treatment of dermatological diseases are being researched. For the treatment of acne, the most prescibred retinoid drug is 13-cis retinoic acid (isotretinoin). It reduces the size and secretion of the sebaceous glands. Although it is known that 40 mg of isotretinoin will break down to an equivalent of 10 mg of ATRA — the mechanism of action of the drug (original brand name Accutane) remains unknown and is a matter of some controversy. Isotretinoin reduces bacterial numbers in both the ducts and skin surface. This is thought to be a result of the reduction in sebum, a nutrient source for the bacteria. Isotretinoin reduces inflammation via inhibition of chemotatic responses of monocytes and neutrophils. Isotretinoin also has been shown to initiate remodeling of the sebaceous glands; triggering changes in gene expression that selectively induce apoptosis. Isotretinoin is a teratogen with a number of potential side-effects. Consequently, its use requires medical supervision.

Retinal/retinol versus retinoic acid

Vitamin A deprived rats can be kept in good general health with supplementation of retinoic acid. This reverses the growth-stunting effects of vitamin A deficiency, as well as early stages of xerophthalmia. However, such rats show infertility (in both male and females) and continued degeneration of the retina, showing that these functions require retinal or retinol, which are intraconvertable but which cannot be recovered from the oxidized retinoic acid. The requirement of retinol to rescue reproduction in vitamin A deficient rats is now known to be due to a requirement for local synthesis of retinoic acid from retinol in testis and embryos.

Deficiency

Vitamin A deficiency is estimated to affect approximately one third of children under the age of five around the world. It is estimated to claim the lives of 670,000 children under five annually. Approximately 250,000–500,000 children in developing countries become blind each year owing to vitamin A deficiency, with the highest prevalence in Southeast Asia and Africa.

Vitamin A deficiency can occur as either a primary or a secondary deficiency. A primary vitamin A deficiency occurs among children and adults who do not consume an adequate intake of provitamin A carotenoids from fruits and vegetables or preformed vitamin A from animal and dairy products. Early weaning from breastmilk can also increase the risk of vitamin A deficiency.

Secondary vitamin A deficiency is associated with chronic malabsorption of lipids, impaired bile production and release, and chronic exposure to oxidants, such as cigarette smoke, and chronic alcoholism. Vitamin A is a fat soluble vitamin and depends on micellar solubilization for dispersion into the small intestine, which results in poor use of vitamin A from low-fat diets. Zinc deficiency can also impair absorption, transport, and metabolism of vitamin A because it is essential for the synthesis of the vitamin A transport proteins and as the cofactor in conversion of retinol to retinal. In malnourished populations, common low intakes of vitamin A and zinc increase the severity of vitamin A deficiency and lead physiological signs and symptoms of deficiency. A study in Burkina Faso showed major reduction of malaria morbidity with combined vitamin A and zinc supplementation in young children.

Due to the unique function of retinal as a visual chromophore, one of the earliest and specific manifestations of vitamin A deficiency is impaired vision, particularly in reduced light – night blindness. Persistent deficiency gives rise to a series of changes, the most devastating of which occur in the eyes. Some other ocular changes are referred to as xerophthalmia. First there is dryness of the conjunctiva (xerosis) as the normal lacrimal and mucus-secreting epithelium is replaced by a keratinized epithelium. This is followed by the build-up of keratin debris in small opaque plaques (Bitot's spots) and, eventually, erosion of the roughened corneal surface with softening and destruction of the cornea (keratomalacia) and total blindness. Other changes include impaired immunity (increased risk of ear infections, urinary tract infections, Meningococcal disease), hyperkeratosis (white lumps at hair follicles), keratosis pilaris and squamous metaplasia of the epithelium lining the upper respiratory passages and urinary bladder to a keratinized epithelium. With relations to dentistry, a deficiency in Vitamin A leads to enamel hypoplasia.

Adequate supply, but not excess vitamin A, is especially important for pregnant and breastfeeding women for normal fetal development. Deficiencies cannot be compensated by postnatal supplementation. Excess vitamin A, which is most common with high dose vitamin supplements, can cause birth defects and therefore should not exceed recommended daily values.

Vitamin A metabolic inhibition as a result of alcohol consumption during pregnancy is the elucidated mechanism for fetal alcohol syndrome and is characterized by teratogenicity closely matching maternal vitamin A deficiency.

Vitamin A supplementation

Global efforts to support national governments in addressing vitamin a deficiency are led by the Global Alliance for Vitamin A (GAVA), which is an informal partnership between A2Z, the Canadian International Development Agency, Helen Keller International, the Micronutrient Initiative, UNICEF, USAID, and the World Bank. Joint GAVA activity is coordinated by the Micronutrient Initiative.

While strategies include intake of vitamin A through a combination of breast feeding and dietary intake, delivery of oral high-dose supplements remain the principal strategy for minimizing deficiency. Studies have shown vitamin A supplementation of children under five who are at risk of deficiency can reduce mortality by 23%. About 75% of the vitamin A required for supplementation activity by developing countries is supplied by the Micronutrient Initiative with support from the Canadian International Development Agency. Food fortification approaches are becoming increasingly feasible but cannot yet ensure coverage levels.

An estimated 1.25 million deaths due to vitamin A deficiency have been averted in 40 countries since 1998. In 2008 it was estimated that an annual investment of US$60 million in vitamin A and zinc supplementation combined would yield benefits of more than US$1 billion per year, with every dollar spent generating benefits of more than US$17. These combined interventions were ranked by the Copenhagen Consensus 2008 as the world's best development investment.

Toxicity

Since vitamin A is fat-soluble, disposing of any excesses taken in through diet is much harder than with water-soluble B vitamins and vitamin C, and vitamin A toxicity is possible.

In general, acute toxicity occurs at doses of 25,000 IU/kg of body weight, with chronic toxicity occurring at 4,000 IU/kg of body weight daily for 6–15 months. However, liver toxicities can occur at levels as low as 15,000 IU per day to 1.4 million IU per day, with an average daily toxic dose of 120,000 IU per day, particularly with excessive consumption of alcohol. In people with renal failure, 4000 IU can cause substantial damage. In addition, excessive alcohol intake can increase toxicity. Children can reach toxic levels at 1,500 IU/kg of body weight.

Excessive vitamin A consumption can lead to nausea, irritability, anorexia (reduced appetite), vomiting, blurry vision, headaches, hair loss, muscle and abdominal pain and weakness, drowsiness, and altered mental status. In chronic cases, hair loss, dry skin, drying of the mucous membranes, fever, insomnia, fatigue, weight loss, bone fractures,

anemia, and diarrhea can all be evident on top of the symptoms associated with less serious toxicity. Some of these symptoms are also common to acne treatment with Isotretinoin. Chronically high doses of vitamin A, and also pharmaceutical retinoids such as 13-cis retinoic acid, can produce the syndrome of pseudotumor cerebri. This syndrome includes headache, blurring of vision and confusion, associated with increased intracerebral pressure. Symptoms begin to resolve when intake of the offending substance is stopped.

An estimated 75% of people in developed nations may be ingesting more than the RDA for vitamin A on a regular basis. Chronic intake of 1500 RAE of preformed vitamin A may be associated with osteoporosis and hip fractures. This may be due to the fact that an excess of vitamin A can block the expression of certain proteins dependent on vitamin K to reduce the efficacy of vitamin D, but has not yet been proven. High vitamin A intake has been associated with spontaneous bone fractures in animals. Cell culture studies have linked increased bone resorption and decreased bone formation with high intakes. This interaction may occur because vitamins A and D may compete for the same receptor and then interact with parathyroid hormone, which regulates calcium. Indeed, a study by Forsmo *et al.* shows a correlation between low bone mineral density and too high intake of vitamin A.

Toxic effects of vitamin A have been shown to significantly affect developing fetuses. Therapeutic doses used for acne treatment have been shown to disrupt cephalic neural cell activity. The fetus is particularly sensitive to vitamin A toxicity during the period of organogenesis. These toxicities only occur with preformed (retinoid) vitamin A (such as from liver). The carotenoid forms (such as beta-carotene as found in carrots), give no such symptoms, except with supplements and chronoic alcoholism, but excessive dietary intake of beta-carotene can lead to carotenodermia, which causes orange-yellow discoloration of the skin.

Smokers and chronic alcohol consumers have been observed to have increased risk of mortality due to lung cancer, esophageal cancer, gastrointestinal cancer and colon cancer. Hepatic (liver) injury been found in human and animal studies where consumption of alcohol is paired with high dose vitamin A and beta-carotene supplementation.

Researchers have succeeded in creating water-soluble forms of vitamin A, which they believed could reduce the potential for toxicity. However, a 2003 study found water-soluble vitamin A was approximately 10 times as toxic as fat-soluble vitamin. A 2006 study found children given water-soluble vitamin A and D, which are typically fat-soluble, suffer from asthma twice as much as a control group supplemented with the fat-soluble vitamins.

Vitamin A and derivatives in medical use

Retinyl palmitate has been used in skin creams, where it is broken down to retinoic acid, which has potent biological activity, as described above.

The retinoids, (for example, 13-cis-retinoic acid), constitute a class of chemical compounds chemically related to retinoic acid, and are used in medicine to modulate gene functions in place of this compound. Like retinoic acid, the related compounds do not have full vitamin A activity, but do have powerful effects on gene expression and epithelial cell differentiation.

Pharmaceutics utilizing mega doses of naturally occurring retinoic acid derivatives are currently in use for cancer, HIV, and dermatological purposes. At high doses, side-effects are similar to vitamin A toxicity. Severe side effects related to vitamin A toxicity, and a small optimal range of use are key obstacles in developing vitamin A-derived pharmaceutics for therapeutic use.

Chapter 3

Vitamin B1 (Thiamine)

Thiamine or **thiamin** or **vitamin B_1** and named as the "thio-vitamine" ("sulfur-containing vitamin") is a water-soluble vitamin of the B complex. First named *aneurin* for the detrimental neurological effects of its lack in the diet, it was eventually assigned the generic descriptor name vitamin B_1. Its phosphate derivatives are involved in many cellular processes. The best-characterized form is thiamine pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. In yeast, TPP is also required in the first step of alcoholic fermentation.

All living organisms use thiamine in their biochemistry, but it is synthesized in bacteria, fungi, and plants. Animals must obtain it from their diet, and, thus, for them it is a vitamin. Insufficient intake in birds produces a characteristic polyneuritis, and in mammals results in a disease called beriberi affecting the peripheral nervous system (polyneuritis) and/or the cardiovascular system, with fatal outcome if not cured by thiamine administration. In less severe deficiency, nonspecific signs include malaise, weight loss, irritability and confusion.

There is still much work devoted to elucidating the exact mechanisms by which thiamine deficiency leads to the specific symptoms observed. New thiamine phosphate derivatives have recently been discovered, emphasizing the complexity of thiamine metabolism and the need for more research in the field.

Thiamine derivatives with improved pharmacokinetics have been discovered and are to be considered more effective in alleviating the symptoms of thiamine deficiency and other thiamine-related conditions such as impaired glucose metabolism in diabetes. These compounds include allithiamine, prosultiamine, fursultiamine, benfotiamine, and sulbutiamine, among others.

History: The discovery of vitamins and the biochemical lesion

Thiamine was the first of the water-soluble vitamins to be described, leading to the discovery of more such trace compounds essential for survival and to the notion of vitamin.

In 1884, Kanehiro Takaki (1849–1920), a surgeon general in the Japanese navy, rejected the previous germ theory for beriberi and attributed the disease to insufficient diet instead. Switching diet on navy ships, he discovered that substituting white rice with brown barley rice will eliminate beriberi (he was nicknamed "Barley Baron" after obtaining peerage). However, he incorrectly attributed the benefit to nitrogen intake as vitamin was unknown substance at the time.

In 1897, Christiaan Eijkman (1858–1930), a military doctor in the Dutch Indies, discovered that fowl fed on a diet of cooked, polished rice developed paralysis, which could be reversed by discontinuing rice polishing. He attributed that to a nerve poison in the endosperm of rice, from which the outer layers of the grain gave protection to the body. Eijkman was awarded the Nobel Prize in Physiology and Medicine in 1929, because his observations led to the discovery of vitamins. An associate, Gerrit Grijns (1865–1944), correctly interpreted the connection between excessive consumption of polished rice and beriberi in 1901: He concluded that rice contains an essential nutrient in the outer layers of the grain that is removed by polishing.

In 1911, Casimir Funk isolated an antineuritic substance from rice bran that he called a "vitamine" (on account of its containing an amino group). Dutch chemists, Barend Coenraad Petrus Jansen (1884–1962) and his closest collaborator Willem Frederik Donath (1889–1957), went on to isolate and crystallize the active agent in 1926, whose structure was determined by Robert Runnels Williams (1886–1965), a US chemist, in 1934. Thiamine (“sulfur-containing vitamin”) was synthesized in 1936 by the same group.

It was first named "aneurin" (for anti-neuritic vitamin). Sir Rudolph Peters, in Oxford, introduced thiamine-deprived pigeons as a model for understanding how thiamine deficiency can lead to the pathological-physiological symptoms of beriberi. Indeed, feeding the pigeons upon polished rice leads to an easily recognizable behavior of head retraction, a condition called opisthotonos. If not treated, the animal will die after a few days. Administration of thiamine at the stage of opithotonos will lead to a complete cure of the animal within 30 min. As no morphological modifications were observed in the brain of the pigeons before and after treatment with thiamine, Peeters introduced the concept of biochemical lesion

When Lohman and Schuster (1937) showed that the diphosphorylated thiamine derivative (thiamine diphosphate, ThDP) was a cofactor required for the oxydative decarboxylation of pyruvate, (a reaction now known to be catalyzed by pyruvate dehydrogenase), the mechanism of action of thiamine in the cellular metabolism seemed to be elucidated. At present, this view seems to be oversimplified: Pyruvate dehydrogenase is only one of

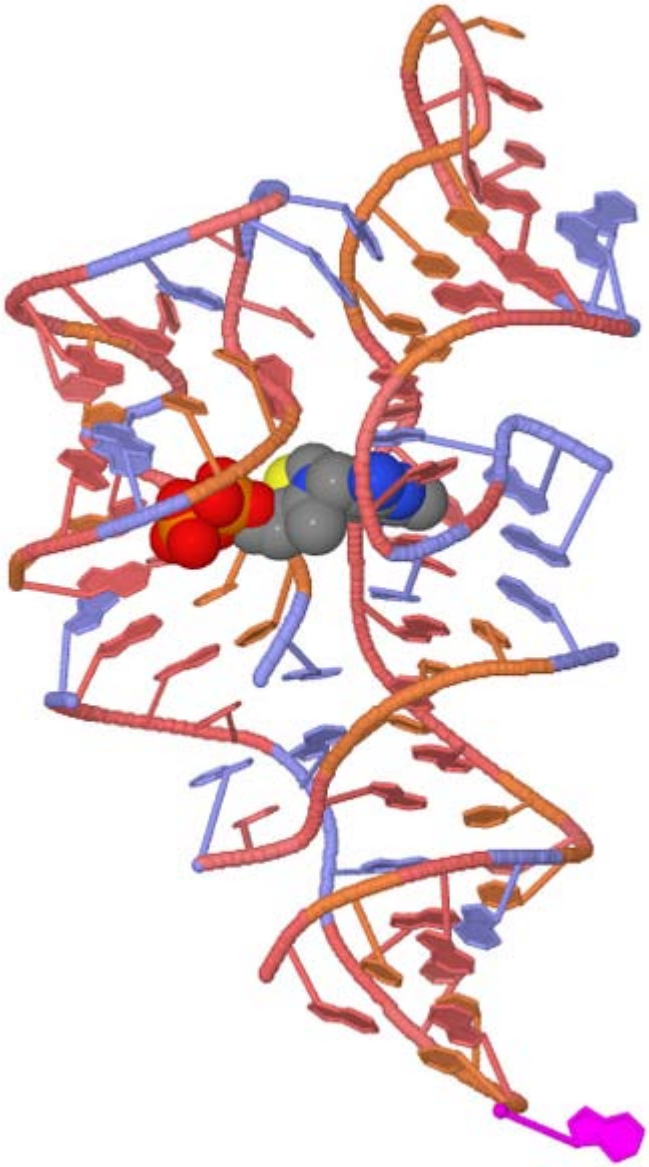
several enzymes requiring thiamine diphosphate as a cofactor; moreover, other thiamine phosphate derivatives have been discovered since then, and they may also contribute to the symptoms observed during thiamine deficiency.

Finally, the mechanism by which the thiamine moiety of ThDP exerts its coenzyme function by proton substitution on position 2 of the thiazolium ring was elucidated by Ronald Breslow in 1958.

Chemical properties

Thiamine is a colorless compound with a chemical formula $C_{12}H_{17}N_4OS$. Its structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether, chloroform, and benzene. It is stable at acidic pH, but is unstable in alkaline solutions. Thiamine is unstable to heat, but stable during frozen storage. It is unstable when exposed to ultraviolet light and gamma irradiation. Thiamine reacts strongly in Maillard-type reactions.

Biosynthesis



A 3D representation of the TPP riboswitch with thiamine bound

Complex thiamine biosynthetic pathways occur in bacteria, some protozoans, plants and fungi. The thiazole and pyrimidine moieties are synthesized separately and then assembled to form ThMP by thiamine-phosphate synthase (EC 2.5.1.3). The exact biosynthetic pathways may differ among organisms. In E. coli and other enterobacteriaceae ThMP may be phosphorylated to the cofactor ThDP by a thiamine-phosphate kinase (ThMP + ATP → ThDP + ADP, EC 2.7.4.16). In most bacteria and in eukaryotes, ThMP is hydrolyzed to thiamine, that may then be pyrophosphorylated to ThDP by thiamine diphosphokinase (thiamine + ATP → ThDP + AMP, EC 2.7.6.2).

The biosynthetic pathways are regulated by riboswitches in all organisms that synthesise thiamine. If there is sufficient thiamine present in the cell then the thiamine binds to the mRNA encoding genes required in the pathway preventing the translation of the enzymes. If there is no thiamine present then there is no inhibition, and the enzymes required for the biosynthesis are produced. The specific riboswitch, the TPP riboswitch, is the only riboswitch identified in both eukaryotic and prokaryotic organisms.

Nutrition

Occurrence in foods

Thiamine is found in a wide variety of foods at low concentrations. Yeast, yeast extract (e.g. Marmite) and pork are the most highly concentrated sources of thiamine. In general, cereal grains are the most important dietary sources of thiamine, by virtue of their ubiquity. Of these, whole grains contain more thiamine than refined grains, as thiamine is found mostly in the outer layers of the grain and in the germ (which are removed during the refining process). For example, 100 g of whole-wheat flour contains 0.55 mg of thiamine, while 100 g of white flour contains only 0.06 mg of thiamine. In the US, processed flour must be enriched with thiamine mononitrate (along with niacin, ferrous iron, riboflavin, and folic acid) to replace that lost in processing. A whole foods diet is therefore recommended for deficiency.

Some other foods rich in thiamine are oatmeal, flax, and sunflower seeds, brown rice, whole grain rye, asparagus, kale, cauliflower, potatoes, oranges, liver (beef, pork and chicken), and eggs.

Thiamine hydrochloride (Betaxin) is a (when by itself) white, crystalline hygroscopic food-additive used to add a brothy/meaty flavor to gravies or soups. It is a natural intermediary resulting from a thiamine-HCl reaction, which precedes hydrolysis and phosphorylation, before it is finally employed (in the form of TPP) in a number of enzymatic amino, fatty acid, and carbohydrate reactions.

Reference Daily Intake and high doses

The RDA in most countries is set at about 1.4 mg. However, tests on female volunteers at daily doses of about 50 mg have claimed an increase in mental acuity. There are no reports available of adverse effects from consumption of excess thiamine by ingestion of food and supplements. Because the data is inadequate for a quantitative risk assessment, no Tolerable Upper Intake Level (UL) can be derived for thiamine.

Antagonists

Thiamine in foods can be degraded in a variety of ways. Sulfites, which are added to foods usually as a preservative, will attack thiamine at the methylene bridge in the structure, cleaving the pyrimidine ring from the thiazole ring. The rate of this reaction is increased under acidic conditions. Thiamine is degraded by thermolabile thiaminases

(present in raw fish and shellfish). Some thiaminases are produced by bacteria. Bacterial thiaminases are cell surface enzymes that must dissociate from the membrane before being activated; the dissociation can occur in ruminants under acidotic conditions. Rumen bacteria also reduce sulfate to sulfite, therefore high dietary intakes of sulfate can have thiamine-antagonistic activities.

Plant thiamine antagonists are heat-stable and occur as both the ortho- and para-hydroxyphenols. Some examples of these antagonists are caffeic acid, chlorogenic acid, and tannic acid. These compounds interact with the thiamine to oxidize the thiazole ring, thus rendering it unable to be absorbed. Two flavonoids, quercetin and rutin, have also been implicated as thiamine antagonists.

Absorption and transport

Absorption

Thiamine is released by the action of phosphatase and pyrophosphatase in the upper small intestine. At low concentrations, the process is carrier mediated and at higher concentrations, absorption occurs via passive diffusion. Active transport is greatest in the jejunum and ileum (it is inhibited by alcohol consumption and by folic deficiency). Decline in thiamine absorption occurs at intakes above 5 mg. The cells of the intestinal mucosa have thiamine pyrophosphokinase activity, but it is unclear whether the enzyme is linked to active absorption. The majority of thiamine present in the intestine is in the pyrophosphorylated form ThDP, but when thiamine arrives on the serosal side of the intestine it is often in the free form. The uptake of thiamine by the mucosal cell is likely coupled in some way to its phosphorylation/dephosphorylation. On the serosal side of the intestine, evidence has shown that discharge of the vitamin by those cells is dependent on Na^+-dependent ATPase.

Bound to serum proteins

The majority of thiamine in serum is bound to proteins, mainly albumin. Approximately 90% of total thiamine in blood is in erythrocytes. A specific binding protein called thiamine-binding protein (TBP) has been identified in rat serum and is believed to be a hormonally regulated carrier protein that is important for tissue distribution of thiamine.

Cellular uptake

Uptake of thiamine by cells of the blood and other tissues occurs via active transport and passive diffusion. About 80% of intracellular thiamine is phosphorylated and most is bound to proteins. In some tissues, thiamine uptake and secretion appears to be mediated by a soluble thiamine transporter that is dependent on Na^+ and a transcellular proton gradient.

Tissue distribution

Human storage of thiamine is about 25 to 30 mg with the greatest concentrations in skeletal muscle, heart, brain, liver, and kidneys. ThMP and free (unphosphorylated) thiamine is present in plasma, milk, cerebrospinal fluid, and likely all extracellular fluids. Unlike the highly phosphorylated forms of thiamine, ThMP and free thiamine are capable of crossing cell membranes. Thiamine contents in human tissues are less than those of other species.

Excretion

Thiamine and its acid metabolites (2-methyl-4-amino-5-pyrimidine carboxylic acid, 4-methyl-thiazole-5-acetic acid and thiamine acetic acid) are excreted principally in the urine.

Thiamine phosphate derivatives and function

Thiamine is mainly the transport form of the vitamin, while the active forms are phosphorylated thiamine derivatives. There are four known natural thiamine phosphate derivatives: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), also sometimes called thiamine pyrophosphate (TPP), thiamine triphosphate (ThTP), and the recently discovered adenosine thiamine triphosphate (AThTP) and adenosine thiamine diphosphate (AThDP).

Thiamine monophosphate

There is no known physiological role of ThMP.

Thiamine diphosphate

The synthesis of thiamine diphosphate (ThDP), also known as *thiamine pyrophosphate* (TPP) or *cocarboxylase*, is catalyzed by an enzyme called thiamine diphosphokinase according to the reaction thiamine + ATP → ThDP + AMP (EC 2.7.6.2). ThDP is a coenzyme for several enzymes that catalyze the transfer of two-carbon units and in particular the dehydrogenation (decarboxylation and subsequent conjugation with coenzyme A) of 2-oxoacids (alpha-keto acids). Examples include:

- Present in most species
  - pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase (also called α-ketoglutarate dehydrogenase)
  - branched-chain α-keto acid dehydrogenase
  - 2-hydroxyphytanoyl-CoA lyase
  - transketolase

- Present in some species:
  - pyruvate decarboxylase (in yeast)

- several additional bacterial enzymes

The enzymes transketolase, pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH) are all important in carbohydrate metabolism. The cytosolic enzyme transketolase is a key player in the pentose phosphate pathway, a major route for the biosynthesis of the pentose sugars deoxyribose and ribose. The mitochondrial PDH and OGDH are part of biochemical pathways that result in the generation of adenosine triphosphate (ATP), which is a major form of energy for the cell. PDH links glycolysis to the citric acid cycle, while the reaction catalyzed by OGDH is a rate-limting step in the citric acid cycle. In the nervous system, PDH is also involved in the production of acetylcholine, a neurotransmitter, and for myelin synthesis.

Thiamine triphosphate

Thiamine triphosphate (ThTP) was long considered a specific neuroactive form of thiamine. However, recently it was shown that ThTP exists in bacteria, fungi, plants and animals suggesting a much more general cellular role. In particular in *E. coli*, it seems to play a role in response to amino acid starvation.

Adenosine thiamine triphosphate

Adenosine thiamine triphosphate (AThTP) or thiaminylated adenosine triphosphate has recently been discovered in *Escherichia coli* where it accumulates as a result of carbon starvation. In *E. coli*, AThTP may account for up to 20% of total thiamine. It also exists in lesser amounts in yeast, roots of higher plants and animal tissue.

Adenosine thiamine diphosphate

Adenosine thiamine diphosphate (AThDP) or thiaminylated adenosine diphosphate exists in small amounts in vertebrate liver, but its role remains unknown.

Deficiency

Thiamine derivatives and thiamine-dependent enzymes are present in all cells of the body, thus, a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system and the heart are particularly sensitive to thiamine deficiency, because of their high oxidative metabolism.

Thiamine deficiency can lead to severe fatigue of eyes and myriad problems including neurodegeneration, wasting, and death. A lack of thiamine can be caused by malnutrition, a diet high in thiaminase-rich foods (raw freshwater fish, raw shellfish, ferns) and/or foods high in anti-thiamine factors (tea, coffee, betel nuts) and by grossly impaired nutritional status associated with chronic diseases, such as alcoholism, gastrointestinal diseases, HIV-AIDS, and persistent vomiting. It is thought that many people with diabetes have a deficiency of thiamine and that this may be linked to some of the complications that can occur.

Well-known syndromes caused by thiamine deficiency include beriberi and Wernicke-Korsakoff syndrome, diseases also common with chronic alcoholism.

Beriberi

Beriberi is a neurological and cardiovascular disease. The three major forms of the disorder are dry beriberi, wet beriberi, and infantile beriberi.

- *Dry beriberi* is characterized principally by peripheral neuropathy consisting of symmetric impairment of sensory, motor, and reflex functions affecting distal more than proximal limb segments and causing calf muscle tenderness.
- *Wet beriberi* is associated with mental confusion, muscular atrophy, edema, tachycardia, cardiomegaly, and congestive heart failure in addition to peripheral neuropathy.
- *Infantile beriberi* occurs in infants breast-fed by thiamin-deficient mothers (who may show no sign of thiamine deficiency). Infants may manifest cardiac, aphonic, or pseudomeningitic forms of the disorder. Infants with cardiac beriberi frequently exhibit a loud piercing cry, vomiting, and tachycardia. Convulsions are not uncommon, and death may ensue if thiamine is not administered promptly.

Following thiamine treatment, rapid improvement occurs, in general, within 24 hours. Improvements of peripheral neuropathy may require several months of thiamine treatment.

Alcoholic brain disease

Nerve cells and other supporting cells (such as glial cells) of the nervous system require thiamine. Examples of neurologic disorders that are linked to alcohol abuse include Wernicke's encephalopathy (WE, Wernicke-Korsakoff syndrome) and Korsakoff's psychosis (alcohol amnestic disorder) as well as varying degrees of cognitive impairment.

Wernicke's encephalopathy is the most frequently encountered manifestation of thiamine deficiency in Western society, though it may also occur in patients with impaired nutrition from other causes, such as gastrointestinal disease, those with HIV-AIDS, and with the injudicious administration of parenteral glucose or hyperalimentation without adequate B-vitamin supplementation. This is a striking neuro-psychiatric disorder characterized by paralysis of eye movements, abnormal stance and gait, and markedly deranged mental function.

Alcoholics may have thiamine deficiency because of the following:

- Inadequate nutritional intake: Alcoholics tend to intake less than the recommended amount of thiamine.
- Decreased uptake of thiamine from the GI tract: Active transport of thiamine into enterocytes is disturbed during acute alcohol exposure.
- Liver thiamine stores are reduced due to hepatic steatosis or fibrosis.

- Impaired thiamine utilization: Magnesium, which is required for the binding of thiamine to thiamine-using enzymes within the cell, is also deficient due to chronic alcohol consumption. The inefficient utilization of any thiamine that does reach the cells will further exacerbate the thiamine deficiency.
- Ethanol *per se* inhibits thiamine transport in the gastrointestinal system and blocks phosphorylation of thiamine to its cofactor form (ThDP).

Korsakoff Psychosis is, in general, considered to occur with deterioration of brain function in patients initially diagnosed with WE. This is an amnestic-confabulatory syndrome characterized by retrograde and anterograde amnesia, impairment of conceptual functions, and decreased spontaneity and initiative.<

Following improved nutrition and the removal of alcohol consumption, some impairments linked with thiamine deficiency are reversed; particularly poor brain functionality, although in more severe cases, Wernicke-Korsakoff syndrome leaves permanent damage.

Thiamine deficiency in poultry

As most feedstuffs used in poultry diets contain enough quantities of vitamins to meet the requirements in this species, deficiencies in this vitamin do not occur with commercial diets. This was, at least, the opinion in the 1960s.

Mature chickens show signs 3 weeks after being fed a deficient diet. In young chicks, it can appear before 2 weeks of age.

Onset is sudden in young chicks. There is anorexia and an unsteady gait. Later on, there are locomotor signs, beginning with an apparent paralysis of the flexor of the toes. The characteristic position is called "stargazing", meaning a chick "sitting on its hocks and the head in opisthotonos".

Response to administration of the vitamin is rather quick, occurring a few hours later.

Differential diagnosis include riboflavin deficiency and avian encephalomyelitis. In riboflavin deficiency, the "curled toes" is a characteristic symptom. Muscle tremor is typical of avian encephalomyelitis. A therapeutic diagnosis can be tried by supplementing thiamine only in the affected bird. If the animals do not respond in a few hours, thiamine deficiency can be excluded.

Thiamine deficiency in ruminants

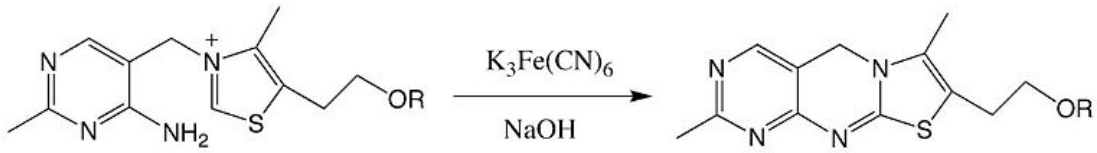
Polioencephalomalacia (PEM) is the most common thiamine deficiency disorder in young ruminant and nonruminant animals. Symptoms of PEM include a profuse, but transient, diarrhea, listlessness, circling movements, star gazing or opisthotonus (head drawn back over neck), and muscle tremors. The most common cause is high-carbohydrate feeds, leading to the overgrowth of thiaminase-producing bacteria, but

dietary ingestion of thiaminase (e.g., in bracken fern), or inhibition of thiamine absorption by high sulfur intake are also possible. Another cause of PEM is *Clostridium sporogenes* or *Bacillus aneurinolyticus* infection. These bacteria produce thiaminases that will cause an acute thiamine deficiency in the affected animal.

Idiopathic paralytic disease in wild birds

Recently, thiamine deficiency has been identified as the cause of a paralytic disease affecting wild birds in the Baltic Sea area dating back to 1982. In this condition, there is difficulty in keeping the wings folded along the side of the body when resting, loss of the ability to fly and voice, with eventual paralysis of the wings and legs and death. It affects primarily 0.5–1 kg sized birds such as the herring gull (Larus argentatus), Common Starling (Sturnus vulgaris) and Common Eider (Somateria mollissima). Researches noted, "Because the investigated species occupy a wide range of ecological niches and positions in the food web, we are open to the possibility that other animal classes may suffer from thiamine deficiency as well."

Analysis and diagnostic testing



R = H	Thiamine	Thiochrome
R = PO_3^{2-}	ThMP	ThcMP
R = $P_2O_6^{3-}$	ThDP	ThcDP
R = $P_3O_9^{4-}$	ThTP	ThcTP
R = $P_3O_8^{3-}$-adénosine	AThTP	AThcTP

Oxidation of thiamine derivatives to fluorescent thiochromes by potassium ferricyanide under alkaline conditions

A positive diagnosis test for thiamine deficiency can be ascertained by measuring the activity of the enzyme transketolase in erythrocytes (Erythrocyte Transketolase Activation Assay). Thiamine, as well as its phosphate derivatives, can also be detected directly in whole blood, tissues, foods, animal feed, and pharmaceutical preparations following the conversion of thiamine to fluorescent thiochrome derivatives (Thiochrome Assay) and separation by high-performance liquid chromatography (HPLC). In recent reports, a number of Capillary Electrophoresis (CE) techniques and in-capillary enzyme reaction methods have emerged as potential alternative techniques for the determination and monitoring of thiamine in samples.

Genetic diseases

Genetic diseases of thiamine transport are rare but serious. Thiamine responsive megaloblastic anemia (TRMA) with diabetes mellitus and sensorineural deafness is an autosomal recessive disorder caused by mutations in the gene SLC19A2, a high affinity thiamine transporter. TRMA patients do not show signs of systemic thiamine deficiency, suggesting redundancy in the thiamine transport system. This has led to the discovery of a second high-affinity thiamine transporter, SLC19A3. Leigh disease (subacute necrotising encephalomyelopathy) is an inherited disorder that affects mostly infants in the first years of life and is invariably fatal. Pathological similarities between Leigh disease and WE led to the hypothesis that the cause was a defect in thiamine metabolism. One of the most consistent findings has been an abnormality of the activation of the pyruvate dehydrogenase complex.

Other disorders in which a putative role for thiamine has been implicated include subacute necrotizing encephalomyelopathy, opsoclonic cerebellopathy (a paraneoplastic syndrome), and Nigerian seasonal ataxia. In addition, several inherited disorders of ThDP-dependent enzymes have been reported, which may respond to thiamine treatment.

Research

Research in the field mainly concerns the mechanisms by which thiamine deficiency leads to neuronal death in relation to Wernicke Korsakoff Psychosis. Another important field concerns the understanding of the molecular mechanisms involved in ThDP catalysis. More recently, research has been devoted to the understanding of the possible non-cofactor roles of other derivatives such as ThTP and AThTP.

Understanding the mechanism by which thiamine deficiency leads to selective neuronal death

Experimentally induced beriberi polyneuropathy in chickens may be a good model for studying these forms of neuropathy in view of diagnosis and treatment. From studies using rat models, a link between thiamine deficiency and colon carcinogenesis was suggested. Rat model is used also in research of Wernicke's encephalopathy. Thiamine deprived mice are a classic model of systemic oxidative stress, used in research of Alzheimer’s disease.

Catalytic mechanisms in thiamine diphosphate-dependent enzymes

A lot of work is devoted to the understanding of the interplay between ThDP and ThDP-dependent enzymes in catalysis.

Non-cofactor roles of thiamine derivatives

Thiamine compounds other than ThDP exist in most cells from many organisms, including bacteria, fungi, plants and animals. Among those compounds are thiamine triphosphate (ThTP) and adenosine thiamine triphosphate (AThTP) are thought to have non-cofactor roles, though at present it is not known to what extent they participate in the symptoms

Persistent carbenes

The production of furoin from furfural is catalyzed by thiamine through a relatively stable carbene (an organic molecule containing unbonded valence electrons pairs at a carbon center). This reaction, studied in 1957 by R. Breslow, was the first evidence for the existence of persistent carbenes.

Chapter 4

Vitamin B2 (Riboflavin)

Riboflavin, also known as **vitamin B_2** or **additive E101**, is an easily absorbed micronutrient with a key role in maintaining health in humans and other animals. It is the central component of the cofactors FAD and FMN, and is therefore required by all flavoproteins. As such, vitamin B_2 is required for a wide variety of cellular processes. It plays a key role in energy metabolism, and for the metabolism of fats, ketone bodies, carbohydrates, and proteins.

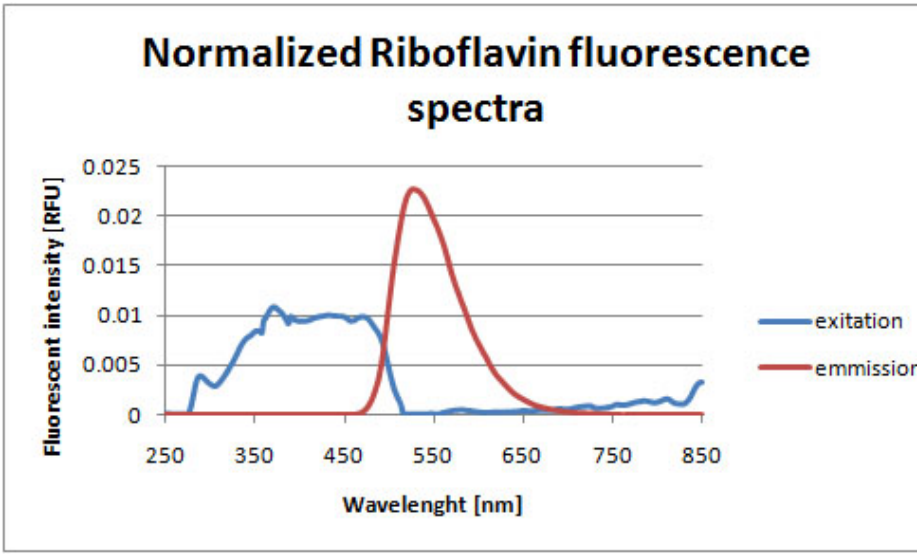
Milk, cheese, leafy green vegetables, liver, kidneys, legumes, tomatoes, yeast, mushrooms, and almonds are good sources of vitamin B_2, but exposure to light destroys riboflavin.

The name "riboflavin" comes from "ribose" (the sugar which forms part of its structure, which in turn is a transposition of arabinose) and "flavin", the ring-moiety which imparts the yellow color to the oxidized molecule (from Latin *flavus*, "yellow"). The reduced form, which occurs in metabolism, is colorless.

Riboflavin is best known visually as the vitamin which imparts the orange color to solid B-vitamin preparations, the yellow color to vitamin supplement solutions, and the unusual fluorescent yellow color to the urine of persons who supplement with high-dose B-complex preparations (no other vitamin imparts any color to urine).

Discovery

Riboflavin powder.



Fluorescent spectra of Riboflavin

Vitamin B was originally considered to have two components, a heat-labile vitamin B_1 and a heat-stable vitamin B_2. In the 1920s, vitamin B_2 was thought to be the factor necessary for preventing pellagra. In 1923, Paul Gyorgy in Heidelberg was investigating egg white injury in rats; the curative factor for this condition was called vitamin H. Since both pellagra and vitamin H deficiency were associated with dermatitis, Gyorgy decided to test the effect of vitamin B_2 on vitamin H deficiency in rats. He enlisted the service of Wagner-Jauregg in Kuhn's laboratory (1). In 1933, Kuhn, Gyorgy, and Wagner found that thiamin-free extracts of yeast, liver, or rice bran prevented the growth failure of rats fed a thiamin-supplemented diet. Further, they noted that a yellow-green fluorescence in each extract promoted rat growth, and that the intensity of fluorescence was proportional to the effect on growth. This observation enabled them to develop a rapid chemical and bioassay to isolate the factor from egg white in 1933, they called it Ovoflavin. The same group then isolated the same preparation (a growth-promoting compound with yellow-green fluorescence) from whey using the same procedure (lactoflavin). In 1934 Kuhn's group identified the structure of so-called flavin and synthesized vitamin B_2.

Biochemical function

Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) function as coenzymes for a wide variety of oxidative enzymes and remain bound to the enzymes during the oxidation-reduction reactions. Flavaflavins can act as oxidizing agents because of their ability to accept a pair of hydrogen atoms. Reduction of isoalloxazine ring (FAD, FMN oxidized form) yields the reduced forms of the flavoproteins (FMNH2 and FADH2).

Mechanism of action as cofactors and flavoproteins

Flavoproteins exhibit a wide range of redox potential and therefore can play a wide variety of roles in intermediary metabolism. Some of these roles are:

- Flavoproteins play very important roles in the electron transport chain
- Decarboxylation of pyruvate and α-ketoglutarate requires FAD
- Fatty acyl CoA dehydrogenase requires FAD in fatty acid oxidation
- FAD is required to the production of pyridoxic acid from pyridoxal (vitamin B_6)
- The primary coenzyme form of vitamin B_6 (pyridoxal phosphate) is FMN dependent
- FAD is required to convert retinal (vitamin A) to retinoic acid
- Synthesis of an active form of folate (5-methyl THF) is FADH2 dependent
- FAD is required to convert tryptophan to niacin (vitamin B_3)
- Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) is also FAD dependent

Riboflavin in food: occurrence, sources and stability

A solution of riboflavin.

Riboflavin is yellow or yellow-orange in color and in addition to being used as a food coloring, it is also used to fortify some foods. It is used in baby foods, breakfast cereals, pastas, sauces, processed cheese, fruit drinks, vitamin-enriched milk products, and some energy drinks. Regarding occurrence and sources of vitamin B_2, yeast extract is considered to be exceptionally rich in vitamin B_2, and liver and kidney are also rich sources. Wheat bran, eggs, meat, milk, and cheese are important sources in diets containing these foods. Cereals grains contain relatively low concentrations of flavins, but are important sources in those parts of the world where cereals constitute the staple diet. The milling of cereals results in considerable loss (up to 60%) of vitamin B_2, so

white flour is enriched in some countries such as USA by addition of the vitamin. The enrichment of bread and ready-to-eat breakfast cereals contributes significantly to the dietary supply of vitamin B_2. Polished rice is not usually enriched, because the vitamin's yellow color would make the rice visually unacceptable to the major rice-consumption populations. However, most of the flavins content of the whole brown rice is retained if the rice is steamed prior to milling. This process drives the flavins in the germ and aleurone layers into the endosperm. Free riboflavin is naturally present in foods along with protein-bound FMN and FAD. Bovine milk contains mainly free riboflavin, with a minor contribution from FMN and FAD. In whole milk, 14% of the flavins are bound noncovalently to specific proteins. Egg white and egg yolk contain specialized riboflavin-binding proteins, which are required for storage of free riboflavin in the egg for use by the developing embryo.

It is difficult to incorporate riboflavin into many liquid products because it has poor solubility in water, hence the requirement for riboflavin-5'-phosphate (E101a), a more expensive but more soluble form of riboflavin.

Riboflavin is generally stable during the heat processing and normal cooking of foods if light is excluded. The alkaline conditions in which riboflavin is unstable are rarely encountered in foodstuffs. Riboflavin degradation in milk can occur slowly in dark during storage in the refrigerator. (7).

Nutrition and recommended dietary allowance

Recommended dietary allowance (RDA)

The latest (1998) RDA recommendations for vitamin B_2 are similar to the 1989 RDA, which for adults, suggested a minimum intake of 1.2 mg for persons whose caloric intake may be > 2,000 Kcal. The current RDAs for riboflavin for adult men and women are 1.3 mg/day and 1.1 mg/day, respectively; the estimated average requirement for adult men and women are 1.1 mg and 0.9 mg, respectively. Recommendations for daily riboflavin intake increase with pregnancy and lactation to 1.4 mg and 1.6 mg, respectively (1in advanced). For infants, the RDA is 0.3-0.4 mg/day and for children it is 0.6-0.9 mg/day.

Riboflavin deficiency

Riboflavin is continuously excreted in the urine of healthy individuals, making deficiency relatively common when dietary intake is insufficient. However, riboflavin deficiency is always accompanied by deficiency of other vitamins.

A deficiency of riboflavin can be primary - poor vitamin sources in one's daily diet - or secondary, which may be a result of conditions that affect absorption in the intestine, the body not being able to use the vitamin, or an increase in the excretion of the vitamin from the body.

In humans, signs and symptoms of riboflavin deficiency (ariboflavinosis) include cracked and red lips, inflammation of the lining of mouth and tongue, mouth ulcers, cracks at the corners of the mouth (angular cheilitis), and a sore throat. A deficiency may also cause dry and scaling skin, fluid in the mucous membranes, and iron-deficiency anemia. The eyes may also become bloodshot, itchy, watery and sensitive to bright light.

Riboflavin deficiency is classically associated with the oral-ocular-genital syndrome. Angular cheilitis, photophobia, and scrotal dermatitis are the classic remembered signs.

In animals, riboflavin deficiency results in lack of growth, failure to thrive, and eventual death. Experimental riboflavin deficiency in dogs results in growth failure, weakness, ataxia, and inability to stand. The animals collapse, become comatose, and die. During the deficiency state, dermatitis develops together with hair loss. Other signs include corneal opacity, lenticular cataracts, hemorrhagic adrenals, fatty degeneration of the kidney and liver, and inflammation of the mucous membrane of the gastrointestinal tract. Post-mortem studies in rhesus monkeys fed a riboflavin-deficient diet revealed about one-third the normal amount of riboflavin was present in the liver, which is the main storage organ for riboflavin in mammals. These overt clinical signs of riboflavin deficiency are rarely seen among inhabitants of the developed countries. However, about 28 million Americans exhibit a common ‘sub-clinical’ stage. characterized by a change in biochemical indices (e.g. reduced plasma erythrocyte glutathione reductase levels). Although the effects of long-term subclinical riboflavin deficiency are unknown, in children this deficiency results in reduced growth. Subclinical riboflavin deficiency has also been observed in women taking oral contraceptives, in the elderly, in people with eating disorders, and in disease states such as HIV, inflammatory bowel disease, diabetes and chronic heart disease. The fact that riboflavin deficiency does not immediately lead to gross clinical manifestations indicates that the systemic levels of this essential vitamin are tightly regulated.

Assessment of riboflavin status

Biochemical tests are essential for confirming clinical cases of riboflavin deficiency and for establishing subclinical deficiencies. Among these tests:

- **Erythrocyte glutathione reductase activity**:

Glutathione reductase is a nicotinamide adenine dinucleotide phosphate (NADPH), a FAD-dependent enzyme, and the major flavoproteins in erythrocyte. The measurement of the activity coefficient of erythrocyte glutathione reductase (EGR) is the preferred method for assessing riboflavin status. It provides a measure of tissue saturation and long-term riboflavin status. In vitro enzyme activity in terms of activity coefficients (AC) is determined both with and without the addition of FAD to the medium. ACs represent a ratio of the enzyme’s activity with FAD to the enzyme’s activity without FAD. An AC of 1.2 to 1.4, riboflavin status is considered low when FAD is added to stimulate enzyme activity. An AC > 1.4 suggests riboflavin deficiency. On the other hand, if FAD is added and AC is < 1.2, then riboflavin status is considered acceptable. Tillotson and Baker

(1972) reported that a decrease in the intakes of riboflavin was associated with increase in EGR AC. in the U.K. study of Norwich elderly (Bailey et al., 1997), initial EGR AC values for both males and females were significantly correlated with those measured 2 years later, suggesting that EGR AC may be a reliable measure of long-term biochemical riboflavin status of individuals. These findings are consistent with earlier studies (Rutishauser et al., 1979).

- **Urinary riboflavin excretion**:

Experimental balance studies indicate that urinary riboflavin excretion rates increase slowly with increasing intakes, until intake level approach 1.0 mg/d, when tissue saturation occurs. At higher intakes, the rate of excretion increases dramatically. Once intakes of 2.5 mg/d are reached, excretion becomes approximately equal to the rate of absorption (Horwitt et al., 1950)(18). At such high intake a significant proportion of the riboflavin intake is not absorbed.If urinary riboflavin excretion is <19 µg/g creatinine (without recent riboflavin intake) or < 40 µg per day are indicative of deficiency.

Clinical uses

Riboflavin has been used in several clinical and therapeutic situations. For over 30 years, riboflavin supplements have been used as part of the phototherapy treatment of neonatal jaundice. The light used to irradiate the infants breaks down not only bilirubin, the toxin causing the jaundice, but also the naturally occurring riboflavin within the infant's blood, so extra supplementation is necessary.

High dose riboflavin appears to be useful alone or along with beta-blockers in the prevention of migraine. A dose of 400 mg daily has been used effecitvely in the prophylaxis of migraines, especially in combination with a daily supplement of magnesium citrate 500 mg and, in some cases, a supplement of coenzyme Q10.

Riboflavin has also been used as a muscle pain reliever.

Riboflavin in combination with UV light has been shown to be effective in reducing the ability of harmful pathogens found in blood products to cause disease. When UV light is applied to blood products containing riboflavin, the nucleic acids in pathogens are damaged, rendering them unable to replicate and cause disease. Riboflavin and UV light treatment has been shown to be effective for inactivating pathogens in platelets and plasma, and is under development for application to whole blood. Because platelets and red blood cells do not contain a nucleus (ie they have no DNA to be damaged). The technique is well-suited for destroying nucleic acid containing pathogens (including viruses, bacteria, parasites, and white blood cells) in blood products.

Recently, riboflavin has been used in a new treatment to slow or stop the progression of the corneal disorder keratoconus. This is called corneal collagen crosslinking (CXL). In corneal crosslinking, riboflavin drops are applied to the patient’s corneal surface. Once

the riboflavin has penetrated through the cornea, ultraviolet A light therapy is applied. This induces collagen crosslinking, which increases the tensile strength of the cornea. The treatment has been shown in several studies to stabilize keratoconus.

Industrial uses

Because riboflavin is fluorescent under UV light, dilute solutions (0.015-0.025% w/w) are often used to detect leaks or to demonstrate coverage in an industrial system such a chemical blend tank or bioreactor.

Good sources

Riboflavin is found naturally in asparagus, popcorn, bananas, persimmons, okra, chard, cottage cheese, milk, yogurt, meat, eggs, fish, and green beans (particularly on the ends), each of which contain at least 0.1 mg of the vitamin per 3–10.5 oz (85–300 g) serving.(5). Riboflavin is destroyed by exposure to ultraviolet light, so milk sold in transparent (glass/plastic) bottles will likely contain less riboflavin than milk sold in opaque containers.

Toxicity

Riboflavin is not toxic when taken orally, as its low solubility keeps it from being absorbed in dangerous amounts from the gut. Although toxic doses can be administered by injection, any excess at nutritionally relevant doses is excreted in the urine, imparting a bright yellow color when in large quantities. In humans, there is no evidence for riboflavin toxicity produced by excessive intakes, though it helps relieve muscle pain. Even when 400 mg/d of riboflavin was given orally to subjects in one study for three months to investigate the efficacy of riboflavin in the prevention of migraine headache, no short-term side effects were reported.

Industrial synthesis

Large cultures of *Micrococcus luteus* growing on pyridine (left)and succinic acid (right). The yellow pigment being produced in the presence of pyridine is riboflavin.

Various biotechnological processes have been developed for industrial scale riboflavin biosynthesis using different microorganisms, including filamentous fungi such as *Ashbya gossypii*, *Candida famata* and *Candida flaveri*, as well as the bacteria *Corynebacterium ammoniagenes* and *Bacillus subtilis*. The latter organism has been genetically modified to both increase the bacteria's production of riboflavin and to introduce an antibiotic (ampicillin) resistance marker, and is now successfully employed at a commercial scale to produce riboflavin for feed and food fortification purposes. The chemical company BASF has installed a plant in South Korea, which is specialized on riboflavin production using *Ashbya gossypii*. The concentrations of riboflavin in their modified strain are so high, that the mycelium has a reddish / brownish color and accumulates riboflavin crystals in the vacuoles, which will eventually burst the mycelium. Riboflavin is sometimes overproduced, possibly as a protective mechanism, by certain bacteria in the presence of high concentrations of hydrocarbons or aromatic compounds. One such organism is *Micrococcus luteus* (American Type Culture Collection strain number ATCC 49442), which develops a yellow color due to production of riboflavin while growing on pyridine, but not when grown on other substrates, such as succinic acid.

Chapter 5

Vitamin B3 (Niacin)

Niacin (also known as **vitamin B_3**, **nicotinic acid** and **vitamin PP**) is an organic compound with the formula $C_6H_5NO_2$ and, depending on the definition used, one of the forty to eighty essential human nutrients. This colorless, water-soluble solid is a derivative of pyridine, with a carboxyl group (COOH) at the 3-position. Other forms of vitamin B_3 include the corresponding amide, nicotinamide ("niacinamide"), where the carboxyl group has been replaced by a carboxamide group ($CONH_2$), as well as more complex amides and a variety of esters. The terms niacin, nicotinamide, and vitamin B_3 are often used interchangeably to refer to any member of this family of compounds, since they have the same biochemical activity.

Niacin cannot be directly converted to nicotinamide, but both compounds could be converted to NAD and NADP *in vivo*. Although the two are identical in their vitamin activity, nicotinamide does not have the same pharmacological effects as niacin, which occur as side effects of niacin's conversion. Nicotinamide does not reduce cholesterol or cause flushing. Nicotinamide may be toxic to the liver at doses exceeding 3 g/day for adults. Niacin is a precursor to NAD+/NADH and NADP+/NADPH, which play essential metabolic roles in living cells. Niacin is involved in both DNA repair, and the production of steroid hormones in the adrenal gland.

Niacin is one of five vitamins associated with a pandemic deficiency disease:

- niacin deficiency (pellagra)
- vitamin C deficiency (scurvy)
- thiamin deficiency (beriberi)
- vitamin D deficiency (rickets)
- vitamin A deficiency.

In larger doses, niacin can reverse atherosclerosis by lowering low-density lipoprotein (LDL) and favorably affecting other compounds.

History

Niacin was first described by Hugo Weidel in 1873 in his studies of nicotine. The original preparation remains useful: The oxidation of nicotine using nitric acid. Niacin was extracted from livers by Conrad Elvehjem, who later identified the active ingredient, then referred to as the "pellagra-preventing factor" and the "anti-blacktongue factor." When the biological significance of nicotinic acid was realized, it was thought appropriate to choose a name to dissociate it from nicotine, to avoid the perception that vitamins or niacin-rich food contains nicotine, or that cigarettes contain vitamins. The resulting name 'niacin' was derived from **ni**cotinic **ac**id + vitam**in.**

Carpenter found in 1951 that niacin in corn is biologically unavailable, and can be released only in very alkaline lime water of pH 11. This process is known as nixtamalization.

Niacin is referred to as vitamin B_3 because it was the third of the B vitamins to be discovered. It has historically been referred to as "vitamin PP" or "vitamin P-P".

Dietary needs

The recommended daily allowance of niacin is 2–12 mg/day for children, 14 mg/day for women, 16 mg/day for men, and 18 mg/day for pregnant or breast-feeding women. The upper limit for adult men and women is 35 mg/day, which is based on flushing as the critical adverse effect.

In general, niacin status is tested through urinary biomarkers, which are believed to be more reliable than plasma levels.

Deficiency

A man with pellagra, which is caused by a chronic lack of vitamin B_3 in the diet

At present, niacin deficiency is rarely seen in developed countries, but it is usually apparent in conditions of poverty, malnutrition, and chronic alcoholism. It also tends to occur in areas where people eat maize (corn, the only grain low in niacin) as a staple food. A special cooking technique called nixtamalization is needed to increase the bioavailability of niacin during maize meal/flour production.

Mild niacin deficiency has been shown to slow metabolism, causing decreased tolerance to cold.

Severe deficiency of niacin in the diet causes the disease pellagra, which is characterized by diarrhea, dermatitis, and dementia, as well as "necklace" lesions on the lower neck, hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue, digestive disturbances, amnesia, delirium, and eventually death, if left untreated. Common psychiatric symptoms of niacin deficiency include irritability, poor concentration, anxiety, fatigue, restlessness, apathy, and depression. Studies have indicated that, in patients with alcoholic pellagra, niacin deficiency may be an important factor influencing both the onset and severity of this condition. Alcoholic patients

typically experience increased intestinal permeability, leading to negative health outcomes.

Hartnup's disease is a hereditary nutritional disorder resulting in niacin deficiency. This condition was first identified in the 1950s by the Hartnup family in London. It is due to a deficit in the intestines and kidneys, making it difficult for the body to break down and absorb dietary tryptophan. The resulting condition is similar to pellagra, including symptoms of red, scaly rash, and sensitivity to sunlight. Oral niacin is given as a treatment for this condition in doses ranging from 40–200 mg, with a good prognosis if identified and treated early. Niacin synthesis is also deficient in carcinoid syndrome, because of metabolic diversion of its precursor tryptophan to form serotonin.

Lipid-modifying effects

In pharmacological doses, niacin has been proven to reverse atherosclerosis by reducing total cholesterol, triglycerides, very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL), and increasing high-density lipoprotein (HDL). It has been proposed that niacin has the ability to lower lipoprotein(a), which is beneficial at reducing thrombotic tendency.

Niacin, prescribed in doses between 1000 and 2000 mg, two to three times daily, blocks the breakdown of fats in adipose tissue. These fats are used to build very-low-density lipoproteins (VLDL) in the liver, which are precursors of low-density lipoprotein (LDL) or "bad" cholesterol. Because niacin blocks the breakdown of fats, it causes a decrease in free fatty acids in the blood and, as a consequence, decreases the secretion of VLDL and cholesterol by the liver.

By lowering VLDL levels, niacin also increases the level of high-density lipoprotein (HDL) or "good" cholesterol in blood, and therefore it is sometimes prescribed for patients with low HDL, who are also at high risk of a heart attack. Whether the increasing of HDL translates into a reduction in clinical events might be answered by two large outcome studies: AIM HIGH and HPS-2 THRIVE. If it does, the current approach to lipid modulation of high-risk patient would be fundamentally changed.

The ARBITER 6-HALTS study, reported at the 2009 annual meeting of the American Heart Association and in the New England Journal of Medicine concluded that, when added to statins, 2000 mg/day of slow-release niacin was more effective than ezetimibe (Zetia) in reducing carotid intima-media thickness, a marker of atherosclerosis.

As of August 2008, a combination of niacin with laropiprant is being tested in a clinical trial. Laropiprant reduces facial flushes induced by niacin.

Toxicity

Pharmacological doses of niacin (1.5 - 6 g per day) often lead to side effects that can include dermatological conditions such as skin flushing and itching, dry skin, and skin

rashes including eczema exacerbation and acanthosis nigricans. These symptoms are generally related to niacin's role as the rate limiting cofactor in the histidine decarboxylase enzyme which converts l-histidine into histamine. H1 and H2 receptor mediated histamine is metabolized via a sequence of mono (or di-) amine oxidase and COMT into methylhistamine which is then conjugated through the liver's CP450 pathways. Persistent flushing and other symptoms may indicate deficiencies in one or more of the cofactors responsible for this enzymatic cascade. Gastrointestinal complaints, such as dyspepsia (indigestion), nausea and liver toxicity fulminant hepatic failure, have also been reported. Side effects of hyperglycemia, cardiac arrhythmias and "birth defects in experimental animals" have also been reported. The flush lasts for about 15 to 30 minutes, and is sometimes accompanied by a prickly or itching sensation, in particular, in areas covered by clothing. This effect is mediated by prostaglandin and can be blocked by taking 300 mg of aspirin half an hour before taking niacin, or by taking one tablet of ibuprofen per day. Taking the niacin with meals also helps reduce this side effect. After several weeks of a consistent dose, most patients no longer flush. Slow- or "sustained"-release forms of niacin have been developed to lessen these side effects. One study showed the incidence of flushing was significantly lower with a sustained release formulation though doses above 2 g per day have been associated with liver damage, in particular, with slow-release formulations. Flushing is often thought to involve histamine, but histamine has been shown not to be involved in the reaction. Prostaglandin (PGD_2) is the primary cause of the flushing reaction, with serotonin appearing to have a secondary role in this reaction.

Although high-dose niacin may also elevate blood sugar, thereby worsening diabetes mellitus. Recent studies show the actual effect on blood sugar is only 5-10%. Patients with diabetes continue taking their anti-diabetes drugs did not experience major blood glucose changes. Thus from the big picture, niacin is recommended as a drug for prevention of CVD in patients with diabetes.

Hyperuricemia is another side effect of taking high-dose niacin, and may exacerbate gout.

Niacin at doses used in lowering cholesterol has been associated with birth defects in laboratory animals, with possible consequences for infant development in pregnant women.

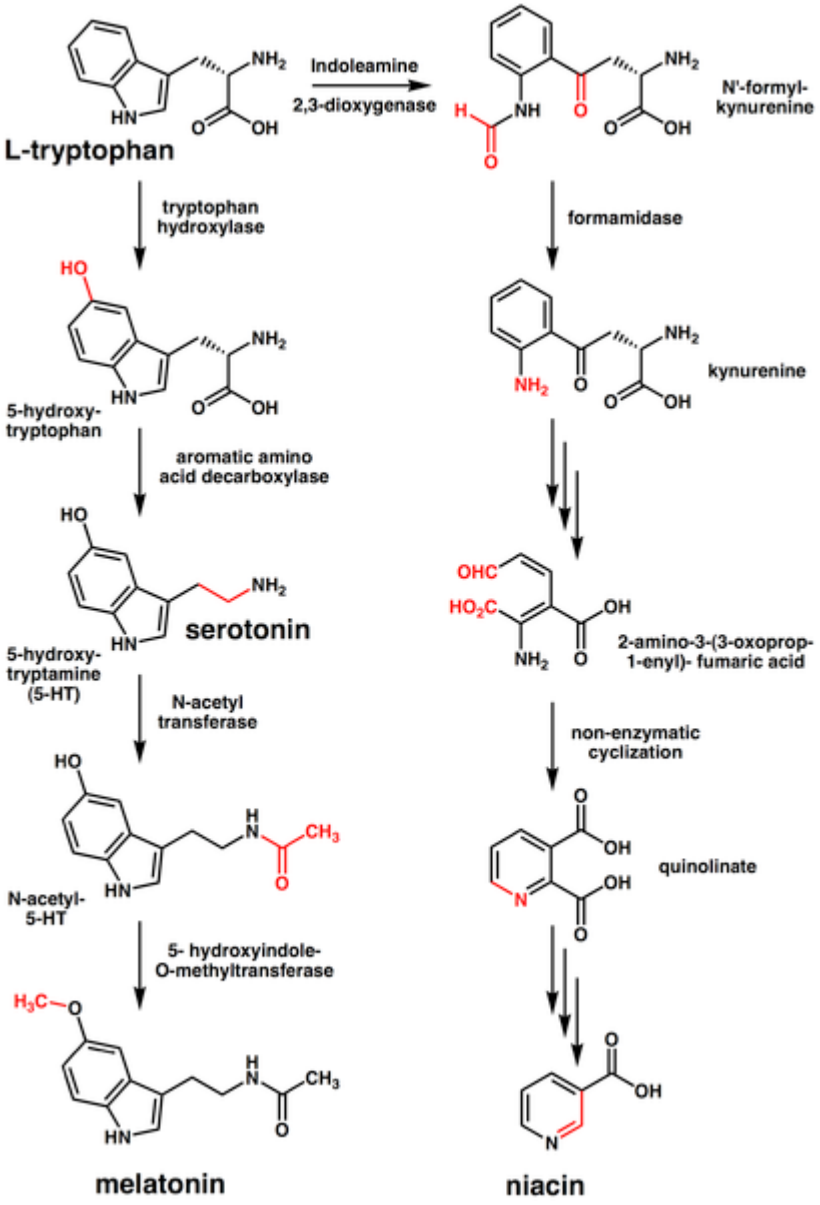
Niacin at extremely high doses can have life-threatening acute toxic reactions. Extremely high doses of niacin can also cause niacin maculopathy, a thickening of the macula and retina, which leads to blurred vision and blindness. This maculopathy is reversible after stopping niacin intake.

Inositol hexanicotinate

One popular form of dietary supplement is inositol hexanicotinate (IHN), which is inositol that has been esterified with niacin on all six of inositol's alcohol groups. IHN is usually sold as "flush-free" or "no-flush" niacin in units of 250, 500, or 1000 mg/tablets or capsules. It is sold as an over-the-counter formulation, and often is marketed and

labeled as niacin, thus misleading consumers into thinking they are getting the active form of the medication. While this form of niacin does not cause the flushing associated with the immediate-release products, the evidence that it has lipid-modifying functions is contradictory, at best. As the clinical trials date from the early 1960s (Dorner, Welsh) or the late 1970s (Ziliotto, Kruse, Agusti), it is difficult to assess them by today's standards. One of the last of those studies affirmed the superiority of inositol and xantinol esters of nicotinic acid for reducing serum free fatty acid, but other studies conducted during the same period found no benefit. Studies explain that this is primarily because "flush-free" preparations do not contain any free nicotinic acid. A more recent placebo-controlled trial was small (n=11/group), but results after three months at 1500 mg/day showed no trend for improvements in total cholesterol, LDL-C, HDL-C or triglycerides. Thus, so far there is not enough evidence to recommend IHN to treat dyslipidemia. Furthermore, the American Heart Association and the National Cholesterol Education Program both take the position that only prescription niacin should be used to treat dyslipidemias, and only under the management of a physician. The reason given is that niacin at effective intakes of 1500–3000 mg/day can also potentially have severe adverse effects. Monitoring of liver enzymes is necessary.

Biosynthesis and chemical synthesis



Biosynthesis

The liver can synthesize niacin from the essential amino acid tryptophan, requiring 60 mg of tryptophan to make one mg of niacin. The 5-membered aromatic heterocycle of tryptophan is cleaved and rearranged with the alpha amino group of tryptophan into the 6-membered aromatic heterocycle of niacin. Riboflavin, Vitamin B6 and iron are required in some of the reacitons involved in the conversion of tryptophan to NAD.

Several million kilograms of niacin are manufactured each year, starting from 3-methylpyridine.

Receptor

The receptor for niacin is a G protein-coupled receptor called HM74A. It couples to the Gi alpha subunit.

Food sources

Niacin is found in variety of foods, including liver, chicken, beef, fish, cereal, peanuts and legumes, and is also synthesized from tryptophan, which is found in meat, dairy and eggs.

Animal products:

- liver, heart and kidney
- chicken
- beef
- fish: tuna, salmon
- milk
- eggs

Fruits and vegetables:

- avocados
- dates
- tomatoes
- leaf vegetables
- broccoli
- carrots
- sweet potatoes
- asparagus

Seeds:

- nuts
- whole grain products
- legumes
- saltbush seeds

Fungi:

- mushrooms
- brewer's yeast

Other:

- Vegemite (from spent brewer's yeast)

Chapter 6

Vitamin B5 (Pantothenic Acid)

Pantothenic acid, also called **pantothenate** or **vitamin B_5** (a B vitamin), is a water-soluble vitamin. For many animals, pantothenic acid is an essential nutrient. Animals require pantothenic acid to synthesize coenzyme-A (CoA), as well as to synthesize and metabolize proteins, carbohydrates, and fats.

Pantothenic acid is the amide between pantoate and beta-alanine. Its name derives from the Greek *pantothen* (πάντοθεν) meaning "from everywhere" and small quantities of pantothenic acid are found in nearly every food, with high amounts in whole-grain cereals, legumes, eggs, meat, royal jelly, avocado, and yogurt . It is commonly found as its alcohol analog, the provitamin panthenol, and as calcium pantothenate. Pantothenic acid is an ingredient in some hair and skin care products.

Biological role

Only the dextrorotatory (D) isomer of pantothenic acid possesses biologic activity. The levorotatory (L) form may antagonize the effects of the dextrorotatory isomer.

Pantothenic acid is used in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group carrier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell. CoA is important in energy metabolism for pyruvate to enter the tricarboxylic acid cycle(TCA cycle) as acetyl-CoA, and for α-ketoglutarate to be transformed to succinyl-CoA in the cycle. CoA is also important in the biosynthesis of many important compounds such as fatty acids, cholesterol, and acetylcholine. CoA is incidentally also required in the formation of ACP, which is also required for fatty acid synthesis in addition to CoA.

Pantothenic acid in the form of CoA is also required for acylation and acetylation, which, for example, are involved in signal transduction and enzyme activation and deactivation, respectively.

Since pantothenic acid participates in a wide array of key biological roles, it is essential to all forms of life. As such, deficiencies in pantothenic acid may have numerous wide-ranging effects, as discussed below.

Sources

Dietary

Small quantities of pantothenic acid are found in most foods. The major food source of pantothenic acid is in meats, although the concentration found in food animals' muscles is only about half that in humans' muscles. Whole grains are another good source of the vitamin, but milling often removes much of the pantothenic acid, as it is found in the outer layers of whole grains. Vegetables, such as broccoli and avocados, also have an abundance of the acid. In animal feeds, the most important sources of the vitamin are rice, wheat brans, alfalfa, peanut meal, molasses, yeasts, and condensed fish solutions. The most significant sources of pantothenic acid in nature are coldwater fish ovaries and royal jelly.

A recent study also suggests that gut bacteria in humans can generate pantothenic acid, but this has not yet been proven.

Supplementation

The derivative of pantothenic acid, pantothenol, is a more stable form of the vitamin and is often used as a source of the vitamin in multivitamin supplements. Another common supplemental form of the vitamin is calcium pantothenate. Calcium pantothenate is often used in dietary supplements because, as a salt, it is more stable than pantothenic acid in the digestive tract, allowing for better absorption.

Possible benefits of supplementation: Doses of 2 g/day of calcium pantothenate may reduce the duration of morning stiffness, degree of disability, and pain severity in rheumatoid arthritis patients. Although the results are inconsistent, supplementation may improve oxygen utilization efficiency and reduce lactic acid accumulation in athletes.

Daily requirement

Pantothenate in the form of 4'phosphopantetheine is considered to be the more active form of the vitamin in the body; however, any derivative must be broken down to pantothenic acid before absorption. 10 mg of calcium pantothenate is equivalent to 9.2 mg of pantothenic acid.

Age group	Age	Requirements
Infants	0–6 months	1.7 mg
Infants	7–12 months	1.8 mg
Children	1–3 years	2 mg
Children	4–8 years	3 mg
Children	9–13 years	4 mg
Adult men and women	14+ years	5 mg
Pregnant women	(vs. 5)	6 mg
Breastfeeding women	(vs. 5)	7 mg

- United Kingdom RDA: 6 mg/day

Absorption

Within most foods, pantothenic acid is in the form of CoA or acyl carrier protein (ACP). For the intestinal cells to absorb this vitamin, it must be converted into free pantothenic acid. Within the lumen of the intestine, CoA and ACP are hydrolyzed into 4'-phosphopantetheine. The 4'-phosphopantetheine is then dephosphorylated into pantetheine. Pantetheinase, an intestinal enzyme, then hydrolyzes pantetheine into free pantothenic acid.

Free pantothenic acid is absorbed into intestinal cells via a saturable, sodium-dependent active transport system. At high levels of intake, when this mechanism is saturated, some pantothenic acid may also be absorbed via passive diffusion. As intake increases 10-fold, however, absorption rate decreases to 10%.

Deficiency

Pantothenic acid deficiency is exceptionally rare and has not been thoroughly studied. In the few cases where deficiency has been seen (victims of starvation and limited volunteer trials), nearly all symptoms can be reversed with the return of pantothenic acid.

Symptoms of deficiency are similar to other vitamin B deficiencies. There is impaired energy production, due to low CoA levels, which could cause symptoms of irritability, fatigue, and apathy. Acetylcholine synthesis is also impaired; therefore, neurological symptoms can also appear in deficiency; they include numbness, paresthesia, and muscle cramps. Deficiency in pantothenic acid can also cause hypoglycemia, or an increased sensitivity to insulin. Insulin receptors are acylated with palmitic acid when they do not want to bind with insulin. Therefore, more insulin will bind to receptors when acylation decreases, causing hypoglycemia. Additional symptoms could include restlessness, malaise, sleep disturbances, nausea, vomiting, and abdominal cramps. In a few rare circumstances, more serious (but reversible) conditions have been seen, such as adrenal insufficiency and hepatic encephalopathy.

One study noted painful burning sensations of the feet were reported in tests conducted on volunteers. Deficiency of pantothenic acid may explain similar sensations reported in malnourished prisoners of war.

Deficiency symptoms in other nonruminant animals include disorders of the nervous, gastrointestinal, and immune systems, reduced growth rate, decreased food intake, skin lesions and changes in hair coat, and alterations in lipid and carbohydrate metabolism.

Toxicity

Toxicity of pantothenic acid is unlikely. In fact, no Tolerable Upper Level Intake (UL) has been established for the vitamin. Large doses of the vitamin, when ingested, have no reported side effects and massive doses (e.g., 10 g/day) may only yield mild intestinal distress, and diarrhea at worst.

There are also no adverse reactions known following parenteral or topical application of the vitamin.

Uses

Given pantothenic acid's prevalence among living things and the limited body of studies in deficiency, many uses of pantothenic acid have been the subject of research.

Testicular torsion

Testicular torsion can severely affect fertility if it occurs. One study on a rat model indicated a treatment of 500 mg of dexpanthenol/kg body weight 30 minutes prior to detorsion can greatly decrease the risk of infertility after torsion. Pantothenic acid has the ability to spare reduced glutathione levels. Reactive oxygen species play a role in testicular atrophy, which glutathione counteracts.

Diabetic ulceration

Foot ulceration is a problem commonly associated with diabetes, which often leads to amputation. A preliminary study completed by Abdelatif, Yakoot and Etmaan indicated that perhaps a royal jelly and panthenol ointment can help cure the ulceration. People with foot ulceration or deep tissue infection in the study had a 96% and 92% success rate of recovery. While these results appear promising, they need to be validated, as this was a pilot study; it was not a randomized, placebo-controlled, double-blind study.

Hypolipidemic effects

Pantothenic acid derivatives, panthenol, phosphopantethine and pantethine, have also been seen to improve the lipid profile in the blood and liver. In this mouse model, they injected 150 mg of the derivative/kg body weight. All three derivatives were able to effectively lower low-density lipoprotein (LDL), as well as triglyceride (TG) levels;

panthenol was able to lower total cholesterol, and pantethine was able to lower LDL-cholesterol in the serum. The decrease in LDL is significant, as it is related to a decrease the risk of myocardial infarction and stroke. In the liver, panthenol was the most effective, as it lowered TG, total cholesterol, free cholesterol and cholesterol-ester levels.

Wound healing

A study in 1999 showed pantothenic acid has an effect on wound healing *in vitro*. Wiemann and Hermann found cell cultures with a concentration of 100 μg/mL calcium D-pantothenate increased migration, and the fibers ran directionally with several layers, whereas the cell cultures without pantothenic acid healed in no orderly motion, and with fewer layers. Cell proliferation or cell multiplication was found to increase with pantothenic acid supplementation. Finally, increased concentrations of two proteins, both of which have yet to be identified, were found in the supplemented culture, but not in the control. Further studies are needed to determine whether these effects will stand *in vivo*.

Hair care

Mouse models identified skin irritation and loss of hair color as possible results of severe pantothenic acid deficiency. As a result, the cosmetic industry began adding pantothenic acid to various cosmetic products, including shampoo. These products, however, showed no benefits in human trials. Despite this, many cosmetic products still advertise pantothenic acid additives.

Acne

Following from discoveries in mouse trials, in the late 1990s, a small study was published promoting the use of pantothenic acid to treat acne vulgaris.

According to a study published in 1995 by Dr. Lit-Hung Leung, high doses of vitamin B_5 resolved acne and decreased pore size. Dr. Leung also proposed a mechanism, stating that CoA regulates both hormones and fatty acids, and without sufficient quantities of pantothenic acid, CoA will preferentially produce androgens. This causes fatty acids to build up and be excreted through sebaceous glands, causing acne. Leung's study gave 45 Asian males and 55 Asian females varying doses of 10-20g of pantothenic acid (100000% of the US Daily Value), 80% orally and 20% through topical cream. Leung noted improvement of acne within one week to one month of the start of the treatment.

Obesity

In a report published in 1997 by Dr. Lit-Hung Leung, it was hypothesized that pantothenic acid also has an effect on weight management. Dr. Leung proposed that those who were deficient in pantothenic acid would feel the effects of hunger and weakness more strongly. To access fat storages in the body in times of fasting or dieting requires CoA. Diets high in pantothenic acid produce more CoA. In a study done on 100 Chinese individuals from age range 15-55 it was observed that on a diet of 1000 calories a day

and 10 mg pantothenic acid, the dieters could lose on average 1.2 kg/week with lessened effects from hunger or weakness. Ketone bodies in urine indicated that some dieters required more than 10 mg of pantothenic acid a day. The possibilities of pantothenic acid in weight management have not been fully explored, but remain an area of research.

Diabetic peripheral polyneuropathy

Twenty-eight out of 33 patients (84.8%) previously treated with alpha-lipoic acid for peripheral polyneuropathy reported further improvement after combination with pantothenic acid. The theoretical basis for this is that both substances intervene at different sites in pyruvate metabolism and are, thus, more effective than one substance alone. Additional clinical findings indicated diabetic neuropathy may occur in association with a latent prediabetic metabolic disturbance, and that the symptoms of neuropathy can be favorably influenced by the described combination therapy, even in poorly controlled diabetes.

Ruminant nutrition

No dietary requirement for pantothenic acid has been established as synthesis of pantothenic acid by ruminal microorganisms appears to be 20 to 30 times more than dietary amounts. Net microbial synthesis of pantothenic acid in the rumen of steer calves has been estimated to be 2.2 mg/kg of digestible organic matter consumed per day. The degradation of dietary intake of pantothenic acid is considered to be 78 percent. Supplementation of pantothenic acid at 5 to 10 times theoretic requirements did not improve performance of feedlot cattle

Chapter 7

Vitamin B_6

Vitamin B_6 is a water-soluble vitamin and is part of the vitamin B complex group. Several forms of the vitamin are known, but pyridoxal phosphate (PLP) is the active form and is a cofactor in many reactions of amino acid metabolism, including transamination, deamination, and decarboxylation. PLP also is necessary for the enzymatic reaction governing the release of glucose from glycogen.

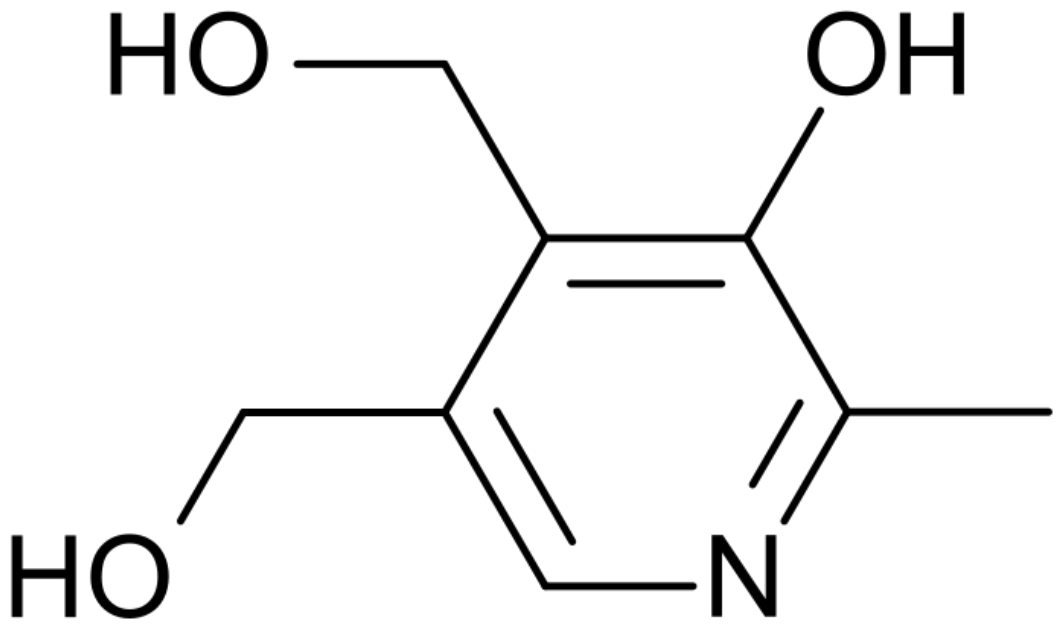
History

Vitamin B_6 is a water-soluble compound that was discovered in the 1930s during nutrition studies on rats. In 1934, a Hungarian physician, Paul György discovered a substance that was able to cure a skin disease in rats (dermititis acrodynia), this substance he named vitamin B_6. In 1938, Lepkovsky isolated vitamin B_6 from rice bran. Harris and Folkers in 1939 determined the structure of pyridoxine, and, in 1945, Snell was able to show that there are two forms of vitamin B_6, pyridoxal and pyridoxamine. Vitamin B_6 was named pyridoxine to indicate its structural homology to pyridine. All three forms of vitamin B_6 are precursors of an activated compound known as pyridoxal 5'-phosphate (PLP), which plays a vital role as the co-factor of a large number of essential enzymes in the human body.

Enzymes dependent on PLP focus a wide variety of chemical reactions mainly involving amino acids. The reactions carried out by the PLP-dependent enzymes that act on amino acids include transfer of the amino group, decarboxylation, racemization, and beta- or gamma-elimination or replacement. Such versatility arises from the ability of PLP to covalently bind the substrate, and then to act as an electrophilic catalyst, thereby stabilizing different types of carbanionic reaction intermediates.

Overall, the Enzyme Commission has catalogued more than 140 PLP-dependent activities, corresponding to ~4% of all classified activities.

Forms



Pyridoxine

Seven forms of this vitamin are known:

- Pyridoxine (PN), the form that is most commonly given as vitamin B_6 supplement
- Pyridoxine 5'-phosphate (PNP)
- Pyridoxal (PL)
- Pyridoxal 5'-phosphate (PLP), the metabolically active form (sold as 'P-5-P' vitamin supplement)
- Pyridoxamine (PM)
- Pyridoxamine 5'-phosphate (PMP)
- 4-Pyridoxic acid (PA), the catabolite which is excreted in the urine

All forms except PA can be interconverted.

Functions

Pyridoxal phosphate, the metabolically active form of vitamin B_6, is involved in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and function and gene expression. Pyridoxal phosphate generally serves as a coenzyme for many reactions and can help facilitate decarboxylation, transamination, racemization, elimination, replacement and beta-group interconversion reactions. The liver is the site for vitamin B_6 metabolism.

Amino acid metabolism

Pyridoxal phosphate (PLP) is a cofactor in transaminases that can catabolize amino acids. PLP is also an essential component of two enzymes that converts methionine to cysteine via two reactions. Low vitamin B_6 status will result in decreased activity of these enzymes. PLP is also an essential cofactor for enzymes involved in the metabolism of selenomethionine to selenohomocysteine and then from selenohomocysteine to hydrogen selenide. Vitamin B_6 is also required for the conversion of tryptophan to niacin and low vitamin B_6 status will impair this conversion. PLP is also used to create physiologically active amines by decarboxylation of amino acids. Some notable examples of this include: histidine to histamine, tryptophan to serotonin, glutamate to gamma-aminobutyric acid (GABA), and dihydroxyphenylalanine to dopamine.

Gluconeogenesis

Vitamin B_6 also plays a role in gluconeogenesis. Pyridoxal phosphate can catalyze transamination reactions that are essential for the providing amino acids as a substrate for gluconeogenesis. Also, vitamin B_6 is a required coenzyme of glycogen phosphorylase, the enzyme that is necessary for glycogenolysis to occur.

Lipid metabolism

Vitamin B_6 is an essential component of enzymes that facilitate the biosynthesis of sphingolipids. Particularly, the synthesis of ceramide requires PLP. In this reaction serine is decarboxylated and combined with palmitoyl-CoA to form sphinganine which is combined with a fatty acyl CoA to form dihydroceramide. Dihydroceramide is then further desaturated to form ceramide. In addition, the breakdown of sphingolipids is also dependent on vitamin B_6 since S1P lyase, the enzyme responsible for breaking down sphingosine-1-phosphate, is also PLP dependent.

Metabolic functions

The primary role of vitamin B_6 is to act as a coenzyme to many other enzymes in the body that are involved predominantly in metabolism. This role is performed by the active form, pyridoxal phosphate. This active form is converted from the two other natural forms founds in food: pyridoxal, pyridoxine and pyridoxamine.

Vitamin B_6 is involved in the following metabolic processes:

- amino acid, glucose and lipid metabolism
- neurotransmitter synthesis
- histamine synthesis
- hemoglobin synthesis and function
- gene expression

Amino acid metabolism

Pyridoxal phosphate is involved in almost all amino acid metabolism, from synthesis to breakdown.

1. Transamination: transaminase enzymes needed to break down amino acids are dependent on the presence of pyridoxal phosphate. The proper activity of these enzymes are crucial for the process of moving amine groups from one amino acid to another.

2. Transsulfuration: Pyridoxal phosphate is a coenzyme needed for the proper function of the enzymes cystathionine synthase and cystathionase. These enzymes work to transform methionine into cysteine.

3. Selenoamino acid metabolism: Selenomethionine is the primary dietary form of selenium. Pyridoxal phosphate is needed as a cofactor for the enzymes that allow selenium to be used from the dietary form. Pyridoxal phosphate also plays a cofactor role in releasing selenium from selenohomocysteine to produce hydrogen selenide. This hydrogen selenide can then be used to incorporate selenium into selenoproteins.

4. Vitamin B_6 is also required for the conversion of tryptophan to niacin and low vitamin B_6 status will impair this conversion.

Neurotransmitter synthesis

Pyridoxal phosphate-dependent enzymes play a role in the biosynthesis of four important neurotransmitters: serotonin, epinephrine, norepinephrine and gamma-aminobutyric acid. Serine racemase, which synthesizes the neuromodulator D-serine, is also a pyridoxal phosphate-dependent enzyme.

Histamine synthesis

Pyridoxal phosphate is involved in the metabolism of histamine.

Hemoglobin synthesis and function

Pyridoxal phosphate aids in the synthesis of heme and can also bind to two sites on hemoglobin to enhance the oxygen binding of hemoglobin.

Gene expression

It transforms homocysteine into cistation then into cysteine. Pyridoxal phosphate has been implicated in increasing or decreasing the expression of certain genes. Increased intracellular levels of the vitamin will lead to a decrease in the transcription of glucocorticoid hormones. Also, vitamin B_6 deficiency will lead to the increased expression of albumin mRNA. Also, pyridoxal phosphate will influence gene expression

of glycoprotein IIb by interacting with various transcription factors. The result is inhibition of platelet aggregation.

Dietary reference intakes

Life Stage Group	RDA/AI*	UL
Infants	(mg/day)	(mg/day)
0–6 months	0.1*	ND
7–12 months	0.3*	ND
Children		
1-3 yrs	0.5	30
4-8 yrs	0.6	40
Males		
9-13 yrs	1.0	60
14-18 yrs	1.3	80
19-50 yrs	1.3	100
50- >70 yrs	1.7	100
Females		
9-13 yrs	1.0	60
13-18 yrs	1.2	80
19-50 yrs	1.3	100
50- >70 yrs	1.5	100
Pregnancy		
<18 yrs	1.9	80
19-50 yrs	1.9	100
Lactation		
<18 yrs	2.0	80
19-50 yrs	2.0	100

The Institute of Medicine notes that "No adverse effects associated with Vitamin B_6 from food have been reported. This does not mean that there is no potential for adverse effects resulting from high intakes. Because data on the adverse effects of Vitamin B_6 are limited, caution may be warranted. Sensory neuropathy has occurred from high intakes of supplemental forms."

Food sources

Vitamin B_6 is widely distributed in foods in both its free and bound forms. Good sources include meats, whole grain products, vegetables, nuts and bananas. Cooking, storage and processing losses of vitamin B_6 vary and in some foods may be more than 50%, depending on the form of vitamin present in the food. Plant foods lose the least during processing as they contain mostly pyridoxine which is far more stable than the pyridoxal or pyridoxamine found in animal foods. For example, milk can lose 30-70% of its vitamin B_6 content when dried. Vitamin B_6 is found in the germ and aleurone layer of

grains and milling results to the reduction of this vitamin in white flour. Freezing and canning are other food processing methods that results in the loss of vitamin B_6 in foods.

Absorption and excretion

Vitamin B_6 is absorbed in the jejunum and ileum via passive diffusion. With the capacity for absorption being so great, animals are able to absorb quantities much greater than what is needed for physiological demands. The absorption of pyridoxal phosphate and pyridoxamine phosphate involves their dephosphorylation catalyzed by a membrane-bound alkaline phosphatase. Those products and non-phosphorylated vitamers in the digestive tract are absorbed by diffusion, which is driven by trapping of the vitamin as 5'-phosphates through the action of phosphorylation (by a pyridoxal kinase) in the jejunal mucosa. The trapped pyridoxine and pyridoxamine are oxidized to pyridoxal phosphate in the tissue.

The products of vitamin B_6 metabolism are excreted in the urine; the major product of which is 4-pyridoxic acid. It has been estimated that 40-60% of ingested vitamin B_6 is oxidized to 4-pyridoxic acid. Several studies have shown that 4-pyridoxic acid is undetectable in the urine of vitamin B_6 deficient subjects, making it a useful clinical marker to assess the vitamin B_6 status of an individual. Other products of vitamin B_6 metabolism that are excreted in the urine when high doses of the vitamin have been given include pyridoxal, pyridoxamine, and pyridoxine and their phosphates. A small amount of vitamin B_6 is also excreted in the feces.

Deficiencies

The classic clinical syndrome for B_6 deficiency is a seborrhoeic dermatitis-like eruption, atrophic glossitis with ulceration, angular cheilitis, conjunctivitis, intertrigo, and neurologic symptoms of somnolence, confusion, and neuropathy.

While severe vitamin B_6 deficiency results in dermatologic and neurologic changes, less severe cases present with metabolic lesions associated with insufficient activities of the coenzyme pyridoxal phosphate. The most prominent of the lesions is due to impaired tryptophan-niacin conversion. This can be detected based on urinary excretion of xanthurenic acid after an oral tryptophan load. Vitamin B_6 deficiency can also result from impaired transsulfuration of methionine to cysteine. The pyridoxal phosphate-dependent transaminases and glycogen phosphorylase provide the vitamin with its role in gluconeogenesis, so deprivation of vitamin B_6 results in impaired glucose tolerance.

A deficiency of vitamin B_6 alone is relatively uncommon and often occurs in association with other vitamins of the B complex. The elderly and alcoholics have an increased risk of vitamin B_6 deficiency, as well as other micronutrient deficiencies. Renal patients undergoing dialysis may experience vitamin B_6 deficiency. Also, patients with liver disease, rheumatoid arthritis and those infected with HIV also appear to be at risk, despite adequate dietary intakes. The availability of vitamin B_6 to the body can be affected by certain drugs such as anticonvulsants and corticosteroids. The drug isoniazid (used in the

treatment of tuberculosis), and cycloserine, penicillamine, and hydrocortisone all interfere with vitamin B_6 metabolism. These drugs may form a complex with vitamin B_6 that is inhibitory for pyridoxal kinase, or they may positively displace PLP from binding sites.

Clinical assessment of vitamin B_6

The biochemical assessment of vitamin B_6 status is essential, as the clinical signs and symptoms of vitamin B_6 deficiency are very nonspecific. The three biochemical tests most widely used are the activation coefficient for the erythrocyte enzyme aspartate aminotransferase, plasma pyridoxal phosphate (PLP) concentrations, and the urinary excretion of vitamin B_6 degradation products, specifically urinary pyridoxic acid. Of these, plasma PLP is probably the best single measure because it reflects tissue stores. When plasma pyridoxal phosphate is less than 10nmol/L, it is indicative of vitamin B_6 deficiency. Urinary 4-pyridoxic acid is also an indicator of vitamin B_6 deficiency. Urinary 4-pyridoxic of less than 3.0 mmol/day is suggestive of vitamin B_6 deficiency.

Toxicity

Adverse effects have only been documented from vitamin B_6 supplements and never from food sources. Here we, only discusses the safety of the common supplemental form of vitamin B_6 pyridoxine. Toxicologic animal studies identify specific destruction of the dorsal root ganglia which is documented in human cases of overdosage of pyridoxine. Although vitamin B_6 is a water-soluble vitamin and is excreted in the urine, doses of pyridoxine in excess of the RDI over long periods of time thus result in painful and ultimately irreversible neurological problems.

The primary symptoms are pain and numbness of the extremities, and in severe cases difficulty walking. Sensory neuropathy typically develops at doses of pyridoxine in excess of 1,000 mg per day. However, there have been a few case reports of individuals who developed sensory neuropathies at doses of less than 500 mg daily over a period of months. None of the studies, in which an objective neurological examination was performed, found evidence of sensory nerve damage at intakes of pyridoxine below 200 mg/day. This condition is usually reversible when supplementation is stopped.

Existing authorisations and valuations vary considerably worldwide. In 1993 the European Community Scientific Committee on Food defines intakes of 50 mg vitamin B6 per day as harmful and established tolerable upper intake level of 25 mg/day for adults in 2000.

The Expert Group on Vitamins and Minerals of the Food Standard Agency UK (UK EVM) derived a safe upper level (SUL) of 10 mg/day for a 60 kg adult in 2003.

The tolerable upper limit has been set by the US FDA at 100 mg/day in 2000. The nutrient reference values in Australia and New Zealand recommend an upper limit of 50 mg a day in adults. "The same figure was set for pregnancy and lactation as there is no

evidence of teratogenicity at this level. The UL was set based on metabolic body size and growth considerations for all other ages and life stages except infancy. It was not possible to set a UL for infants, so intake is recommended in the form of food, milk or formula." "The ULs were set using results of studies involving long-term oral administration of pyridoxine at doses of less than 1g/day (Berger & Schaumburg 1984, Bernstein & Lobitz 1988, Dalton 1985, Dalton & Dalton 1987, Del Tredici et al 1985, FNB:IOM 1998, Parry & Bredesen 1985). A NOAEL of 200 mg/day was identified from the studies of Bernstein & Lobitz (1988) and Del Tredici et al (1985). These studies involved subjects who had generally been on the supplements for 5 to 6 months or less. The study of Dalton and Dalton (1987), however, suggested that symptoms might take substantially longer than this to appear. In this latter retrospective survey, subjects who reported symptoms had been on supplements for 2.9 years on average. Those reporting no symptoms had taken supplements for 1.9 years."

Because there have been no placebo-controlled studies showing therapeutic benefits of high doses of pyridoxine, and the well documented occurrence of significant toxic effects there is little reason to exceed the RDI using supplements unless under medical supervision e.g. in treatment of primary hyperoxaluria.

Oncology

Vitamin B_6 intake is inversely associated with the risk of colorectal cancer.

Preventive roles and therapeutic uses

Vitamin B_6 has been used to treat nausea and vomiting in early pregnancy for decades, commonly in conjunction with other medications such as metoclopramide or doxylamine. Alone, it has been found safe and effective, though any woman's prenatal caregiver must help guide treatment for these symptoms.

At least one preliminary study has found that this vitamin may increase dream vividness or the ability to recall dreams. It is thought that this effect may be due to the role this vitamin plays in the conversion of tryptophan to serotonin.

The intake of vitamin B_6, from either diet or supplements, could cut the risk of Parkinson's disease by half according to a prospective study from the Netherlands. "Stratified analyses showed that this association was restricted to smokers," wrote the authors.

Pyridoxine has a role in preventing heart disease. Without enough pyridoxine, a compound called homocysteine builds up in the body. Homocysteine damages blood vessel linings, setting the stage for plaque buildup when the body tries to heal the damage. Vitamin B_6 prevents this buildup, thereby reducing the risk of heart attack. Pyridoxine lowers blood pressure and blood cholesterol levels and keeps blood platelets from sticking together. All of these properties work to keep heart disease at bay.

Nutritional supplementation with high dose vitamin B_6 and magnesium is one of the most popular alternative medicine choices for autism but randomised control trials have had mixed results and small sample sizes mean that no conclusions can be drawn as to the efficacy of this treatment.

Some studies suggest that the vitamin B_6-magnesium combination can also help attention deficit disorder, citing improvements in hyperactivity, hyperemotivity/aggressiveness and improved school attention.

A lack of the vitamin may play a role in sensitivity to monosodium glutamate (MSG), a flavor enhancer. This sensitivity can cause headaches, pain and tingling of the upper extremities, nausea, and vomiting. In both of these syndromes, supplementation of pyridoxine alleviates symptoms only when people were deficient in the vitamin to begin with.

If people are marginally deficient in vitamin B_6, they may be more susceptible to carpal tunnel syndrome. Carpal tunnel syndrome is characterized by pain and tingling in the wrists after performing repetitive movements or otherwise straining the wrist on a regular basis. Vitamin B_6 has been shown in at least two small-scale clinical studies to have a beneficial effect on carpal tunnel syndrome, particularly in cases where no trauma or overuse etiology for the CTS is known.

Vitamin B_6 has long been publicized as a cure for premenstrual syndrome (PMS). Study results conflict as to which symptoms are eased, but most of the studies confirm that women who take B_6 supplements have reductions in bloating, breast pain, and premenstrual acne flare, a condition in which pimples break out about a week before a woman's period begins.There is strong evidence that pyridoxine supplementation, starting ten days before the menstrual period, prevents most pimples from forming. This effect is due to the vitamin's role in hormone and prostaglandin regulation. Skin blemishes are typically caused by a hormone imbalance, which vitamin B_6 helps to regulate.

Mental depression is another condition which may result from low vitamin B_6 intake. Because of pyridoxine's role in serotonin and other neurotransmitter production, supplementation often helps depressed people feel better, and their mood improves significantly. It may also help improve memory in older adults. However, the effectiveness as treatment for PMS, PMDD, and clinical depression is debatable.

It is also suggested that ingestion of vitamin B_6 can alleviate some of the many symptoms of an alcoholic hangover and morning sickness from pregnancy. This might be due to B_6's mild diuretic effect. Though the mechanism is not known, results show that pyridoxamine has a therapeutic effects in clinical trials for diabetic nephropathy.

Larsson *et al.* have shown that vitamin B_6 intake and pyridoxal phosphate (PLP) levels are inversely related to the risk of colon cancer. While in their study the correlation with B_6 intake was moderate, it was quite dramatic with PLP levels where the risk of colon cancer was nearly decreased in half.

Chapter 8

Vitamin B7 (Biotin)

Biotin is a water-soluble B-complex vitamin (vitamin B_7) that is composed of a ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring. A valeric acid substituent is attached to one of the carbon atoms of the tetrahydrothiophene ring. Biotin is a coenzyme in the metabolism of fatty acids and leucine, and it plays a role in gluconeogenesis.

General overview

Biotin is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids. It plays a role in the citric acid cycle, which is the process by which biochemical energy is generated during aerobic respiration. Biotin not only assists in various metabolic reactions but also helps to transfer carbon dioxide. Biotin may also be helpful in maintaining a steady blood sugar level. Biotin is often recommended for strengthening hair and nails. As a consequence, it is found in many cosmetics and health products for the hair and skin, though it cannot be absorbed through the hair or skin itself.

Biotin deficiency is rare, because, in general, intestinal bacteria produce biotin in excess of the body's daily requirements. For that reason, statutory agencies in many countries, for example the USA and Australia, do not prescribe a recommended daily intake of biotin. However, a number of metabolic disorders in which an individual's metabolism of biotin is abnormal exist; in these disorders, megadoses of biotin, far higher than the average daily intake from food, in general, can mitigate symptoms and correct the underlying metabolic disturbance.

Biochemistry

Biotin D(+) is a cofactor responsible for carbon dioxide transfer in several carboxylase enzymes:

- Acetyl-CoA carboxylase alpha
- Acetyl-CoA carboxylase beta
- Methylcrotonyl-CoA carboxylase
- Propionyl-CoA carboxylase
- Pyruvate carboxylase

and, so, is important in fatty acid synthesis, branched-chain amino acid catabolism, and gluconeogenesis. Biotin covalently attaches to the epsilon-amino group of specific lysine residues in these carboxylases. This biotinylation reaction requires ATP and is catalyzed by holocarboxylase synthetase. The attachment of biotin to various chemical sites can be used as an important laboratory technique to study various processes including protein localization, protein interactions, DNA transcription, and replication. Biotinidase itself is known to be able to biotinylate histone proteins, but little biotin is found naturally attached to chromatin.

Biotin binds very tightly to the tetrameric protein avidin (also streptavidin and neutravidin), with a dissociation constant K_d in the order of 10^{-15}, which is one of the strongest known protein-ligand interactions, approaching the covalent bond in strength. This is often used in different biotechnological applications. Until 2005, very harsh conditions were required to break the biotin-streptavidin bond.

Sources of biotin

Biotin is consumed from a wide range of food sources in the diet, however there are few particularly rich sources. Foods with a relatively high biotin content include raw egg yolk (however, the consumption of egg whites with egg yolks minimizes the effectiveness of egg yolk's biotin in one's body), liver, some vegetables, and peanuts. The dietary biotin intake in Western populations has been estimated to be 35 to 70 μg/d (143–287 nmol/d).

Biotin is also available from supplements. The synthetic process developed by Leo Sternbach and Moses Wolf Goldberg in the 1940s uses fumaric acid as a starting material and is identical to the natural product.

Bioavailability

Biotin is also called vitamin H (the H represents "Haar und Haut", German words for "hair and skin") or vitamin B_7. Studies on the bioavailability of biotin have been conducted in rats and in chicks. From these studies, it was concluded that biotin bioavailability may be low or variable, depending on the type of food being consumed. In general, biotin exists in food as protein bound form or biocytin. Proteolysis by protease is required prior to absorption. This process assists free biotin release from biocytin and protein-bound biotin. The biotin present in corn is readily available; however, most grains have about a 20-40% bioavailability of biotin.

A possible explanation for the wide variability in biotin bioavailability is that it is due to ability of an organism to break various biotin-protein bonds from food. Whether an

organism has an enzyme with the ability to break that bond will determine the bioavailability of biotin from the foodstuff.

Factors that affect biotin requirements

The frequency of marginal biotin status is not known, but the incidence of low circulating biotin levels in alcoholics has been found to be much greater than in the general population. Also, relatively low levels of biotin have been reported in the urine or plasma of patients that have had partial gastrectomy or that have other causes of achlorhydria, burn patients, epileptics, elderly individuals, and athletes. Pregnancy and lactation may be associated with an increased demand for biotin. In pregnancy, this may be due to a possible acceleration of biotin catabolism, whereas, in lactation, the higher demand has yet to be elucidated. Recent studies have shown that marginal biotin deficiency can be present in human gestation, as evidenced by increased urinary excretion of 3-hydroxyisovaleric acid, decreased urinary excretion of biotin and bisnorbiotin, and decreased plasma concentration of biotin. Additionally, smoking may further accelerate biotin catabolism in women.

Deficiency

Biotin deficiency is relatively rare and mild, and can be addressed with supplementation. Such deficiency can be caused by the consumption of raw egg whites (Eating 2 or more uncooked egg whites daily for several months has caused biotin deficiency that is serious enough to produce symptoms), which contain high levels of the protein avidin, which binds biotin strongly. Avidin denaturates upon heating (cooking), while the biotin remains intact.

Symptoms of overt biotin deficiency include:

- Hair loss (alopecia)
- Conjunctivitis
- Dermatitis in the form of a scaly red rash around the eyes, nose, mouth, and genital area.
- Neurological symptoms in adults such as depression, lethargy, hallucination, and numbness and tingling of the extremities.

The characteristic facial rash, together with an unusual facial fat distribution, has been termed the "biotin-deficient face" by some experts. Individuals with hereditary disorders of biotin deficiency have evidence of impaired immune system function, including increased susceptibility to bacterial and fungal infections.

Pregnant women tend to have high risk of biotin deficiency. Research has shown that nearly half of pregnant women have an abnormal increase of 3-hydroxyisovaleric acid, which reflects reduced status of biotin. Numbers of studies reported that this possible biotin deficiency during the pregnancy may cause infants' congenital malformations such as cleft palate. Mice fed with dried raw egg to induce biotin deficiency during the

gestation resulted in up to 100% incidence of the infants' malnourishment. Infants and embryos are more sensitive to the biotin deficiency. Therefore, even a mild level of mother's biotin deficiency that does not reach the appearance of physiological deficiency signs may cause a serious consequence in the infants.

Metabolic disorders

Inherited metabolic disorders characterized by deficient activities of biotin-dependent carboxylases are termed multiple carboxylase deficiency. These include deficiencies in the enzymes holocarboxylase synthetase or biotinidase. Holocarboxylase synthetase deficiency prevents the body's cells from using biotin effectively, and thus interferes with multiple carboxylase reactions. Biochemical and clinical manifestation includes: ketolactic acidosis, organic aciduria, hyperammonemia, skin rash, feeding problems, hypotonia, seizures, developmental delay, alopecia, and coma.

Biotinidase deficiency is not due to inadequate biotin, but rather to a deficiency in the enzymes that process it. Biotinidase catalyzes the cleavage of biotin from biocytin and biotinyl-peptides (the proteolytic degradation products of each holocarboxylase) and thereby recycles biotin. It is also important in freeing biotin from dietary protein-bound biotin. General symptoms include decreased appetite and growth. Dermatologic symptoms include dermatitis, alopecia (hair loss) and achromotrichia (absence or loss of pigment in the hair). Perosis (a shortening and thickening of bones) is seen in the skeleton. Fatty liver and kidney syndrome (FLKS) and hepatic steatosis also can occur.

Uses

Hair problems

The signs and symptoms of biotin deficiency include hair loss that progresses in severity to include loss of eyelashes and eyebrows in severely deficient subjects. Some shampoos are available that contain biotin, but it is doubtful whether they would have any useful effect, as biotin is not absorbed well through the skin.

Cradle cap (seborrheic dermatitis)

Children with a rare inherited metabolic disorder called phenylketonuria (PKU; in which one is unable to break down the amino acid phenylalanine) often develop skin conditions such as eczema and seborrheic dermatitis in areas of the body other than the scalp. The scaly skin changes that occur in people with PKU may be related to poor ability to use biotin. Increasing dietary biotin has been known to improve seborrheic dermatitis in these cases.

Diabetes

Diabetics may also benefit from biotin supplementation. In both insulin-dependent and non-insulin-dependent diabetics, supplementation with biotin can improve blood sugar

control and help lower fasting blood glucose levels, in some studies the reduction in fasting glucose exceeded 50 percent. Biotin can also play a role in preventing the neuropathy often associated with diabetes, reducing both the numbness and tingling associated with poor glucose control.

Toxicity

Animal studies have indicated few, if any, effects due to toxic doses of biotin. This may provide evidence that both animals and humans could tolerate doses of at least an order of magnitude greater than each of their nutritional requirements. There are no reported cases of adverse effects from receiving high doses of the vitamin, in particular, when used in the treatment of metabolic disorders causing sebhorrheic dermatitis in infants.

Laboratory uses

In the laboratory, biotin is often chemically linked to proteins for biochemical assays. Its small size means the biological activity of the protein will most likely be unaffected. This process is called biotinylation. Because both streptavidin and avidin bind biotin with high affinity (K_d of $\sim 10^{-14}$ mol/L) and specificity, biotinylated proteins of interest can be isolated from a sample by exploiting this highly-stable interaction. The sample is incubated with streptavidin/avidin beads, allowing capture of the biotinylated protein of interest. Any other proteins binding to the biotinylated molecule will also stay with the bead and all other unbound proteins can be washed away. However, due to the extremely strong streptavidin-biotin interaction, very harsh conditions are needed to elute the biotinylated protein from the beads (typically 6M GuHCl at pH 1.5), which often will denature the protein of interest. To circumvent this problem, beads conjugated to monomeric avidin can be used, which has a decreased biotin-binding affinity of $\sim 10^{-8}$ mol/L, allowing the biotinylated protein of interest to be eluted with excess free biotin.

ELISAs often make use of biotinylated primary antibodies against the antigen of interest, followed by a detection step using streptavidin conjugated to a reporter molecule, such as Horseradish peroxidase.

Chapter 9

Vitamin B9 (Folic Acid)

Folic acid (also known as **vitamin B_9**, **vitamin B_c** or **folacin**) and **folate** (the naturally occurring form), as well as **pteroyl-L-glutamic acid**, **pteroyl-L-glutamate**, and **pteroylmonoglutamic acid** are forms of the water-soluble vitamin B_9. Folic acid is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver.

Vitamin B_9 (folic acid and folate inclusive) is essential to numerous bodily functions. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reactions involving folate. It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy, as well as in "feeding" some cancers. While a normal diet also high in natural folates may decrease the risk of cancer, there is diverse evidence that high folate intake from supplementation may actually promote some cancers as well as precancerous tumors and lesions. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia.

Folate and folic acid derive their names from the Latin word *folium* (which means "leaf"). Leafy vegetables are a principal source, although in Western diets fortified cereals and bread may be a larger dietary source.

A lack of dietary folic acid leads to folate deficiency which is uncommon in normal Western diets. Failures to replenish one's folates might not manifest themselves as folate deficiency for 4 months because a healthy individual has about 500-20,000 mcg of folate in body stores. This deficiency can result in many health problems, the most notable one being neural tube defects in developing embryos. Common symptoms of folate deficiency include diarrhea, macrocytic anemia with weakness or shortness of breath, nerve damage with weakness and limb numbness (peripheral neuropathy), pregnancy complications, mental confusion, forgetfulness or other cognitive declines, mental depression, sore or swollen tongue, peptic or mouth ulcers, headaches, heart palpitations, irritability, and behavioral disorders. Low levels of folate can also lead to homocysteine

accumulation. DNA synthesis and repair are impaired and this could lead to cancer development. Supplementation in patients with ischaemic heart disease may also lead to increased rates of cancer.

A 2010 opinion article in the *New York Times* named micronutrients, especially folic acid, the "world's most luscious food," since absence of folic acid and a handful of other micronutrients causes otherwise preventable deformities and diseases, especially in fetal development. Folic acid can be used to help treat Alzheimer's disease, depression, anemia, and certain types of cancer.

Dietary reference intake

Because of the difference in bioavailability between supplemented folic acid and the different forms of folate found in food, the dietary folate equivalent (DFE) system was established. One DFE is defined as 1 µg (microgram) of dietary folate, or 0.6 µg of folic acid supplement.

	Women	Pregnant women	Men
RDA	400 µg DFE	600 µg DFE	400 µg DFE
UL	1000 µg DFE	1000 µg DFE	1000 µg DFE

The "*Dietary Reference Intakes*" (DRIs) (created by the National Academy of Sciences,) are a set of reference values used for planning and assessing nutrient intake for healthy people. Two important types of reference values included in the DRIs are **Recommended Dietary Allowances (RDA)**, and **Tolerable Upper Intake Levels** for folate *from fortified foods or supplements* **(UL)**. The RDA is the daily intake sufficient to meet 97-98% of healthy individuals' needs. The UL, on the other hand, is the maximum daily intake from fortified foods or supplements unlikely to result in adverse health effects.

"*It is important to recognize that the UL refers to the amount of synthetic folate (i.e. folic acid) being consumed per day from fortified foods and/or supplements. There is no health risk, and no UL, for natural sources of folate found in food,*" according to the NIH.

Folate in foods and other sources

Certain foods are very high in folate:

- Leafy vegetables such as spinach, asparagus, turnip greens
- Legumes such as dried or fresh beans, peas and lentils
- Egg yolks.
- Baker's yeast
- Fortified grain products (pasta, cereal, bread); some breakfast cereals (ready-to-eat and others) are fortified with 25% to 100% of the recommended dietary allowance (RDA) for folic acid
- Sunflower seeds
- Liver and liver products also contain high amounts of folate

- Kidney

Moderate amounts:

- Certain fruits (orange juice, canned pineapple juice, cantaloupe, honeydew melon, grapefruit juice, banana, raspberry, grapefruit and strawberry) and vegetables (beets, corn, tomato juice, vegetable juice, broccoli, brussels sprouts, romaine lettuce and bok choy), beer.

A table of selected food sources of folate and folic acid can be found at the USDA National Nutrient Database for Standard Reference. Folic acid is added to grain products in many countries, and, in these countries, fortified products make up a significant source of the population's folic acid intake. Because of the difference in bioavailability between supplemented folic acid and the different forms of folate found in food, the dietary folate equivalent (DFE) system was established. 1 DFE is defined as 1 μg of dietary folate, or 0.6 μg of folic acid supplement. This is reduced to 0.5 μg of folic acid if the supplement is taken on an empty stomach.

Folic acid naturally found in food is susceptible to high heat and ultraviolet light, and is soluble in water. It is heat-labile in acidic environments and may also be subject to oxidation.

Some meal replacement products do not meet the folate requirements as specified by the RDAs.

Spinach

Aspargus

Beans

Peas

Lentils

Egg yolk

Baker's yeast

Fortified grain

Sunflower seeds

Conversion to biologically active derivatives

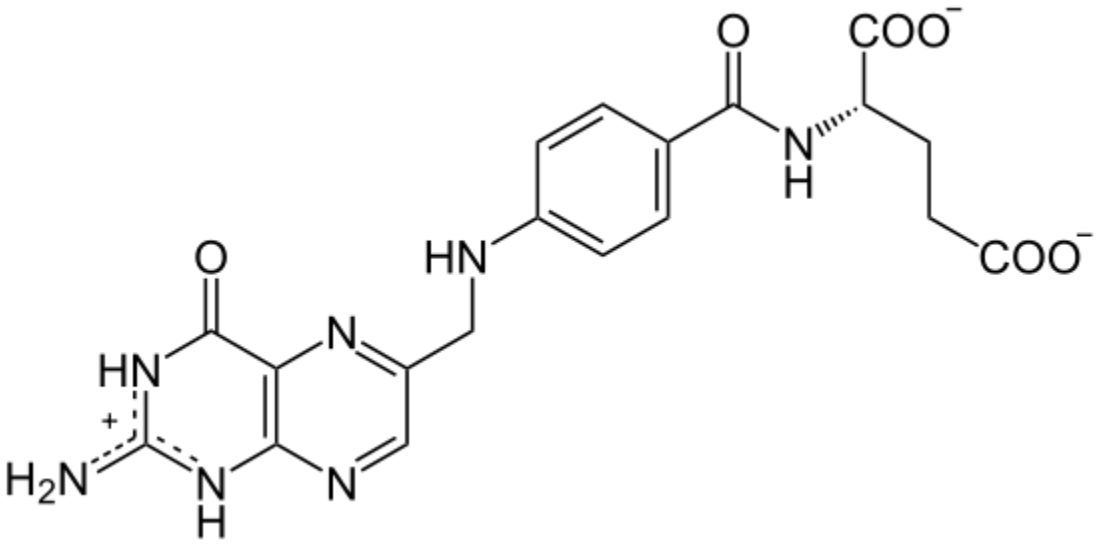
All the biological functions of folic acid are performed by tetrahydrofolate and other derivatives. Their biological availability to the body depends upon dihydrofolate reductase action in the liver. This action is unusually slow in humans, being less than 2% of that in rats. Moreover, in contrast to rats, an almost-5-fold variation in the activity of this enzyme exists between humans. Due to this low activity, it has been suggested this limits the conversion of folic acid into its biologically active forms "when folic acid is consumed at levels higher than the Tolerable Upper Intake Level (1 mg/d for adults)."

History

In the 1920s, scientists believed folate deficiency and anemia were the same condition. A key observation by researcher Lucy Wills in 1931 led to the identification of folate as the nutrient needed to prevent anemia during pregnancy. Dr. Wills demonstrated anemia could be reversed with brewer's yeast. Folate was identified as the corrective substance in brewer's yeast in the late 1930s, and was first isolated in and extracted from spinach leaves by Mitchell and others in 1941. Bob Stokstad isolated the pure crystalline form in 1943, and was able to determine its chemical structure while working at the Lederle Laboratories of the American Cyanamid Company. This historical research project, of obtaining folic acid in a pure crystalline form in 1945, was done by the team called the "folic acid boys," under the supervision and guidance of Director of Research Dr.

Yellapragada Subbarao, at the Lederle Lab, Pearl River, NY. This research subsequently led to the synthesis of the antifolate aminopterin, the first-ever anticancer drug, the clinical efficacy was proven by Dr. S. Farber in 1948. In the 1950s and 1960s, scientists began to discover the biochemical mechanisms of action for folate. In 1960, experts first linked folate deficiency to neural tube defects. In the late 1990s, US scientists realized, despite the availability of folate in foods and in supplements, there was still a challenge for people to meet their daily folate requirements, which is when the US implemented the folate fortification program.

Biological roles



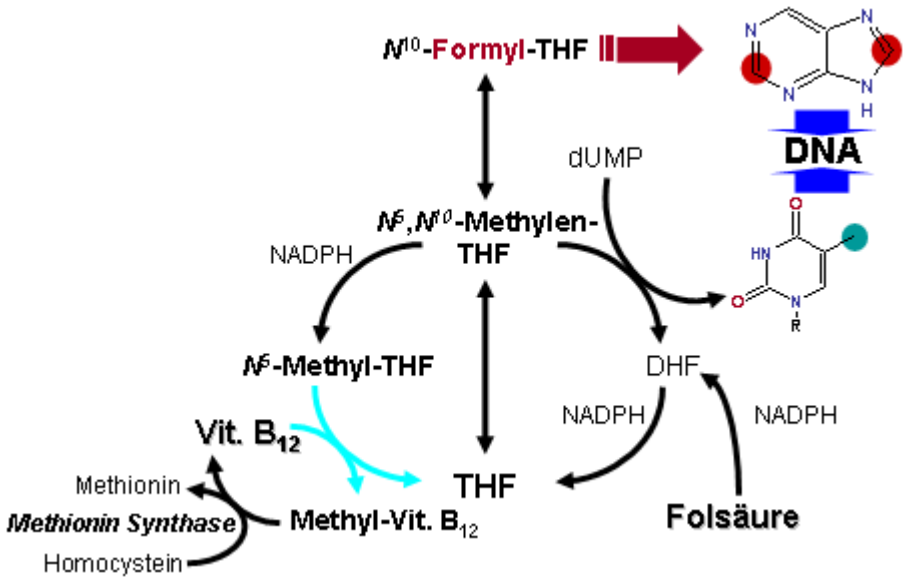
A diagram of the chemical structure of folate

DNA and cell division

Folate is necessary for the production and maintenance of new cells, for DNA synthesis and RNA synthesis, and for preventing changes to DNA, and, thus, for preventing cancer. It is especially important during periods of rapid cell division and growth, such as infancy and pregnancy. Folate is needed to carry one-carbon groups for methylation reactions and nucleic acid synthesis (the most notable one being thymine, but also purine bases). Thus, folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and neoplasms the most because of rapid cell division. RNA transcription, and subsequent protein synthesis, are less affected by folate deficiency, as the mRNA can be recycled and used again (as opposed to DNA synthesis, where a new genomic copy must be created). Since folate deficiency limits cell division, erythropoiesis, production of red blood cells, is hindered and leads to megaloblastic anemia, which is characterized by large immature red blood cells. This pathology results from persistently thwarted attempts at normal DNA replication, DNA repair, and cell division, and produces abnormally large red cells called megaloblasts (and hypersegmented neutrophils) with abundant cytoplasm capable of RNA and protein synthesis, but with clumping and fragmentation of nuclear chromatin. Some of these large cells, although immature

(reticulocytes), are released early from the marrow in an attempt to compensate for the anemia. Both adults and children need folate to make normal red and white blood cells and prevent anemia. Deficiency of folate in pregnant women has been implicated in neural tube defects (NTD); therefore, many developed countries have implemented mandatory folic acid fortification in cereals, etc. It must be noted that NTDs occur early in pregnancy (first month), therefore women must have abundant folate upon conception. Folate is required to make red blood cells and white blood cells and folate deficiency may lead to anemia, which further leads to fatigue and weakness and inability to concentrate.

Biochemistry of DNA base and amino acid production



Metabolism of folic acid to produce methyl-vitamin B_{12}

In the form of a series of tetrahydrofolate (THF) compounds, folate derivatives are substrates in a number of single-carbon-transfer reactions, and also are involved in the synthesis of dTMP (2′-deoxythymidine-5′-phosphate) from dUMP (2′-deoxyuridine-5′-phosphate). It is a substrate for an important reaction that involves vitamin B_{12} and it is necessary for the synthesis of DNA, and so required for all dividing cells.

The pathway leading to the formation of tetrahydrofolate (FH_4) begins when folate (F) is reduced to dihydrofolate (DHF) (FH_2), which is then reduced to THF. Dihydrofolate reductase catalyses the last step. Vitamin B_3 in the form of NADPH is a necessary cofactor for both steps of the synthesis.

Methylene-THF (CH_2FH_4) is formed from THF by the addition of methylene groups from one of three carbon donors: formaldehyde, serine, or glycine. Methyl tetrahydrofolate (CH_3-THF) can be made from methylene-THF by reduction of the methylene group with NADPH. It is important to note that Vitamin B_{12} is the only acceptor of methyl-THF. There is also only one acceptor for methyl-B_{12}, which is homocysteine in a reaction catalyzed by homocysteine methyltransferase. This is

important because a defect in homocysteine methyltransferase or a deficiency of B_{12} can lead to a methyl-trap of THF and a subsequent deficiency. Thus, a deficiency in B_{12} can generate a large pool of methyl-THF that is unable to undergo reactions and will mimic folate deficiency. Another form of THF, formyl-THF or folinic acid results from oxidation of methylene-THF or is formed from formate donating formyl group to THF. Finally, histidine can donate a single carbon to THF to form methenyl-THF.

In other words:

folate → dihydrofolate → tetrahydrofolate ↔ methylene-THF → methyl-THF

Overview of drugs that interfere with folate reactions

A number of drugs interfere with the biosynthesis of folic acid and THF. Among them are the dihydrofolate reductase inhibitors such as trimethoprim, pyrimethamine, and methotrexate; the sulfonamides (competitive inhibitors of 4-aminobenzoic acid in the reactions of dihydropteroate synthetase).

Valproic acid, one of the most commonly prescribed anticonvulsants that is also used to treat certain psychological conditions, is a known inhibitor of folic acid, and as such, has been shown to cause neural tube defects and cases of spina bifida and cognitive impairment in the newborn. Because of this considerable risk, those mothers who must continue to use valproic acid or its derivatives during pregnancy to control their condition (as opposed to stopping the drug or switching to another drug or to a lesser dose) should take folic acid supplements under the direction and guidance of their health care providers.

The National Health and Nutrition Examination Survey (NHANES III 1988–91) and the Continuing Survey of Food Intakes by Individuals (1994–96 CSFII) indicated most adults did not consume adequate folate. However, the folic acid fortification program in the United States has increased folic acid content of commonly eaten foods such as cereals and grains, and as a result, diets of most adults now provide recommended amounts of folate equivalents.

Health issues

Human reproduction

Folic acid is an important nutrient for women who may become pregnant, because a woman's blood levels of folate fall during pregnancy due to an increased maternal RBC synthesis in the first half of the pregnancy and fetal demands in the second half. The first four weeks of pregnancy (when most women do not even realize they are pregnant) require folic acid for proper development of the brain, skull, and spinal cord. Serious birth defects such as neural tube defects are less likely to occur when women take 0.4 mg of folic acid daily. Adequate folate intake during the preconception period, the time right before and just after a woman becomes pregnant, helps protect against a number of

congenital malformations, including neural tube defects (which are the most notable birth defects that occur from folate deficiency). Neural tube defects (NTDs) result in malformations of the spine (spina bifida), skull, and brain (anencephaly). The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthy diet prior to and during the first month following conception. The protective effect of folate during pregnancy goes beyond NTDs. Supplementation with folic acid has been shown to reduce the risk of congenital heart defects, cleft lip, limb defects, and urinary tract anomalies. Women who could become pregnant are advised to eat foods fortified with folic acid or take supplements in addition to eating folate-rich foods to reduce the risk of some serious birth defects. Having enough folic acid supplements in the months before pregnancy is very important to prevent neural tube defects. Taking 400 micrograms of synthetic folic acid daily from fortified foods and/or supplements has been suggested. The RDA for folate equivalents for pregnant women is 600-800 micrograms, twice the normal RDA of 400 micrograms for women who are not pregnant.

A study published by Milunski et al. has indicated women who take folic acid supplements during the course of pregnancy can dramatically reduce the prevalence of infant neural tube defects by 74%, from 3.5 to 0.9 defects per 1000 births.

Although the recommended folic acid intake for women planning for pregnancy is 400 micrograms per day, the mechanisms and reasons why folic acid prevents birth defects is unknown. It is hypothesized that the insulin-like growth factor 2 gene is differentially methylated and these changes in IGF2 result in improved intrauterine growth and development.

Folate deficiency during pregnancy can increase the risk of preterm delivery, infant low birth weight, and fetal growth retardation. Folate deficiency in the mother increases homocysteine level in the blood, which may lead to spontaneous abortion and pregnancy complications, such as placental abruption and preeclampsia.

Recent studies have been conducted to test the hypothesis that folic acid supplementation reduces the risk of childhood acute lymphoblastic leukemia, but evidence so far has been weak.

Folic acid may also reduce chromosomal defects in sperm to some extent, which may be relevant for men considering to father a child. A benefit is indicated even for more than 700 mcg folate per day, which, though below the tolerable upper intake levels of 1,000 µg/day, was 1.8 times the recommended dietary allowance.

It is estimated approximately 85% of women use folic acid supplements before they become pregnant, but only 18% use enough folic acid supplements to meet the current folic acid requirements due to socio-economic challenges facing some women.

Folic acid supplements may even protect the fetus against disease when the mother is battling a disease or taking medications or smoking during pregnancy.

Heart disease

An estimated 13,500 deaths occur annually due to folate deficiency's effect on coronary artery disease and the risk of ischemic heart disease, and stroke has been reduced by 15% since folate fortification regulations were enforced. Adequate concentrations of folate, vitamin B_{12}, or vitamin B_6 may decrease the circulating level of homocysteine, an amino acid normally found in blood. There is evidence an elevated homocysteine level is an independent risk factor for heart disease and stroke. The evidence suggests high levels of homocysteine may damage coronary arteries or make it easier for blood platelets to clump together and form a clot. However, there is currently no evidence available to suggest lowering homocysteine with vitamins will reduce risk of heart disease. The NORVIT trial suggests folic acid supplementation may do more harm than good.

As of 2006, studies have shown giving folic acid to reduce levels of homocysteine does not result in clinical benefit. One of these studies suggests folic acid in combination with B_{12} may even increase some cardiovascular risks.

However, a 2005 study found 5 mg of folate daily over a three-week period reduced pulse pressure by 4.7 mmHg compared with a placebo, and concluded folic acid is a safe and effective supplement that targets large artery stiffness and may prevent isolated systolic hypertension.

Also, as a result of new research, "heart experts" at Johns Hopkins Medical Center reported in March 2008 in favour of therapeutic folate, although they cautioned that it is premature for people to begin to self-medicate by taking high doses of folic acid."

Hyperhomocysteinemia is a predictor of cardiovascular disease and hypertension among children and folic acid is a safe and effective supplement because it reduces serum homocysteine levels as well as systolic and diastolic blood pressure, thus preventing cardiovascular disease in children.

Folic acid supplements may improve the integrity of the vascular endothelium. Folic acid supplements consumed before and during pregnancy may reduce the risk of heart defects in infants, and may reduce the risk for children to develop metabolic syndrome. Thet may, however, worsen the outcomes in patients with cardiovascular disease such as angina and myocardial infarction.

Stroke

Folic acid appears to reduce the risk of stroke. The reviews indicate the risk of stroke appears to be reduced only in some individuals, but a definite recommendation regarding supplementation beyond the current RDA has not been established for stroke prevention. Observed stroke reduction is consistent with the reduction in pulse pressure produced by folate supplementation of 5 mg per day, since hypertension is a key risk factor for stroke. Folic supplements are inexpensive and relatively safe to use, which is why stroke or

hyperhomocysteinemia patients are encouraged to consume daily B vitamins including folic acid.

Cancer

Folate deficiency decreases intracellular S-adenosylmethionine (SAM), which inhibits cytosine methylation in DNA, activates proto-oncogenes, induces malignant transformations, causes DNA precursor imbalances, misincorporates uracil into DNA, and promotes chromosome breakage; all of these mechanisms increase the risk of prostate cancer development.

The association between folate and cancer appears to be complex and mixed. There are theoretical reasons that folate may help prevent cancer, and a meta-analysis published in 2010 failed to find a statistically significant cancer risk due to folic acid treatments, but a 1995 study found supplementation *increases* rates of cancer.

Some investigations have proposed good levels of folic acid may be related to lower risk of esophageal, stomach, and ovarian cancers, but the benefits of folic acid against cancer may depend on when it is taken and on individual conditions. In addition, folic acid may not be helpful, and could even be **damaging**, in people already suffering from cancer or from a precancerous condition. Likewise, it has been suggested excess folate may promote tumor initiation. Folate has shown to play a dual role in cancer development; *low* folate intake protects against early carcinogenesis, and *high* folate intake **promotes advanced carcinogenesis**. Therefore, public health recommendations should be careful not to encourage too much folate intake.

Diets high in folate are associated with decreased risk of colorectal cancer; some studies show the association is stronger for folate from foods alone than for folate from foods and supplements, while other studies find that folate from supplements is more effective due to greater bioavailability. A 2007 randomized clinical trial found folate supplements did not reduce the risk of colorectal adenomas, and in fact increase the presence of advanced lesions and adenoma multiplicity. Colorectal cancer is the most studied type of cancer in relation to folate and one carbon metabolism. For example, folic acid supplement intake increased advanced colorectal cancer development by 67% in a 14-year European research study involving 520,000 men.

A 2006 prospective study of 81,922 Swedish adults found diets great in folate from foods, but not from supplements, were associated with a reduced risk of pancreatic cancer.

Most epidemiologic studies suggest diets high in folate are associated with decreased risk of breast cancer, but results are not uniformly consistent. One broad cancer screening trial reported a potential harmful effect of much folate intake on breast cancer risk, suggesting routine folate supplementation should not be recommended as a breast cancer preventive, but a 2007 Swedish prospective study found much folate intake was associated with a lower incidence of postmenopausal breast cancer. A 2008 study has shown no significant

effect of folic acid on overall risk of total invasive cancer or breast cancer among women. Folate intake may not have any effect on the risk of breast cancer but may have an effect for women who consume at least 15 g/d of alcohol. Folate intake of more than 300 μg/d may reduce the risk of breast cancer in women who consume alcohol.

Most research studies associate high dietary folate intake with a reduced risk of prostate cancer; however, in men, folic acid supplementation appears to **double the risk of prostate cancer.** Recently, a clinical trial showed daily supplementation of 1 mg of folic acid increased the risk of prostate cancer, while dietary and plasma folate levels among vitamin nonusers actually decreased the risk of prostate cancer. A Finnish study consisting of 29,133 older male smokers observed prostate cancer risk had no relationship with serum folate levels.

The reason high levels of folic acid may increase cancer is because of its role in nucleotide synthesis (proliferating neoplastic cells need this and folate receptors are increased in cancers). Folate's role in DNA methylation is important in prostate cancer. Unmetabolized folic acid is associated with a reduction in natural killer cell cytotoxicity, which reduces the immune system's ability to defend against malignant cells. However, the study also showed dietary baseline intake of folate may have an inverse relationship to prostate cancer occurrence.

Although the relationship between folate and prostate cancer is not yet clear, suicide gene studies show a target vector for folate to prostate and nasopharyngeal cancer cells. Growth of tumor cells is significantly inhibited when a folate-linked nanoparticle is injected intratumorally. The mechanism might be the interference of transfection and communication failures of intracellular gap junctions.

The cancer drug methotrexate is designed to inhibit the metabolism of folic acid. Folic acid may interact unexpectedly with the cancer drug fluorouracil. The exact mechanism of interaction is unknown.

The low dihydrofolate reductase activity in the liver of humans compared to other animals and so the low conversion of folic acid into its active derivatives might be due to the control of this enzyme by transcription factors, such as E2F-1 involved in cell proliferation. It has been suggested "the low level of DHFR, and the other proteins under the control of E2F-1, in humans may have evolved to hinder the development of cancer. If this is the case, other animals with slow tissue turnover rates, possibly related to long life span, might also have low DHFR activity."

Folic acid supplements prevent mistakes (inserting uracils into the DNA, for example) from occurring during DNA replication and repair. This is a proposed mechanism for folic acid's protection against colorectal cancer.

Folic acid supplements stimulate the PI3k/Akt signaling cascade, which leads to improved cell survival, but this could be beneficial or harmful for the body because

cancer cells may use this pathway to survive. Folic acid may also reduce the levels of PTEN (a tumor suppressor gene), making this relationship even more controversial.

Antifolates

Folate is important for cells and tissues that rapidly divide. Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. The antifolate methotrexate is a drug often used to treat cancer because it inhibits the production of the active form of THF from the inactive dihydrofolate (DHF). However, methotrexate can be toxic, producing side effects, such as inflammation in the digestive tract that make it difficult to eat normally. Also, bone marrow depression (inducing leukopenia and thrombocytopenia), and acute renal and hepatic failure have been reported.

Folinic acid, under the drug name leucovorin,a form of folate (formyl-THF), can help "rescue" or reverse the toxic effects of methotrexate. Folinic acid is *not* the same as folic acid. Folic acid supplements have little established role in cancer chemotherapy. There have been cases of severe adverse effects of accidental substitution of folic acid for folinic acid in patients receiving methotrexate cancer chemotherapy. It is important for anyone receiving methotrexate to follow medical advice on the use of folic or folinic acid supplements. The supplement of folinic acid in patients undergoing methotrexate treatment is to give cells dividing less rapidly enough folate to maintain normal cell functions. The amount of folate given will be depleted by rapidly dividing cells (cancer) very fast and so will not negate the effects of methotrexate.

Obesity

Folic acid increases lipolysis in adipocytes, and may have a role in the prevention of obesity and type 2 diabetes. This mechanism involves the beta adrenoceptors in the adbdominal adipocytes. Folic acid supplements may reduce the accumulation of cholesterol in the liver and in the blood; this may be due to folic acid's role in incorporating cholesterol into bile acid. Folic acid supplements have been shown to increase bile acid production and flow.

Depression

Some evidence links a shortage of folate with depression. Limited evidence from randomised controlled trials showed using folic acid in addition to antidepressants, specifically SSRIs, may have benefits. Research at the University of York and Hull York Medical School has found a link between depression and low levels of folate. One study by the same team involved 15,315 subjects. However, the evidence is probably too limited at present for this to be a routine treatment recommendation. Folic acid supplementation affects noradrenaline and serotonin receptors within the brain which could be the cause of folic acid's possible ability to act as an antidepressant.

Memory and mental agility

In a three-year trial on 818 people over the age of 50, short-term memory, mental agility, and verbal fluency were all found to be better among people who took 800 micrograms of folic acid daily, twice the current RDA, than those who took placebo. The study was reported in *The Lancet* on 20 January 2007.

Schizophrenia

Folate deficiency may increase the risk of schizophrenia because, by increasing homocysteine levels, folate also increases interleukin 6 and tumor necrosis factor alpha levels, and these two cytokines are involved in the development of schizophrenia. The exact mechanisms involved in the development of schizophrenia are not entirely clear, but may have something to do with DNA methylation and one carbon metabolism, and these are the precise roles of folate in the body.

Allergic diseases

There is a relationship between folic acid and allergic diseases. In one study that examined the relationship between serum folate levels and markers of atopy, wheeze, and asthma in 8,083 subjects, serum folate levels were found to be inversely related to IgE level, atopy, and wheeze in a dose-response relationship. Increased folate levels were also associated with decreased risk of doctor-diagnosed asthma. Folic acid supplementation during late pregnancy is associated with an increased risk of childhood asthma, increased risk of persistent asthma, and poorer respiratory function in young children.

Rheumatoid arthritis

Folic acid in a dose of of 5–27 mg per week is used to protect patients with rheumatoid arthritis who are taking methotrexate from the toxic effects of this drug.

Fertility

Folate is necessary for fertility in both men and women. In men, it contributes to spermatogenesis. In women, on the other hand, it contributes to oocyte maturation, implantation, placentation, in addition to the general effects of folic acid and pregnancy. Therefore, it is necessary to receive sufficient amounts through the diet to avoid subfertility. Also, polymorphisms in genes of enzymes involved in folate metabolism could be one reason for fertility complications in some women with unexplained infertility.

Renal disease

Folic acid supplements may reduce the risk of children developing renal diseases or injuries, such as microalbuminuria.

Type 1 diabetes mellitus

Type 1 diabetes mellitus patients have lower plasma levels of folic acid, and may benefit from folic acid supplements or folic acid-fortified food products.

Macular degeneration

A substudy of the Women's Antioxidant and Folic Acid Cardiovascular Study published in 2009 reported use of a nutritional supplement containing folic acid, pyridoxine, and cyanocobalamin decreased the risk of developing age-related macular degeneration by 34.7%.

Bone health

Folate deficiency has been hypothesized to lead to elevated homocysteine levels, which, in turn, leads to an increased risk of bone fractures, osteoporosis, and reduction in bone mineral density, but research studies so far show controversial results. It aids in bone health by promoting the regeneration of bone marrow.

Menopause

Folic acid supplements help relieve hot flashes in postmenopausal women. Just as in estrogen hormone replacement therapy, folic acid interacts with neurotransmitters (norepinephrine, serotonin) in the brain to reduce hot flashes.

Infectious disease

Folate deficiency is linked to anemia-causing *Plasmodium falciparum* malaria in areas such as Colombia, where malaria has reached endemic proportions.

Bone loss in Parkinson's disease (PD)

Folate lowers homocysteine levels, which, in turn, prevents bone loss in Parkinson's disease (PD) patients taking levodopa (a psychoactive drug taken to treat Parkinson's disease). Improvements in bone health include increased BMD at the lumbar spine, total femur, and femur shaft.

Folic acid supplements and masking of B_{12} deficiency

There has been concern about the interaction between vitamin B_{12} and folic acid. The National Institutes of Health has found that "Large amounts of **folic acid** can mask the damaging effects of vitamin B_{12} deficiency by correcting the megaloblastic anemia caused by vitamin B_{12} deficiency without correcting the neurological damage that also occurs", there are also indications that "high serum folate levels might not only mask vitamin B_{12} deficiency, but could also exacerbate the anemia and worsen the cognitive

symptoms associated with vitamin B_{12} deficiency". Due to the fact that in the United States legislation has required enriched flour to contain folic acid to reduce cases of fetal neural-tube defects consumers may be injesting more than they realize. To counter the masking effect of B_{12} deficency the NIH recommends "folic acid intake from fortified food and supplements should not exceed 1,000 micrograms (1000 μg = 1 mg) daily in healthy adults."

In fact, to date the evidence such masking actually occurs is scarce, and there is no evidence folic acid fortification in Canada or the U.S. has increased the prevalence of vitamin B_{12} deficiency or its consequences. However, one recent study has demonstrated high folic or folate levels, when combined with low B_{12} levels, are associated with significant cognitive impairment among the elderly.

In any case, it is important for older adults to be aware of the relationship between folic acid and vitamin B_{12}, because they are at greater risk of having a B_{12} deficiency. For this reason, a physician may wish to check the vitamin B_{12} status of patients 50 years of age or older before prescribing them a supplement that contains folic acid.

Overdose Risks

The risk of toxicity from folic acid is low, because folate is a water-soluble vitamin and is regularly removed from the body through urine. The Institute of Medicine has established a tolerable upper intake level (UL) for folate of 1 mg for adult men and women, and a UL of 800 μg for pregnant and lactating (breast-feeding) women less than 18 years of age. Supplemental folic acid should not exceed the UL to prevent folic acid from masking symptoms of vitamin B_{12} deficiency.

Research suggests high levels of folic acid can interfere with some antimalarial treatments.

A 10,000-patient study at Tufts University in 2007 concluded excess folic acid worsens the effects of B_{12} deficiency and, in fact, may affect the absorption of B_{12}.

A study at the University of Adelaide concluded the intake of folic acid supplements during late pregnancy increases the risk of babies developing childhood asthma by 30%, although researchers emphasized that their finding did not contradict recommendations to supplement folic acid in first trimester, when no additional risk was found.

Elderly population

There are benefits and risks of food folic acid fortification for elderly populations. Elevated exposure to folic acid due to fortification can improve folate and homocysteine levels, but can also mask symptoms of vitamin B_{12} deficiency. A study where 747 subjects aged 67 to 96 years were measured for B vitamin and homocysteine status showed diets with folic acid fortification of 140 μg/100 g of grain product decreased homocysteine level and heart disease risk. However, Canada's food supply is fortified

with 150 μg/100 g of grain, and much of the elderly population also takes a supplement that includes a folic acid component of 400 μg. Therefore, it is important not to consume quantities over the recommended DRI.

Folate deficiency

Folate deficiency may lead to glossitis, diarrhea, depression, confusion, anemia, and fetal neural tube defects and brain defects (during pregnancy). Folate deficiency is accelerated by alcohol consumption Folate deficiency is diagnosed by analyzing CBC and plasma vitamin B_{12} and folate levels. CBC may indicate megaloblastic anemia but this could also be a sign of vitamin B_{12} deficiency. A serum folate of 3 μg/L or lower indicates deficiency. Serum folate level reflects folate status but erythrocyte folate level better reflects tissue stores after intake. An erythrocyte folate level of 140 μg/L or lower indicates inadequate folate status. Increased homocysteine level suggests tissue folate deficiency but homocysteine is also affected by vitamin B_{12} and vitamin B_6, renal function, and genetics. One way to differentiate between folate deficiency from vitamin B_{12} deficiency is by testing for methylmalonic acid levels. Normal MMA levels indicate folate deficiency and elevated MMA levels indicate vitamin B_{12} deficiency. Folate deficiency is treated with supplemental oral folate of 400 to 1000 μg per day. This treatment is very successful in replenishing tissues, even if deficiency was caused by malabsorption. Patients with megaloblastic anemia need to be tested for vitamin B_{12} deficiency before folate treatment, because if the patient has vitamin B_{12} deficiency, folate supplementation can remove the anemia, but can also worsen neurologic problems. Morbidly obese patients with BMIs of greater than 50 are more likely to develop folate deficiency. Patients with celiac disease have a higher chance of developing folate deficiency. Cobalamin deficiency may lead to folate deficiency, which, in turn, increases homocysteine levels and finally may result in the development of cardiovascular disease or birth defects.

Iron-folic acid supplementation risk for children

Some studies show iron-folic acid supplementation in children under 5 may result in increased mortality due to malaria; this has prompted the World Health Organization to alter their iron-folic acid supplementation policies for children in malaria-prone areas, such as India.

Dietary fortification

In the USA many grain products are fortified with folic acid.

Since the discovery of the link between insufficient folic acid and neural tube defects, governments and health organizations worldwide have made recommendations concerning folic acid *supplementation* for women intending to become pregnant.

This has led to the introduction in many countries of *fortification*, where folic acid is added to flour with the intention of benefiting all from the associated rise in blood folate levels. This is controversial, with issues having been raised concerning individual liberty, and the masking effect of folate fortification on pernicious anaemia (vitamin B_{12} deficiency). However, several western countries now fortify their flour, along with a

number of Middle Eastern countries and Indonesia. Mongolia and a number of former Soviet republics are among those having widespread voluntary fortification; about five more countries (including Morocco, the first African country) have agreed, but not yet implemented, fortification. To date, no EU country has yet mandated fortification.

Folates can be produced by engineering *Lactococcus lactis* strains using a rodent depletion-repletion bioassay, and the bioavailabilities of these folates are comparable with those of commercial folic acid currently being used for food fortification. These engineered folates can potentially help alleviate the effects of folate deficiency in the diet. Hematologic studies show an improvement in megaloblastic anemia after the addition of *L. lactis* strains; this again suggests lactic acid bacteria can potentially reverse some of the harm done by folate deficiency by acting as an essential, bioavailable vitamin.

Effects of fortification and plasma folate and homocysteine levels

A study has shown folate fortification will substantially increase in folate status, in particular, for the elderly. In the study group, the subjects that did not use vitamin supplements has increased folate concentrations of 4.6 ng/mL to 10.0 ng/mL (11 to 23 nmol/L) ($P<0.001$) from the base-line visit to the follow-up visit. The prevalence of low folate concentrations (<3 ng/mL [7 nmol/L]) decreased from 22.0% to 1.7% ($P< 0.001$). The mean total homocysteine concentration has decreased from a value of 10.1 μmol/L to 9.4 μmol/L during this period ($P<0.001$), while the prevalence of high homocysteine concentrations (>13 μmol/L) has been reduced from 18.7% to 9.8% ($P<0.001$). To further clarify the study methods, there were no statistically significant changes in concentrations of folate or homocysteine for the control group.

Australia

There has been previous debate in Australia regarding the inclusion of folic acid in products such as bread and flour.

Australia and New Zealand have jointly agreed to fortification though the Food Standards Australia New Zealand. Australia will fortify all flour from 18 September 2009. Although the food standard covers both Australia and New Zealand, an Australian government official has stated it is up to New Zealand to decide whether to implement it there, and they will watch with interest.

The requirement is 0.135 mg of folate per 100g of bread.

Canada

In 2003, a Hospital for Sick Children, University of Toronto research group published findings showing the fortification of flour with folic acid in Canada has resulted in a dramatic decrease in neuroblastoma, an early and very dangerous cancer in young children. In 2009, further evidence from McGill University showed a 6.2% decrease per year in the birth prevalence of severe congenital heart defects.

Folic acid used in fortified foods is a synthetic form called pteroylmonoglutamate. It is in its oxidized state and contains only one conjugated glutamate residue. Folic acid therefore enters via a different carrier system from naturally occurring folate, and this may have different effects on folate binding proteins and its transporters. Folic acid has a higher bioavailability than natural folates and are rapidly absorbed across the intestine, therefore it is important to consider the Dietary Folate Equivalent (DFE) when calculating one's intake. Natural occurring folate is equal to 1 DFE, however 0.6 µg of folic acid is equal to 1 DFE.

Folic acid food fortification became mandatory in Canada in 1998, with the fortification of 150 µg of folic acid per 100 grams of enriched flour and uncooked cereal grains. The purpose of fortification was to decrease the risk of neural tube defects in newborns. It is important to fortify grains because it is a widely eaten food and the neural tube closes in the first four weeks of gestation, often before many women even know they are pregnant. Canada's fortification program has been successful with a decrease of neural tube defects by 19% since its introduction. A seven-province study from 1993 to 2002 showed a reduction of 46% in the overall rate of neural tube defects after folic acid fortification was introduced in Canada. The fortification program was estimated to raise a person's folic acid intake level by 70–130 µg/day, however an increase of almost double that amount was actually observed. This could be from the fact that many foods are over fortified by 160–175% the predicted value. In addition, much of the elder population take supplements that adds 400 µg to their daily folic acid intake. This is a concern because 70-80% of the population have detectable levels of unmetabolized folic acid in their blood and high intakes can accelerate the growth of preneoplasmic lesions. It is still unknown the amount of folic acid supplementation that might cause harm.

Folic acid supplementation promotion in Canada

According to a Canadian survey, 58% of women said they took a folic acid containing multivitamin or a folic acid supplement as early as three months before becoming pregnant. Women in higher income households and with more years of school education are using more folic acid supplements before pregnancy. Women with planned pregnancies and who are over the age of 25 are more likely to use folic acid supplements. Canadian public health efforts are focused on promoting awareness of the importance of folic acid supplementation for all women of childbearing age and decreasing socio-economic inequalities by providing practical folic acid support to vulnerable groups of women.

New Zealand

New Zealand was planning to fortify bread (excluding organic and unleavened varieties) from 18 September 2009, but has opted to wait until more research is done.

The Association of Bakers and the Green Party have opposed mandatory fortification, describing it as "mass medication". Food Safety Minister Kate Wilkinson reviewed the decision to fortify in July 2009, citing links between overconsumption of folate with

cancer . The New Zealand Government is reviewing whether it will continue with the mandatory introduction of folic acid to bread.

United Kingdom

There has been previous debate in the United Kingdom regarding the inclusion of folic acid in products such as bread and flour.

The Food Standards Agency has recommended fortification.

United States

The United States Public Health Service recommends an extra 0.4 mg/day, which can be taken as a pill. However, many researchers believe supplementation in this way can never work effectively enough, since about half of all pregnancies in the U.S. are unplanned, and not all women will comply with the recommendation. Approximately 53% of the US population uses dietary supplements and 35% uses dietary supplements containing folic acid. Men consume more folate (in dietary folate equivalents) than women, and non-Hispanic whites have higher folate intakes than Mexican Americans and non-Hispanic blacks. Twenty nine percent of black women have inadequate intakes of folate. The age group consuming the most folate and folic acid is the >50 group. Only 5% of the population exceeds the Tolerable Upper Intake Level.

In 1996, the United States Food and Drug Administration (FDA) published regulations requiring the addition of folic acid to enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products. This ruling took effect on January 1, 1998, and was specifically targeted to reduce the risk of neural tube birth defects in newborns. There are concerns that the amount of folate added is insufficient . In October 2006, the Australian press claimed that U.S. regulations requiring fortification of grain products were being interpreted as disallowing fortification in non-grain products, specifically Vegemite (an Australian yeast extract containing folate). The FDA later said the report was inaccurate, and no ban or other action was being taken against Vegemite.

As a result of the folic acid fortification program, fortified foods have become a major source of folic acid in the American diet. The Centers for Disease Control and Prevention in Atlanta, Georgia used data from 23 birth defect registries covering about half of United States births, and extrapolated their findings to the rest of the country. These data indicate since the addition of folic acid in grain-based foods as mandated by the FDA, the rate of neural tube defects dropped by 25% in the United States. The results of folic acid fortification on the rate of neural tube defects in Canada have also been positive, showing a 46% reduction in prevalence of NTDs; the magnitude of reduction was proportional to the prefortification rate of NTDs, essentially removing geographical variations in rates of NTDs seen in Canada before fortification.

When the U.S. Food and Drug Administration set the folic acid fortification regulation in 1996, the projected increase in folic acid intake was 100 μg/d. Data from a study with

1480 subjects showed that folic acid intake increased by 190 µg/d and total folate intake increased by 323 µg dietary folate equivalents (DFE)/d. Folic acid intake above the upper tolerable intake level (1000 µg folic acid/d) increased only among those individuals consuming folic acid supplements as well as folic acid found in fortified grain products. Taken together, folic acid fortification has led to a bigger increase in folic acid intake than first projected.

Future directions for research

- Identifying polymorphisms or mutations in genes involved in the synthesis of thymidylate, purines, regulatory proteins, or substrates involved in folate and homocysteine metabolism, such as serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase, rather than simply focusing on 5,10-methylenetetrahydrofolate reductase as the main cause .
- Direct and indirect evaluation of the adverse effects of greatly increased folic acid intakes
- Human studies looking into whether folate will interfere with the effectiveness of antifolate treatments and the possibility that it may support cancer growth.
- More epidemiologic and clinical studies need to be done on human tumor samples to fully understand the role of folate receptor alpha in tumor etiology, progression, and patient survival.

Chapter 10

Vitamin B_{12}

Vitamin B_{12}, **vitamin B12** or **vitamin B-12**, also called **cobalamin**, is a water soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production. As the largest and most structurally complicated vitamin, it can be produced industrially only through bacterial fermentation-synthesis.

Vitamin B_{12} consists of a class of chemically-related compounds (vitamers), all of which have vitamin activity. It contains the biochemically rare element cobalt. Biosynthesis of the basic structure of the vitamin in nature is only accomplished by simple organisms such as some bacteria and algae, but conversion between different forms of the vitamin can be accomplished in the human body. A common synthetic form of the vitamin, cyanocobalamin, does not occur in nature, but is used in many pharmaceuticals and supplements, and as a food additive, because of its stability and lower cost. In the body it is converted to the physiological forms, methylcobalamin and adenosylcobalamin, leaving behind the cyanide, albeit in minimal concentration. More recently, hydroxocobalamin (a form produced by bacteria), methylcobalamin, and adenosylcobalamin can also be found in more expensive pharmacological products and food supplements. The extra utility of these is currently debated.

Vitamin B_{12} was discovered from its relationship to the disease pernicious anemia, which is an autoimmune disease that destroys parietal cells in the stomach that secrete intrinsic factor. Intrinsic factor is crucial for the normal absorption of B_{12}, so a lack of intrinsic

factor, as seen in pernicious anemia, causes a vitamin B_{12} deficiency. Many other subtler kinds of vitamin B_{12} deficiency and their biochemical effects have since been elucidated.

Terminology

The names **vitamin B_{12}** or **vitamin B12** or **vitamin B-12**, which are sometimes shortened to **B_{12}** or **B12**, and the alternative name cobalamin generally refer to all forms of the vitamin. Some medical practitioners have suggested that its use be split into two different categories, however.

- In a broad sense, B_{12} refers to a group of cobalt-containing vitamer compounds known as cobalamins: these include cyanocobalamin (an artifact formed from using activated charcoal, which always contains trace cyanide, to purify hydroxycobalamin), hydroxocobalamin (another medicinal form, produced by bacteria), and finally, the two naturally occurring cofactor forms of B_{12} in the human body: 5'-deoxyadenosylcobalamin (adenosylcobalamin—AdoB12), the cofactor of Methylmalonyl Coenzyme A mutase (MUT), and methylcobalamin (MeB_{12}), the cofactor of 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR).
- The term B_{12} may be properly used to refer to cyanocobalamin, the principal B_{12} form used for foods and in nutritional supplements. This ordinarily creates no problem, except perhaps in rare cases of eye nerve damage, where the body is only marginally able to use this form due to high cyanide levels in the blood due to cigarette smoking, and thus requires cessation of smoking, or else B_{12} given in another form, for the optic symptoms to abate. However, tobacco amblyopia is a rare enough condition that debate continues about whether or not it represents a peculiar B_{12} deficiency which is resistant to treatment with cyanocobalamin.

Finally, so-called **pseudo-B_{12}** refers to B_{12}-like substances which are found in certain organisms, including *Spirulina* (a cyanobacterium) and some algae. These substances are active in tests of B_{12} activity by highly sensitive antibody-binding serum assay tests, which measure levels of B_{12} and B_{12}-like compounds in blood. However, these substances do not have B_{12} biological activity for humans, a fact which may pose a danger to vegans and others on limited diets who do not ingest B_{12} producing bacteria, but who nevertheless may show normal "B_{12}" levels in the standard immunoassay which has become the normal medical method for testing for B_{12} deficiency.

Structure

Vitamin B_{12} is a collection of cobalt and corrin ring molecules which are defined by their particular vitamin function in the body. All of the substrate cobalt-corrin molecules from which B_{12} is made, must be synthesized by bacteria. However, after this synthesis is complete, the body has a limited power to convert any form of B_{12} to another, by means of enzymatically removing certain prosthetic chemical groups from the cobalt atom. The various forms (vitamers) of B_{12} are all deeply red colored, due to the color of the cobalt-corrin complex.

Cyanocobalamin is one such "vitamer" in this B complex, because it can be metabolized in the body to an active co-enzyme form. However, the cyanocobalamin form of B_{12} does not occur in nature normally, but is a byproduct of the fact that other forms of B_{12} are avid binders of cyanide (-CN) which they pick up in the process of activated charcoal purification of the vitamin after it is made by bacteria in the commercial process. Since the cyanocobalamin form of B_{12} is easy to crystallize and is not sensitive to air-oxidation, it is typically used as a form of B_{12} for food additives and in many common multivitamins. However, this form is not perfectly synonymous with B_{12}, in as much as a number of substances (vitamers) have B_{12} vitamin activity and can properly be labeled vitamin B_{12}, and cyanocobalamin is but one of them. (Thus, all cyanocobalamin is vitamin B_{12}, but not all vitamin B_{12} is cyanocobalamin).

Hydroxocobalamin is another form of B_{12} commonly encountered in pharmacology, but which is not normally present in the human body. Hydroxocobalamin is sometimes denonoted B_{12a}. This form of B_{12} is the form produced by bacteria, and is what is converted to cyanocobalmin in the commercial charcoal filtration step of production. Hydroxocobalamin has an avid affinity for cyanide ion and has been used as an antidote to cyanide poisoning. It is supplied typically in water solution for injection. Hydroxocobalamin is thought to be converted to the active enzymic forms of B_{12} more easily than cyanocobalamin, and since it is little more expensive than cyanocobalamin, and has longer retention times in the body, has been used for vitamin replacement in situations where added reassurance of activity is desired. Intramuscular administration of hydroxocobalamin is also the preferred treatment for pediatric patients with intrinsic cobalamin metabolic diseases, for vitamin B_{12} deficient patients with tobacco amblyopia (which is thought to perhaps have a component of cyanide poisoning from cyanide in cigarette smoke); and for treatment of patients with pernicious anemia who have optic neuropathy.

B_{12} is the most chemically complex of all the vitamins. The structure of B_{12} is based on a corrin ring, which is similar to the porphyrin ring found in heme, chlorophyll, and cytochrome. The central metal ion is cobalt. Four of the six coordination sites are provided by the corrin ring, and a fifth by a dimethylbenzimidazole group. The sixth coordination site, the center of reactivity, is variable, being a cyano group (-CN), a hydroxyl group (-OH), a methyl group ($-CH_3$) or a 5'-deoxyadenosyl group (here the C5' atom of the deoxyribose forms the covalent bond with Co), respectively, to yield the four B_{12} forms mentioned above. Historically, the covalent C-Co bond is one of first examples of carbon-metal bonds to be discovered in biology. The hydrogenases and, by necessity, enzymes associated with cobalt utilization, involve metal-carbon bonds.

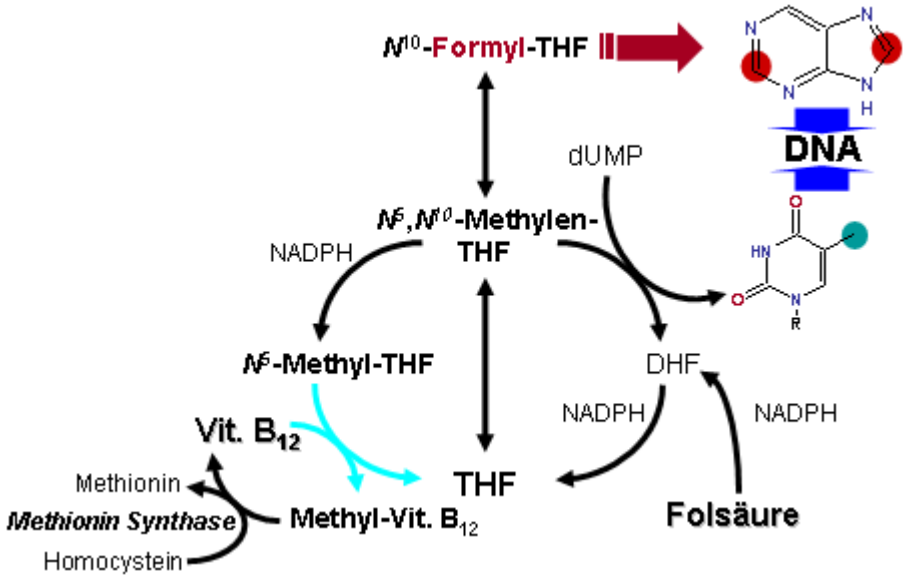
Synthesis

Neither plants nor animals are independently capable of constructing vitamin B_{12}. Only bacteria have the enzymes required for its synthesis. The total synthesis of B_{12} was reported by Robert Burns Woodward and Albert Eschenmoser in 1972, and remains one of the classic feats of organic synthesis. Species from the following genera are known to synthesize B_{12}: *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Bacillus*,

Clostridium, *Corynebacterium*, *Flavobacterium*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcus* and *Xanthomonas*.

Industrial production of B_{12} is through fermentation of selected microorganisms. *Streptomyces griseus*, a bacterium once thought to be a yeast, was the commercial source of vitamin B_{12} for many years. The species *Pseudomonas denitrificans* and *Propionibacterium shermanii* are more commonly used today. These are frequently grown under special conditions to enhance yield, and at least one company, Rhône-Poulenc of France, which has merged into Sanofi-Aventis, used genetically engineered versions of one or both of these species.

Functions



Metabolism of folic acid. The role of Vitamin B_{12} is seen at bottom-left.

Vitamin B_{12} is normally involved in the metabolism of every cell of the body, especially affecting the DNA synthesis and regulation but also fatty acid synthesis and energy production. However, many (though not all) of the effects of functions of B_{12} can be replaced by sufficient quantities of folic acid (vitamin B_9), since B_{12} is used to regenerate folate in the body. Most vitamin B_{12} deficiency symptoms are actually folate deficiency symptoms, since they include all the effects of pernicious anemia and megaloblastosis, which are due to poor synthesis of DNA when the body does not have a proper supply of folic acid for the production of thymine. When sufficient folic acid is available, all known B_{12} related deficiency syndromes normalize, save those narrowly connected with the vitamin B_{12}-dependent enzymes MUT, and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), also known as methionine synthase; and the buildup of their respective substrates (methylmalonic acid, MMA) and homocysteine.

Coenzyme B_{12}'s reactive C-Co bond participates in three main types of enzyme-catalyzed reactions.

1. **Isomerases**. Rearrangements in which a hydrogen atom is directly transferred between two adjacent atoms with concomitant exchange of the second substituent, X, which may be a carbon atom with substituents, an oxygen atom of an alcohol, or an amine.
2. **Methyltransferases**. Methyl ($-CH_3$) group transfers between two molecules.
3. **Dehalogenases**. Reactions in which a halogen atom is removed from an organic molecule. Enzymes in this class have not been identified in humans.

In humans, two major coenzyme B_{12}-dependent enzyme families corresponding to the first two reaction types, are known. These are typified by the following two enzymes:

1. MUT is an isomerase which uses the $AdoB_{12}$ form and reaction type 1 to catalyze a carbon skeleton rearrangement (the X group is -COSCoA). MUT's reaction converts MMl-CoA to Su-CoA, an important step in the extraction of energy from proteins and fats. This functionality is lost in vitamin B_{12} deficiency, and can be measured clinically as an increased methylmalonic acid (MMA) level. Unfortunately, an elevated MMA, though sensitive to B_{12} deficiency, is probably overly sensitive, and not all who have it actually have B_{12} deficiency. For example, MMA is elevated in 90–98% of patients with B_{12} deficiency; however 20–25% of patients over the age of 70 have elevated levels of MMA, yet 25–33% of them do not have B_{12} deficiency. For this reason, assessment of MMA levels is not routinely recommended in the elderly. There is no "gold standard" test for B_{12} deficiency because as a B_{12} deficiency occurs, serum values may be maintained while tissue B_{12} stores become depleted. Therefore, serum B_{12} values above the cut-off point of deficiency do not necessarily indicate adequate B_{12} status The MUT function cannot be affected by folate supplementation, which is necessary for myelin synthesis and certain other functions of the central nervous system. Other functions of B_{12} related to DNA synthesis related to MTR dysfunction can often be corrected with supplementation with the vitamin folic acid, but not the elevated levels of homocysteine, which is normally converted to methionine by MTR.
2. MTR, also known as methionine synthase, is a methyltransferase enzyme, which uses the MeB_{12} and reaction type 2 to catalyze the conversion of the amino acid homocysteine (Hcy) back into methionine (Met). This functionality is lost in vitamin B_{12} deficiency, and can be measured clinically as an increased homocysteine level *in vitro*. Increased homocysteine can also be caused by a folic acid deficiency, since B_{12} helps to regenerate the tetrahydrofolate (THF) active form of folic acid. Without B_{12}, folate is trapped as 5-methyl-folate, from which THF cannot be recovered unless a MTR process reacts the 5-methyl-folate with homocysteine to produce methionine and THF, thus decreasing the need for fresh sources of THF from the diet. THF may be produced in the conversion of homocysteine to methionine, or may be obtained in the diet. It is converted by a non-B_{12}-dependent process to 5,10-methylene-THF, which is involved in the

synthesis of thymine. Reduced availability of 5,10-methylene-THF results in problems with DNA synthesis, and ultimately in ineffective production cells with rapid turnover, in particular blood cells, and also intestinal wall cells which are responsible for absorption. The failure of blood cell production results in the once-dreaded and fatal disease, pernicious anemia. All of the DNA synthetic effects, including the megaloblastic anemia of pernicious anemia, resolve if sufficient folate is present (since levels of 5,10-methylene-THF still remain adequate with enough dietary folate). Thus the best-known "function" of B_{12} (that which is involved with DNA synthesis, cell-division, and anemia) is actually a facultative function which is mediated by B_{12}-conservation of an active form of folate which is needed for efficient DNA production. Other cobalamin-requiring methyltransferase enzymes are also known in bacteria, such as Me-H4-MPT, coenzyme M methyl transferase.

Specific MUT and MTR failure syndromes, even with excess folate

If folate is present in quantity, then of the two absolutely vitamin B_{12}-dependent enzyme-family reactions in humans, the MUT-family reactions show the most direct and characteristic secondary effects, focusing on the nervous system. This is because the MTR (methyltransferase-type) reactions are involved in regenerating folate, and thus are less evident when folate is in good supply.

Since the late 1990s, folic acid has begun to be added to fortify flour in many countries, so folate deficiency is now more rare. At the same time, since DNA synthetic-sensitive tests for anemia and erythrocyte size are routinely done in even simple medical test clinics (so that these folate-mediated biochemical effects are more often directly detected), the MTR-dependent effects of B_{12} deficiency are becoming apparent not as anemia due to DNA-synthetic problems (as they were classically), but now mainly as a simple and less obvious elevation of homocysteine in the blood and urine (homocysteinuria). This condition may result in long term damage to arteries and in clotting (stroke and heart attack), but this effect is difficult to separate from other common processes associated with atherosclerosis and aging.

The specific myelin damage resulting from B_{12} deficiency, even in the presence of adequate folate and methionine, is more specifically and clearly a vitamin deficiency problem. It has been connected to B_{12} most directly by reactions related to MUT, which is absolutely required to convert methylmalonyl coenzyme A into succinyl coenzyme A. Failure of this second reaction to occur results in elevated levels of MMA, a myelin destabilizer. Excessive MMA will prevent normal fatty acid synthesis, or it will be incorporated into fatty acid itself rather than normal malonic acid. If this abnormal fatty acid subsequently is incorporated into myelin, the resulting myelin will be too fragile, and demyelination will occur. Although the precise mechanism(s) are not known with certainty, the result is subacute combined degeneration of central nervous system and spinal cord. Whatever the cause, it is known that B_{12} deficiency causes neuropathies, even if folic acid is present in good supply, and therefore anemia is not present.

Vitamin B_{12}-dependent MTR reactions may also have neurological effects, through an indirect mechanism. Adequate methionine (which, like folate, must otherwise be obtained in the diet, if it is not regenerated from homocysteine by a B_{12} dependent reaction) is needed to make S-adenosyl-methionine (SAMe), which is in turn necessary for methylation of myelin sheath phospholipids. Although production of SAMe is not B_{12} dependent, help in recycling for provision of one adequate substrate for it (the essential amino acid methionine) is assisted by B_{12}. In addition, SAMe is involved in the manufacture of certain neurotransmitters, catecholamines and in brain metabolism. These neurotransmitters are important for maintaining mood, possibly explaining why depression is associated with B_{12} deficiency. Methylation of the myelin sheath phospholipids may also depend on adequate folate, which in turn is dependent on MTR recycling, unless ingested in relatively high amounts.

Human absorption and distribution

The human physiology of vitamin B_{12} is complex, and therefore is prone to mishaps leading to vitamin B_{12} deficiency. Unlike most nutrients, absorption of vitamin B_{12} actually begins in the mouth, where small amounts of unbound crystalline B_{12} can be absorbed through the mucous membrane. Protein-bound vitamin B_{12} must be released from the proteins by the action of digestive proteases in both the stomach and small intestine. Gastric acid releases the vitamin from food particles, therefore antacid and acid-blocking medications (especially proton-pump inhibitors) may inhibit absorption of B_{12}. In addition some elderly people produce less stomach acid as they age thereby increasing their probability of B_{12} deficiencies.

B_{12} taken in a low-solubility, non-chewable supplement pill form may bypass the mouth and stomach and not mix with gastric acids, but these are not necessary for the absorption of free B_{12} not bound to protein.

Once the B_{12} is freed from the proteins in food, R-proteins, such as haptocorrins and cobalaphilins, are secreted, which bind to free vitamin B_{12} to form a B_{12}-R complex. Also in the stomach, intrinsic factor (IF), a protein synthesized by gastric parietal cells, is secreted in response to histamine, gastrin and pentagastrin, as well as the presence of food. If this step fails due to gastric parietal cell atrophy (the problem in pernicious anemia), sufficient B_{12} is not absorbed later on, unless administered orally in relatively massive doses (0.5 to 1 mg/day). Due to the complexity of B_{12} absorption, geriatric patients, many of whom are hypoacidic due to reduced parietal cell function, have an increased risk of B_{12} deficiency.

In the duodenum, proteases digest R-proteins and release B_{12}, which then binds to IF, to form a complex (IF/B_{12}). B_{12} must be attached to IF for it to be absorbed, as receptors on the enterocytes in the terminal ileum of the small bowel only recognize the B_{12}-IF complex; in addition, intrinsic factor protects the vitamin from catabolism by intestinal bacteria. Therefore, absorption of food vitamin B_{12} requires an intact and functioning stomach, exocrine pancreas, intrinsic factor, and small bowel. Problems with any one of these organs makes a vitamin B_{12} deficiency possible. Individuals who lack intrinsic

factor have a decreased ability to absorb B_{12}. This results in 80–100% excretion of oral doses in the feces versus 30–60% excretion in feces as seen in individuals with adequate IF.

Once the IF/B_{12} complex is recognized by specialized ileal receptors, it is transported into the portal circulation. The vitamin is then transferred to transcobalamin II (TC-II/B_{12}), which serves as the plasma transporter. Hereditary defects in production of the transcobalamins and their receptors may produce functional deficiencies in B_{12} and infantile megaloblastic anemia, and abnormal B_{12} related biochemistry, even in some cases with normal blood B_{12} levels. For the vitamin to serve inside cells, the TC-II/B_{12} complex must bind to a cell receptor, and be endocytosed. The transcobalamin-II is degraded within a lysosome, and free B_{12} is finally released into the cytoplasm, where it may be transformed into the proper coenzyme, by certain cellular enzymes .

The total amount of vitamin B_{12} stored in body is about 2–5 mg in adults. Around 50% of this is stored in the liver. Approximately 0.1% of this is lost per day by secretions into the gut, as not all these secretions are reabsorbed. Bile is the main form of B_{12} excretion; however, most of the B_{12} secreted in the bile is recycled via enterohepatic circulation. Due to the extremely efficient enterohepatic circulation of B_{12}, the liver can store several years' worth of vitamin B_{12}; therefore, nutritional deficiency of this vitamin is rare. How fast B_{12} levels change depends on the balance between how much B_{12} is obtained from the diet, how much is secreted and how much is absorbed. B_{12} deficiency may arise in a year if initial stores are low and genetic factors unfavourable, or may not appear for decades. In infants, B_{12} deficiency can appear much more quickly.

History

B_{12} deficiency is the cause of pernicious anemia, an anemic disease that was usually fatal and had unknown etiology when it was first described in medicine. The cure, and B_{12}, were discovered by accident. George Whipple had been doing experiments in which he induced anemia in dogs by bleeding them, and then fed them various foods to observe which diets allowed them fastest recovery from the anemia produced. In the process, he discovered that ingesting large amounts of liver seemed to most-rapidly cure the anemia of blood loss. Thus, he hypothesized that liver ingestion might treat pernicious anemia. He tried this and reported some signs of success in 1920.

After a series of careful clinical studies, George Richards Minot and William Murphy set out to partly isolate the substance in liver which cured anemia in dogs, and found that it was iron. They also found that an entirely different liver substance cured pernicious anemia in humans, that had no effect on dogs under the conditions used. The specific factor treatment for pernicious anemia, found in liver juice, had been found by this coincidence. Minot and Murphy reported these experiments in 1926. This was the first real progress with this disease. Despite this discovery, for several years patients were still required to eat large amounts of raw liver or to drink considerable amounts of liver juice.

In 1928, the chemist Edwin Cohn prepared a liver extract that was 50 to 100 times more potent than the natural liver products. The extract was the first workable treatment for the disease. For their initial work in pointing the way to a working treatment, Whipple, Minot, and Murphy shared the 1934 Nobel Prize in Physiology or Medicine.

These events in turn eventually led to discovery of the soluble vitamin, called vitamin B_{12}, in the liver juice. The vitamin in liver extracts was not isolated until 1948 by the contributions of chemists Mary Shaw Shorb and Karl A. Folkers of the United States and Alexander R. Todd of Great Britain. In 1947, while working for the Poultry Science Department at the University of Maryland Mary Shorb (in a collaborative project with Folkers and Merck) was provided with a $400 grant to develop the LLD assay. The LLD assay led to the purification and characterization of Vitamin B12 as it caused rapid isolation of the anti-pernicious anemia factor. For this discovery, in 1949 the Mead Johnson Award from the American Society of Nutritional Sciences was given to Mary Shorb and Karl Folkers.

The chemical structure of the molecule was determined by Dorothy Crowfoot Hodgkin and her team in 1956, based on crystallographic data. Eventually, methods of producing the vitamin in large quantities from bacteria cultures were developed in the 1950s, and these led to the modern form of treatment for the disease.

Symptoms and damage from deficiency

Vitamin B_{12} deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be experienced. However, these symptoms by themselves are too nonspecific to diagnose deficiency of the vitamin.

Vitamin B_{12} deficiency can also cause symptoms of mania and psychosis.

Vitamin B_{12} deficiency has the following pathomorphology and symptoms:

Pathomorphology: A spongiform state of neural tissue along with edema of fibers and deficiency of tissue. The myelin decays, along with axial fiber. In later phases, fibric sclerosis of nervous tissues occurs. Those changes apply to dorsal parts of the spinal cord and to pyramidal tracts in lateral cords. The pathophysiologic state of the spinal cord is called subacute combined degeneration of spinal cord.

In the brain itself, changes are less severe: They occur as small sources of nervous fibers decay and accumulation of astrocytes, usually subcortically located, and also round hemorrhages with a torus of glial cells. Pathological changes can be noticed as well in the posterior roots of the cord and, to lesser extent, in peripheral nerves.

Clinical symptoms: The main syndrome of vitamin B_{12} deficiency is Biermer's disease (pernicious anemia). It is characterized by a triad of symptoms:

1. Anemia with bone marrow promegaloblastosis (megaloblastic anemia)
2. Gastrointestinal symptoms
3. Neurological symptoms

Each of those symptoms can occur either alone or along with others. The neurological complex, defined as *myelosis funicularis*, consists of the following symptoms:

1. Impaired perception of deep touch, pressure and vibration, abolishment of sense of touch, very annoying and persistent paresthesias
2. Ataxia of dorsal cord type
3. Decrease or abolishment of deep muscle-tendon reflexes
4. Pathological reflexes — Babinski, Rossolimo and others, also severe paresis

During the course of disease, mental disorders can occur. These include irritability, focus/concentration problems, depressive state with suicidal tendencies, and paraphrenia complex. These symptoms may not reverse after correction of hematological abnormalities, and the chance of complete reversal decreases with the length of time the neurological symptoms have been present.

Sources

Foods

Ultimately, animals must obtain vitamin B_{12} directly or indirectly from bacteria, and these bacteria may inhabit a section of the gut which is posterior to the section where B_{12} is absorbed. Thus, herbivorous animals must either obtain B_{12} from bacteria in their rumens, or (if fermenting plant material in the hindgut) by reingestion of cecotrope fæces.

Vitamin B_{12} is found in foods that come from animals, including fish and shellfish, meat (especially liver), poultry, eggs, milk, and milk products. Eggs are often mentioned as a good B_{12} source, but they also contain a factor that blocks absorption. Certain insects such as termites contain B_{12} produced by their gut bacteria, in a way analogous to ruminant animals. An NIH Fact Sheet lists a variety of food sources of vitamin B_{12}.

While lacto-ovo vegetarians usually get enough B_{12} through consuming dairy products, vegans will lack B_{12} unless they consume B_{12}-containing dietary supplements or B_{12}-fortified foods. Examples of fortified foods include fortified breakfast cereals, fortified soy products, fortified energy bars, and fortified nutritional yeast. According to the UK Vegan Society, the present consensus is that any B_{12} present in plant foods is likely to be unavailable to humans because B_{12} analogues can compete with B_{12} and inhibit metabolism.

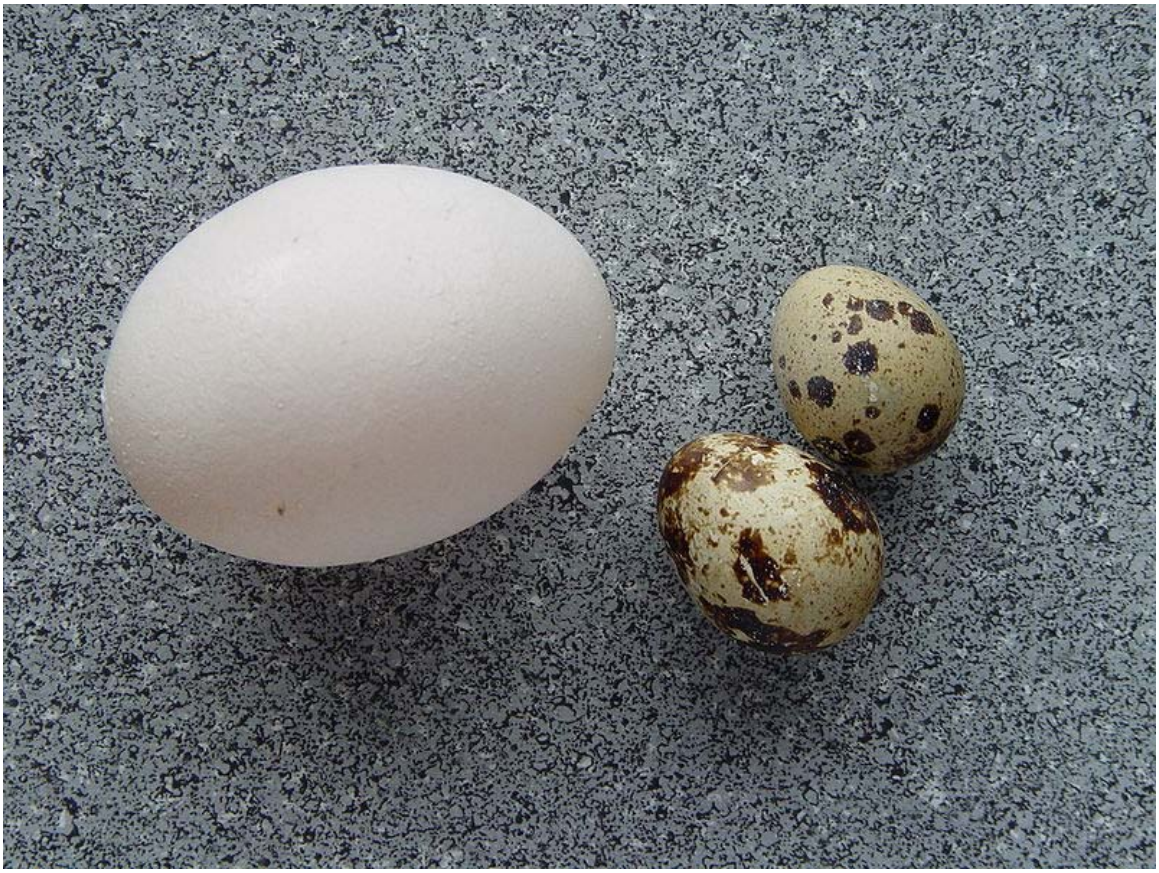
Claimed sources of B_{12} that have been shown to be inadequate or unreliable through direct studies of vegans include laver (a seaweed), barley grass, and human intestinal bacteria (human colonic bacteria produce B_{12}, but it cannot be absorbed from the colon).

Good Sources of Vitamin B_{12}

Food	µg vitamin B_{12}/100g
Panfried beef liver	83.1
Simmered turkey giblets	33.2
Braunschweiger pork liver sausage	20.1
Raw pacific oysters	16.0
Cooked Alaska king crab	11.5
Raw clams	11.3
Simmered chicken giblets	9.4
Swiss cheese	3.34
Beef (uncooked sirloin)	1.15
Egg (raw, whole chicken's egg)	0.89
Whole cow's milk	0.45
Raw chicken breast	0.20

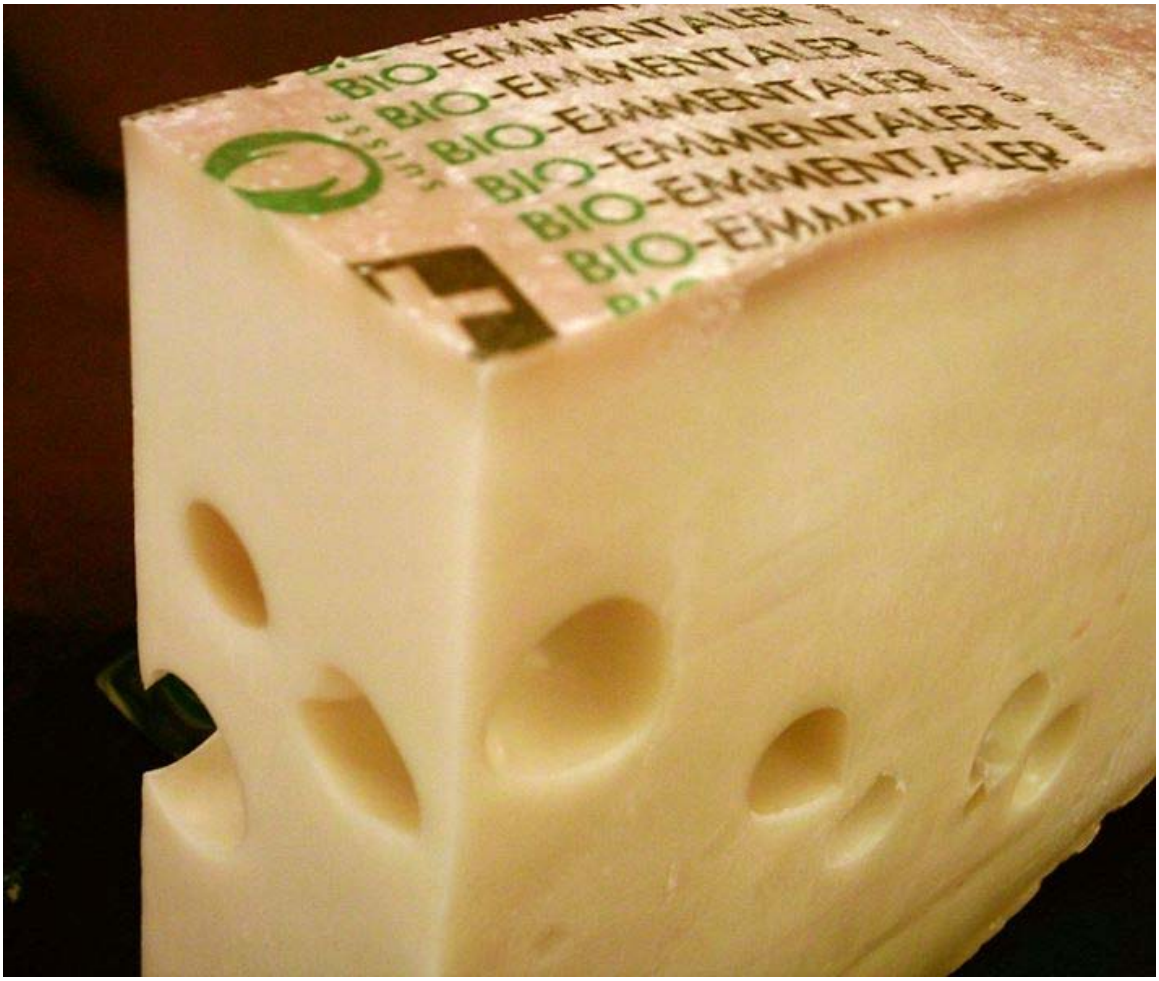
Fish

Shellfish



Egg

Milk



Milk products

Supplements

Vitamin B_{12} is provided as a supplement in many processed foods, and is also available in vitamin pill form, including multi-vitamins. Vitamin B_{12} can be supplemented in healthy subjects also by liquid, transdermal patch, nasal spray, or injection and is available singly or in combination with other supplements.

Cyanocobalamin is converted to its active forms, first hydroxocobalamin and then methylcobalamin and adenosylcobalamin in the liver.

The sublingual route, in which B_{12} is presumably or supposedly absorbed more directly under the tongue, has not proven to be necessary or helpful, though there are a number of lozenges, pills, and even a lollipop designed for sublingual absorption. A 2003 study found no significant difference in absorption for serum levels from oral vs. sublingual delivery of 0.5 mg of cobalamin. Sublingual methods of replacement are effective only because of the typically high doses (0.5 mg), which are swallowed, not because of placement of the tablet. As noted below, such very high doses of oral B_{12} may be

effective as treatments, even if gastro-intestinal tract absorption is impaired by gastric atrophy (pernicious anemia).

Injection and patches are sometimes used if digestive absorption is impaired, but there is evidence that this course of action may not be necessary with modern high potency oral supplements (such as 0.5 to 1 mg or more). Even pernicious anemia can be treated entirely by the oral route. These supplements carry such large doses of the vitamin that 1% to 5% of high oral doses of free crystalline B_{12} is absorbed along the entire intestine by passive diffusion.

However, if the patient has inborn errors in the methyltransfer pathway (cobalamin C disease, combined methylmalonic aciduria and homocystinuria), treatment with intravenous, intramuscular hydroxocobalamin or transdermal B_{12} is needed.

Cyanocobalamin is also sometimes added to beverages including Diet Coke Plus and many energy drinks.

Non-cyano forms as supplements

Recently sublingual methylcobalamin has become available in 1 mg tablets. Such tablets have higher bioavailability than the older cyanocobalamin. No cyanide is released with methylcobolamin, although the amount of cyanide (2% of the weight, or 20 *micrograms* cyanide in a 1 mg cyanocobalamin tab) is far less than ingested in many natural foods. Although the safety of cyanocobalamin has not been seriously questioned, the safety of the other types is also well-established.

Recommendations

The Dietary Reference Intake for an adult ranges from 2 to 3 µg per day.

Vitamin B_{12} is believed to be safe when used orally in amounts that do not exceed the recommended dietary allowance (RDA). The RDA for vitamin B_{12} in pregnant women is 2.6 µg per day and 2.8 µg during lactation periods. There is insufficient reliable information available about the safety of consuming greater amounts of vitamin B_{12} during pregnancy.

The Vegan Society, the Vegetarian Resource Group, and the Physicians Committee for Responsible Medicine, among others, recommend that vegans either consistently eat foods fortified with B_{12} or take a daily or weekly B_{12} supplement. Fortified breakfast cereals are a particularly valuable source of vitamin B_{12} for vegetarians and vegans. In addition, adults age 51 and older are recommended to consume B_{12} fortified food or supplements to meet the RDA, because they are a population at an increased risk of deficiency.

Allergies

Vitamin B_{12} supplements in theory should be avoided in people sensitive or allergic to cobalamin, cobalt, or any other product ingredients. However, direct allergy to a vitamin or nutrient is extremely rare, and if reported, other causes should be sought.

Side effects, contraindications, and warnings

- Vitamin B_{12} has extremely low toxicity and even taking it in enormous doses appears not to be harmful to healthy individuals.
- Hematologic: Peripheral vascular thrombosis has been reported. Treatment of vitamin B_{12} deficiency can unmask polycythemia vera, which is characterized by an increase in blood volume and the number of red blood cells. The correction of megaloblastic anemia with vitamin B_{12} can result in fatal hypokalemia and gout in susceptible individuals, and it can obscure folate deficiency in megaloblastic anemia. Caution is warranted.
- Leber's disease: Vitamin B_{12} in the form of cyanocobalamin is contraindicated in early Leber's disease, which is hereditary optic nerve atrophy. Cyanocobalamin can cause severe and swift optic atrophy, but other forms of vitamin B_{12} are available. However, the sources of this statement are not clear, while an opposing view concludes: "The clinical picture of optic neuropathy associated with vitamin B_{12} deficiency shows similarity to that of Leber's disease optic neuropathy. Both involve the nerve fibres of the papillomacular bundle. The present case reports suggest that optic neuropathy in patients carrying a primary LHON mtDNA mutation may be precipitated by vitamin B_{12} deficiency. Therefore, known carriers should take care to have an adequate dietary intake of vitamin B_{12} and malabsorption syndromes like those occurring in familial pernicious anaemia or after gastric surgery should be excluded."

Other medical uses

Hydroxycobalamin, or hydoxocobalamin, also known as vitamin B_{12}a, is used in Europe both for vitamin B_{12} deficiency and as a treatment for cyanide poisoning, sometimes with a large amount (5–10 g) given intravenously, and sometimes in combination with sodium thiosulfate. The mechanism of action is straightforward: the hydroxycobalamin hydroxide ligand is displaced by the toxic cyanide ion, and the resulting harmless B_{12} complex is excreted in urine. In the United States, the Food and Drug Administration approved (in 2006) the use of hydroxocobalamin for acute treatment of cyanide poisoning.

High vitamin B_{12} level in elderly individuals may protect against brain atrophy or shrinkage, associated with Alzheimer's disease and impaired cognitive function.

Vitamin B_{12} enhances the phase-response of circadian melatonin rhythm to a single bright light exposure in humans. Sleep disturbances may occur because B_{12} may be involved in the regulation of the sleep wake cycle by the pineal gland (through melatonin).

Topical application of vitamin B_{12} has been shown to be an effective treatment for psoriasis.

Interactions

Interactions with drugs

- Alcohol (ethanol): Excessive alcohol intake lasting longer than two weeks can decrease vitamin B_{12} absorption from the gastrointestinal tract.

- Aminosalicylic acid (para-aminosalicylic acid, PAS, Paser): Aminosalicylic acid can reduce oral vitamin B_{12} absorption, possibly by as much as 55%, as part of a general malabsorption syndrome. Megaloblastic changes, and occasional cases of symptomatic anemia have occurred, usually after doses of 8 to 12 g/day for several months. Vitamin B_{12} levels should be monitored in people taking aminosalicylic acid for more than one month.

- Antibiotics: An increased bacterial load can bind significant amounts of vitamin B_{12} in the gut, preventing its absorption. In people with bacterial overgrowth of the small bowel, antibiotics such as metronidazole (Flagyl) can actually improve vitamin B_{12} status. The effects of most antibiotics on gastrointestinal bacteria are unlikely to have clinically significant effects on vitamin B_{12} levels.
- Hormonal contraception: The data regarding the effects of oral contraceptives on vitamin B_{12} serum levels are conflicting. Some studies have found reduced serum levels in oral contraceptive users, but others have found no effect despite use of oral contraceptives for up to 6 months. When oral contraceptive use is stopped, normalization of vitamin B_{12} levels usually occurs. Lower vitamin B_{12} serum levels seen with oral contraceptives probably are not clinically significant.

- Chloramphenicol (Chloromycetin): Limited case reports suggest that chloramphenicol can delay or interrupt the reticulocyte response to supplemental vitamin B_{12} in some patients. Blood counts should be monitored closely if this combination cannot be avoided.
- Cobalt irradiation: Cobalt irradiation of the small bowel can decrease gastrointestinal (GI) absorption of vitamin B_{12}.
- Colchicine: Colchicine in doses of 1.9 to 3.9 mg/day can disrupt normal intestinal mucosal function, leading to malabsorption of several nutrients, including vitamin B_{12}. Lower doses do not seem to have a significant effect on vitamin B_{12} absorption after 3 years of colchicine therapy. The significance of this interaction is unclear. Vitamin B_{12} levels should be monitored in people taking large doses of colchicine for prolonged periods.

- Colestipol (Colestid), cholestyramine (Questran): These resins used for sequestering bile acids to decrease cholesterol, can decrease gastrointestinal (GI) absorption of vitamin B_{12}. It is unlikely this interaction will deplete body stores of vitamin B_{12} unless there are other factors contributing to deficiency. In a group of

children treated with cholestyramine for up to 2.5 years, there was not any change in serum vitamin B_{12} levels. Routine supplements are not necessary.

- H_2-receptor antagonists: include cimetidine (Tagamet), famotidine (Pepcid), nizatidine (Axid), and ranitidine (Zantac). Reduced secretion of gastric acid and pepsin produced by H_2 blockers can reduce absorption of protein-bound (dietary) vitamin B_{12}, but not of supplemental vitamin B_{12}. Gastric acid is needed to release vitamin B_{12} from protein for absorption. Clinically significant vitamin B_{12} deficiency and megaloblastic anemia are unlikely, unless H_2 blocker therapy is prolonged (2 years or more), or the person's diet is poor. It is also more likely if the person is rendered achlorhydric (with complete absence of gastric acid secretion), which occurs more frequently with proton pump inhibitors than H_2 blockers. Vitamin B_{12} levels should be monitored in people taking high doses of H_2 blockers for prolonged periods.
- Metformin (Glucophage): Metformin may reduce serum folic acid and vitamin B_{12} levels. These changes can lead to hyperhomocysteinemia, adding to the risk of cardiovascular disease in people with diabetes. There are also rare reports of megaloblastic anemia in people who have taken metformin for five years or more. Reduced serum levels of vitamin B_{12} occur in up to 30% of people taking metformin chronically. However, clinically significant deficiency is not likely to develop if dietary intake of vitamin B_{12} is adequate. Deficiency can be corrected with vitamin B_{12} supplements even if metformin is continued. The metformin-induced malabsorption of vitamin B_{12} is reversible by oral calcium supplementation. The general clinical significance of metformin upon B_{12} levels is as yet unknown.
- Neomycin: Absorption of vitamin B_{12} can be reduced by neomycin, but prolonged use of large doses is needed to induce pernicious anemia. Supplements are not usually needed with normal doses.
- Nicotine: Nicotine can reduce serum vitamin B_{12} levels. The need for vitamin B_{12} supplementation in smokers has not been adequately studied.
- Nitrous oxide: Nitrous oxide inactivates the cobalamin form of vitamin B_{12} by oxidation. Symptoms of vitamin B_{12} deficiency, including sensory neuropathy, myelopathy, and encephalopathy, can occur within days or weeks of exposure to nitrous oxide anesthesia in people with subclinical vitamin B_{12} deficiency. Symptoms are treated with high doses of vitamin B_{12}, but recovery can be slow and incomplete. People with normal vitamin B_{12} levels have sufficient vitamin B_{12} stores to make the effects of nitrous oxide insignificant, unless exposure is repeated and prolonged (such as recreational use). Vitamin B_{12} levels should be checked in people with risk factors for vitamin B_{12} deficiency prior to using nitrous oxide anesthesia. Chronic nitrous oxide B_{12} poisoning (usually from use of nitrous oxide as a recreational drug), however, may result in B_{12} functional deficiency even with normal measured blood levels of B_{12}.
- Phenytoin (Dilantin), phenobarbital, primidone (Mysoline): These anticonvulsants have been associated with reduced vitamin B_{12} absorption, and reduced serum and cerebrospinal fluid levels in some patients. This may contribute to the megaloblastic anemia, primarily caused by folate deficiency, associated with these drugs. It is also suggested that reduced vitamin B_{12} levels may contribute to

the neuropsychiatric side effects of these drugs. Patients should be encouraged to maintain adequate dietary vitamin B_{12} intake. Folate and vitamin B_{12} status should be checked if symptoms of anemia develop.

- Proton pump inhibitors (PPIs): The PPIs include omeprazole (Prilosec, Losec), lansoprazole (Prevacid), rabeprazole (Aciphex), pantoprazole (Protonix, Pantoloc), and esomeprazole (Nexium). The reduced secretion of gastric acid and pepsin produced by PPIs can reduce absorption of protein-bound (dietary) vitamin B_{12}, but not supplemental vitamin B_{12}. Gastric acid is needed to release vitamin B_{12} from protein for absorption. Reduced vitamin B_{12} levels may be more common with PPIs than with H2-blockers, because they are more likely to produce achlorhydria (complete absence of gastric acid secretion). However, clinically significant vitamin B_{12} deficiency is unlikely, unless PPI therapy is prolonged (2 years or more) or dietary vitamin intake is low. Vitamin B_{12} levels should be monitored in people taking high doses of PPIs for prolonged periods.
- Zidovudine (AZT, Combivir, Retrovir): Reduced serum vitamin B_{12} levels may occur when zidovudine therapy is started. This adds to other factors that cause low vitamin B_{12} levels in people with HIV, and might contribute to the hematological toxicity associated with zidovudine. However, the data suggest vitamin B_{12} supplements are not helpful for people taking zidovudine.

Interactions with herbs and dietary supplements

- Folic acid: Folic acid, particularly in large doses, can mask vitamin B_{12} deficiency by completely correcting hematological abnormalities. In vitamin B_{12} deficiency, folic acid can produce complete resolution of the characteristic megaloblastic anemia, while allowing potentially irreversible neurological damage (from continued inactivity of methylmalonyl mutase) to progress. Thus, vitamin B_{12} status should be determined before folic acid is given as monotherapy.
- Potassium: Potassium supplements can reduce absorption of vitamin B_{12} in some people. This effect has been reported with potassium chloride and, to a lesser extent, with potassium citrate. Potassium might contribute to vitamin B_{12} deficiency in some people with other risk factors, but routine supplements are not necessary.

Chapter 11

Vitamin C

Vitamin C or **L-ascorbic acid** or **L-ascorbate** is an essential nutrient for humans and certain other animal species, in which it functions as a vitamin. In living organisms, ascorbate is an antioxidant, since it protects the body against oxidative stress. It is also a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that cause the most severe symptoms of scurvy when they are dysfunctional. In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries.

Ascorbate (an ion of ascorbic acid) is required for a range of essential metabolic reactions in all animals and plants. It is made internally by almost all organisms; notable mammalian group exceptions are most or all of the order chiroptera (bats), guinea pigs, capybaras, and one of the two major primate suborders, the Anthropoidea (Haplorrhini) (tarsiers, monkeys and apes, including human beings). Ascorbic acid is also not synthesized by some species of birds and fish. All species that do not synthesize ascorbate require it in the diet. Deficiency in this vitamin causes the disease scurvy in humans. It is also widely used as a food additive.

Scurvy has been known since ancient times. People in many parts of the world assumed it was caused by a lack of fresh plant foods. The British Navy started giving sailors lime juice to prevent scurvy in 1795. Ascorbic acid was finally isolated in 1932 and commercially "synthesized" (this included a fermentation step in bacteria) in 1934. The uses and recommended daily intake of vitamin C are matters of ongoing debate, with RDI ranging from 45 to 95 mg/day. Proponents of megadosage propose from 200 mg to more than 2000 mg/day. The fraction of vitamin C in the diet that is absorbed and the rate at which the excess is eliminated from the body vary strongly with the dose. Large, randomized clinical trials on the effects of high doses on the general population have not been conducted.

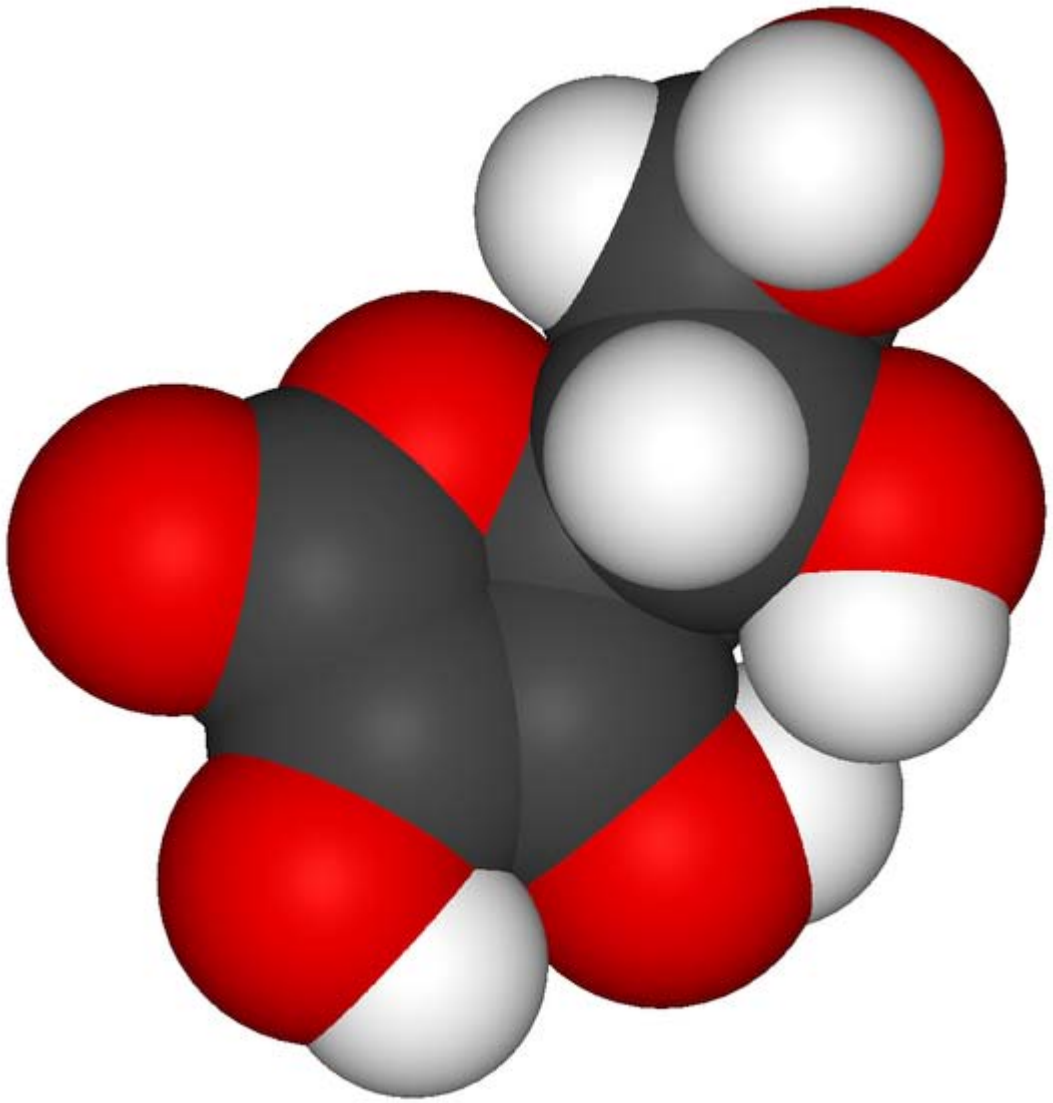
Routine vitamin C supplementation does not reduce the incidence or severity of the common cold in the general population, though the largest analyses suggest supplementation may slightly reduce common cold duration.

Biological significance

Vitamin C is purely the L-enantiomer of ascorbate; the opposite D-enantiomer has no physiological significance. Both forms are mirror images of the same molecular structure. When L-ascorbate, which is a strong reducing agent, carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate. L-dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by enzymes and glutathione. During this process semidehydroascorbic acid radical is formed. Ascorbate free radical reacts poorly with oxygen, and thus, will not create a superoxide. Instead two semidehydroascorbate radicals will react and form one ascorbate and one dehydroascorbate. With the help of glutathione, dehydroxyascorbate is converted back to ascorbate. The presence of glutathione is crucial since it spares ascorbate and improves antioxidant capacity of blood. Without it dehydroxyascorbate could not convert back to ascorbate.

L-Ascorbate is a weak sugar acid structurally related to glucose that naturally occurs attached either to a hydrogen ion, forming ascorbic acid, or to a metal ion, forming a mineral ascorbate.

Biosynthesis in different species



Model of a vitamin C molecule. Black is carbon, red is oxygen, and white is hydrogen

The vast majority of animals and plants are able to synthesize their own vitamin C, through a sequence of four enzyme-driven steps, which convert glucose to vitamin C. The glucose needed to produce ascorbate in the liver (in mammals and perching birds) is extracted from glycogen; ascorbate synthesis is a glycogenolysis-dependent process. In reptiles and birds the biosynthesis is carried out in the kidneys.

Among the animals that have lost the ability to synthesise vitamin C are simians and tarsiers, which together make up one of two major primate suborders, the anthropoidea, also called haplorrhini. This group includes humans. The other more primitive primates

(strepsirrhini) have the ability to make vitamin C. Synthesis does not occur in a number of species (perhaps all species) in the small rodent family caviidae that includes guinea pigs and capybaras, but occurs in other rodents (rats and mice do not need vitamin C in their diet, for example). A number of species of passerine birds also do not synthesise, but not all of them, and those that don't are not clearly related; there is a theory that the ability was lost separately a number of times in birds. All tested families of bats, including major insect and fruit-eating bat families, cannot synthesise vitamin C. A trace of GLO was detected in only 1 of 34 bat species tested, across the range of 6 families of bats tested.

These animals all lack the L-gulonolactone oxidase (GULO) enzyme, which is required in the last step of vitamin C synthesis, because they have a differing non-synthesising gene for the enzyme (Pseudogene ΨGULO). A similar non-functional gene however, is present in the genome of the guinea pigs and in primates, including humans. Some of these species (including humans) are able to make do with the lower levels available from their diets by recycling oxidised vitamin C.

Most simians consume the vitamin in amounts 10 to 20 times higher than that recommended by governments for humans. This discrepancy constitutes much of the basis of the controversy on current recommended dietary allowances. It is countered by arguments that humans are very good at conserving dietary vitamin C, and are able to maintain blood levels of vitamin C comparable with other simians, on a far smaller dietary intake.

An adult goat, a typical example of a vitamin C-producing animal, will manufacture more than 13 g of vitamin C per day in normal health and the biosynthesis will increase "manyfold under stress". Trauma or injury has also been demonstrated to use up large quantities of vitamin C in humans. Some microorganisms such as the yeast *Saccharomyces cerevisiae* have been shown to be able to synthesize vitamin C from simple sugars.

Vitamin C in evolution

Venturi and Venturi suggested that the antioxidant action of ascorbic acid developed first in the plant kingdom when, about 500 million years ago (Mya), plants began to adapt to antioxidant-mineral-deficient fresh-waters of estuaries. Some biologists suggested that many vertebrates had developed their metabolic adaptive strategies in estuary environment. In this theory, some 400–300 Mya, when living plants and animals first began the move from the sea to rivers and land, environmental iodine deficiency was a challenge to the evolution of terrestrial life. In plants, animals and fishes, the terrestrial diet became deficient in many essential antioxidant marine micronutrients, including iodine, selenium, zinc, copper, manganese, iron, etc. Freshwater algae and terrestrial plants, in replacement of marine antioxidants, slowly optimized the production of other endogenous antioxidants such as ascorbic acid, polyphenols, carotenoids, tocopherols etc., some of which became essential “vitamins” in the diet of terrestrial animals (vitamins C, A, E, etc.).

Ascorbic acid or vitamin C is a common enzymatic cofactor in mammals used in the synthesis of collagen. Ascorbate is a powerful reducing agent capable of rapidly scavenging a number of reactive oxygen species (ROS). Freshwater teleost fishes also require dietary vitamin C in their diet or they will get scurvy. The most widely recognized symptoms of vitamin C deficiency in fishes are scoliosis, lordosis and dark skin coloration. Freshwater salmonids also show impaired collagen formation, internal/fin haemorrhage, spinal curvature and increased mortality. If these fishes are housed in seawater with algae and phytoplankton, then vitamin supplementation seems to be less important, it is presumed because of the availability of other, more ancient, antioxidants in natural marine environment.

Some scientists have suggested that loss of the vitamin C biosynthesis pathway may have played a role in the theory of rapid evolutionary changes, leading to hominids and the emergence of human beings. However, another theory based on the theory of evolution is that the loss of ability to make vitamin C in simians may have occurred much farther back in evolutionary history than the emergence of humans or even apes, since it evidently occurred rather soon after the appearance of the first primates, yet sometime after the split of early primates into its two major suborders haplorrhini (which cannot make vitamin C) and its sister suborder of non-tarsier prosimians, the strepsirrhini ("wet-nosed" primates), which retained the ability to make vitamin C. According to molecular clock dating, these two suborder primate branches parted ways about 63 to 60 Mya Approximately three to five million years later (58 Mya), only a short time afterward from an evolutionary perspective, the infraorder Tarsiiformes, whose only remaining family is that of the tarsier (Tarsiidae), branched off from the other haplorrhines. Since tarsiers also cannot make vitamin C, this implies the mutation had already occurred, and thus must have occurred between these two marker points (63 to 58 Mya).

It has been noted that the loss of the ability to synthesize ascorbate strikingly parallels the inability to break down uric acid, also a characteristic of primates. Uric acid and ascorbate are both strong reducing agents. This has led to the suggestion that, in higher primates, uric acid has taken over some of the functions of ascorbate.

Absorption, transport, and disposal

Ascorbic acid is absorbed in the body by both active transport and simple diffusion. Sodium-Dependent Active Transport—Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs)—are the two transporters required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbate across plasma membrane. GLUT1 and GLUT3 are the two glucose transporters, and transfer only dehydroascorbic acid form of Vitamin C. Although dehydroascorbic acid is absorbed in higher rate than ascorbate, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to ascorbate. Thus, SVCTs appear to be the predominant system for vitamin C transport in the body.

SVCT2 is involved in vitamin C transport in almost every tissue, the notable exception being red blood cells, which lose SVCT proteins during maturation. "SVCT2 knockout"

animals genetically engineered to lack this functional gene, die shortly after birth, suggesting that SVCT2-mediated vitamin C transport is necessary for life.

With regular intake the absorption rate varies between 70 to 95%. However, the degree of absorption decreases as intake increases. At high intake (12g), fractional human absorption of ascorbic acid may be as low as 16%; at low intake (<20 mg) the absorption rate can reach up to 98%. Ascorbate concentrations over renal re-absorption threshold pass freely into the urine and are excreted. At high dietary doses (corresponding to several hundred mg/day in humans) ascorbate is accumulated in the body until the plasma levels reach the renal resorption threshold, which is about 1.5 mg/dL in men and 1.3 mg/dL in women. Concentrations in the plasma larger than this value (thought to represent body saturation) are rapidly excreted in the urine with a half-life of about 30 minutes. Concentrations less than this threshold amount are actively retained by the kidneys, and the excretion half-life for the remainder of the vitamin C store in the body thus increases greatly, with the half-life lengthening as the body stores are depleted. This half-life rises until it is as long as 83 days by the onset of the first symptoms of scurvy.

Although the body's maximal store of vitamin C is largely determined by the renal threshold for blood, there are many tissues that maintain vitamin C concentrations far higher than in blood. Biological tissues that accumulate over 100 times the level in blood plasma of vitamin C are the adrenal glands, pituitary, thymus, corpus luteum, and retina. Those with 10 to 50 times the concentration present in blood plasma include the brain, spleen, lung, testicle, lymph nodes, liver, thyroid, small intestinal mucosa, leukocytes, pancreas, kidney and salivary glands.

Ascorbic acid can be oxidized (broken down) in the human body by the enzyme L-ascorbate oxidase. Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by this enzyme and removed.

Deficiency

Scurvy is an avitaminosis resulting from lack of vitamin C, since without this vitamin, the synthesised collagen is too unstable to perform its function. Scurvy leads to the formation of brown spots on the skin, spongy gums, and bleeding from all mucous membranes. The spots are most abundant on the thighs and legs, and a person with the ailment looks pale, feels depressed, and is partially immobilized. In advanced scurvy there are open, suppurating wounds and loss of teeth and, eventually, death. The human body can store only a certain amount of vitamin C, and so the body stores are depleted if fresh supplies are not consumed. The time frame for onset of symptoms of scurvy in unstressed adults switched to a completely vitamin C free diet, however, may range from one month to more than six months, depending on previous loading of vitamin C.

It has been shown that smokers who have diets poor in vitamin C are at a higher risk of lung-borne diseases than those smokers who have higher concentrations of vitamin C in the blood.

Nobel prize winner Linus Pauling and Dr. G. C. Willis have asserted that chronic long term low blood levels of vitamin C (chronic scurvy) is a cause of atherosclerosis.

Western societies generally consume far more than sufficient Vitamin C to prevent scurvy. In 2004, a Canadian Community health survey reported that Canadians of 19 years and above have intakes of vitamin C from food of 133 mg/d for males and 120 mg/d for females; these are higher than the RDA recommendations.

Notable human dietary studies of experimentally-induced scurvy have been conducted on conscientious objectors during WW II in Britain, and on Iowa state prisoner "volunteers" in the late 1960s. These studies both found that all obvious symptoms of scurvy previously induced by an experimental scorbutic diet with extremely low vitamin C content could be completely reversed by additional vitamin C supplementation of only 10 mg a day. In these experiments, there was no clinical difference noted between men given 70 mg vitamin C per day (which produced blood level of vitamin C of about 0.55 mg/dl, about 1/3 of tissue saturation levels), and those given 10 mg per day. Men in the prison study developed the first signs of scurvy about 4 weeks after starting the vitamin C free diet, whereas in the British study, six to eight months were required, possibly due to the pre-loading of this group with a 70 mg/day supplement for six weeks before the scorbutic diet was fed.

Men in both studies on a diet devoid, or nearly devoid, of vitamin C had blood levels of vitamin C too low to be accurately measured when they developed signs of scurvy, and in the Iowa study, at this time were estimated (by labeled vitamin C dilution) to have a body pool of less than 300 mg, with daily turnover of only 2.5 mg/day, implying a instantaneous half-life of 83 days by this time (elimination constant of 4 months).

Moderately higher blood levels of vitamin C measured in healthy persons have been found to be prospectively correlated with decreased risk of cardiovascular disease and ischaemic heart disease, and an increase life expectancy. The same study found an inverse relationship between blood vitamin C levels and cancer risk in men, but not in women. An increase in blood level of 20 micromol/L of vitamin C (about 0.35 mg/dL, and representing a theoretical additional 50 grams of fruit and vegetables per day) was found epidemiologically to reduce the all-cause risk of mortality, four years after measuring it, by about 20%. However, because this was not an intervention study, causation could not be proven, and vitamin C blood levels acting as a proxy marker for other differences between the groups could not be ruled out. However, the four-year long and prospective nature of the study did rule out proxy effect from any vitamin C lowering effects of immediately-terminal illness, or near-end-of-life poor health.

Studies with much higher doses of vitamin C, usually between 200 and 6000 mg/day, for the treatment of infections and wounds have shown inconsistent results. Combinations of antioxidants seem to improve wound healing.

History of human understanding

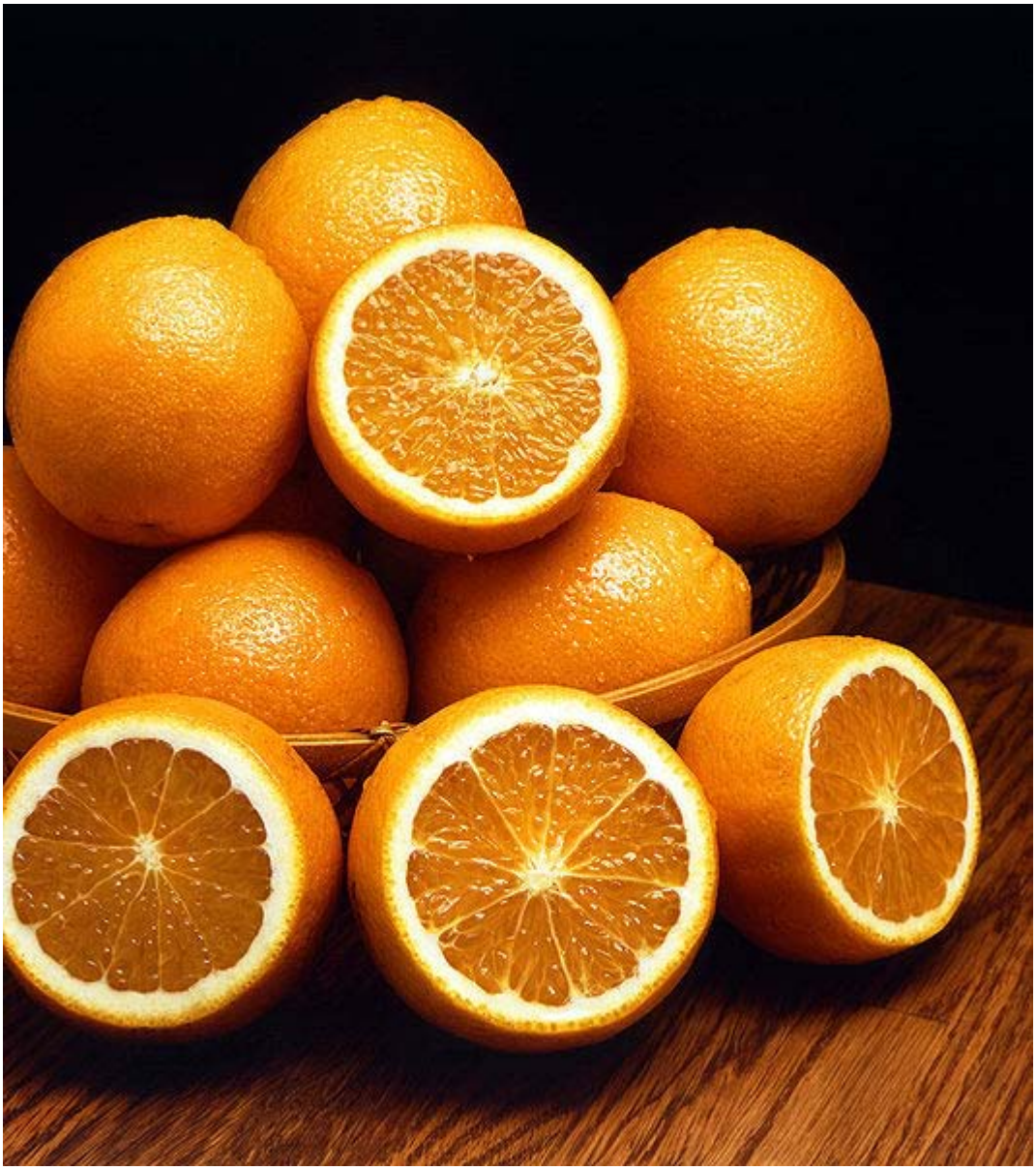
James Lind, a British Royal Navy surgeon who, in 1747, identified that a quality in fruit prevented the disease of scurvy in what was the first recorded controlled experiment.

The need to include fresh plant food or raw animal flesh in the diet to prevent disease was known from ancient times. Native people living in marginal areas incorporated this into their medicinal lore. For example, spruce needles were used in temperate zones in infusions, or the leaves from species of drought-resistant trees in desert areas. In 1536, the French explorer Jacques Cartier, exploring the St. Lawrence River, used the local natives' knowledge to save his men who were dying of scurvy. He boiled the needles of the arbor vitae tree to make a tea that was later shown to contain 50 mg of vitamin C per 100 grams.

Throughout history, the benefit of plant food to survive long sea voyages has been occasionally recommended by authorities. John Woodall, the first appointed surgeon to the British East India Company, recommended the preventive and curative use of lemon juice in his book, *The Surgeon's Mate*, in 1617. The Dutch writer, Johann Bachstrom, in 1734, gave the firm opinion that *"scurvy is solely owing to a total abstinence from fresh vegetable food, and greens, which is alone the primary cause of the disease."*

Scurvy had long been a principal killer of sailors during the long sea voyages. According to Jonathan Lamb, "In 1499, Vasco da Gama lost 116 of his crew of 170; In 1520, Magellan lost 208 out of 230;...all mainly to scurvy."

While the earliest documented case of scurvy was described by Hippocrates around the year 400 BC, the first attempt to give scientific basis for the cause of this disease was by a ship's surgeon in the British Royal Navy, James Lind. Scurvy was common among those with poor access to fresh fruit and vegetables, such as remote, isolated sailors and soldiers. While at sea in May 1747, Lind provided some crew members with two oranges and one lemon per day, in addition to normal rations, while others continued on cider, vinegar, sulfuric acid or seawater, along with their normal rations. In the history of science, this is considered to be the first occurrence of a controlled experiment comparing results on two populations of a factor applied to one group only with all other factors the same. The results conclusively showed that citrus fruits prevented the disease. Lind published his work in 1753 in his *Treatise on the Scurvy*.



Citrus fruits were one of the first sources of vitamin C available to ships' surgeons.

Lind's work was slow to be noticed, partly because his *Treatise* was not published until six years after his study, and also because he recommended a lemon juice extract known as *rob*. Fresh fruit was very expensive to keep on board, whereas boiling it down to juice allowed easy storage but destroyed the vitamin (especially if boiled in copper kettles). Ship captains concluded wrongly that Lind's other suggestions were ineffective because those juices failed to prevent or cure scurvy.

It was 1795 before the British navy adopted lemons or lime as standard issue at sea. Limes were more popular, as they could be found in British West Indian Colonies, unlike lemons, which were not found in British Dominions, and were therefore more expensive. This practice led to the American use of the nickname "limey" to refer to the British.

Captain James Cook had previously demonstrated and proven the principle of the advantages of carrying "Sour krout" on board, by taking his crews to the Hawaiian Islands and beyond without losing any of his men to scurvy. For this otherwise unheard of feat, the British Admiralty awarded him a medal.

The name *antiscorbutic* was used in the eighteenth and nineteenth centuries as general term for those foods known to prevent scurvy, even though there was no understanding of the reason for this. These foods included but were not limited to: lemons, limes, and oranges; sauerkraut, cabbage, malt, and portable soup.

Even before the antiscorbutic substance was identified, there were indications that it was present in amounts sufficient to prevent scurvy, in nearly all fresh (uncooked and uncured) foods, including raw animal-derived foods. In 1928, the Arctic anthropologist Vilhjalmur Stefansson attempted to prove his theory of how the Eskimos are able to avoid scurvy with almost no plant food in their diet, despite the disease's striking European Arctic explorers living on similar high-cooked-meat diets. Stefansson theorised that the natives get their vitamin C from fresh meat that is minimally cooked. Starting in February 1928, for one year he and a colleague lived on an exclusively minimally-cooked meat diet while under medical supervision; they remained healthy. Later studies done after vitamin C could be quantified in mostly-raw traditional food diets of the Yukon, Inuit, and Métís of the Northern Canada, showed that their daily intake of vitamin C averaged between 52 and 62 mg/day, an amount approximately the dietary reference intake (DRI), even at times of the year when little plant-based food were eaten.

Discovery

Albert Szent-Györgyi, pictured here in 1948, was awarded the 1937 Nobel Prize in Medicine "for his discoveries in connection with the biological combustion processes, with special reference to vitamin C and the catalysis of fumaric acid".

In 1907, the needed biological-assay model to isolate and identify the antiscorbutic factor was discovered. Axel Holst and Theodor Frølich, two Norwegian physicians studying shipboard beriberi contracted aboard ship's crews in the Norwegian Fishing Fleet, wanted a small test mammal to substitute for the pigeons then used in beriberi research. They fed guinea pigs their test diet of grains and flour, which had earlier produced beriberi in their pigeons, and were surprised when classic scurvy resulted instead. This was a serendipitous choice of model. Until that time, scurvy had not been observed in any

organism apart from humans, and had been considered an exclusively human disease. (Pigeons, as seed-eating birds, were also later found to make their own vitamin C.) Holst and Frølich found they could cure the disease in guinea pigs with the addition of various fresh foods and extracts. This discovery of a clean animal experimental model for scurvy, made even before the essential idea of *vitamins* in foods had even been put forward, has been called the single most important piece of vitamin C research.

In 1912, the Polish American biochemist Casimir Funk, while researching beriberi in pigeons, developed the concept of vitamins to refer to the non-mineral micronutrient that are essential to health. The name is a blend of "vital", due to the vital biochemical role they play, and "amines" because Funk thought that all these materials were chemical amines. Although the "e" was dropped after skepticism that all these compounds were amines, the word vitamin remained as a generic name for them. One of the *vitamins* was thought to be the anti-scorbutic factor in foods discovered by Holst and Frølich. In 1928, this vitamin was referred to as "water-soluble C," although its chemical structure had still not been determined.

From 1928 to 1933, the Hungarian research team of Joseph L. Svirbely and Albert Szent-Györgyi and the American worker Charles Glen King, first identified the anti-scorbutic factor, calling it "ascorbic acid" for its vitamin activity. Szent-Györgyi had isolated the chemical hexuronic acid from animal adrenal glands at the Mayo clinic, and suspected it to be the antiscorbutic factor, but could not prove it without a biological assay. At the same time, for five years, King's laboratory at the University of Pittsburgh had been trying to isolate the antiscorbutic factor in lemon juice, using the model of scorbutic guinea pigs, which developed scurvy when not fed fresh foods, but were cured by lemon juice. They had also considered hexuronic acid, but had been put off the trail when a coworker made the explicit (and mistaken) experimental claim that this substance was not the antiscorbutic substance.

Finally, in late 1931, Szent-Györgyi gave Svirbely, a former worker in King's lab who had recently joined Szent-Györgyi's lab, the last of this hexuronic acid, with the suggestion that it might be the anti-scorbutic factor. By the spring of 1932, King's laboratory had proven this, but published the result without giving Szent-Györgyi credit for it, leading to a bitter dispute over priority claims (in reality it had taken a teamwork effort by both groups, since Szent-Györgyi was unwilling to do the difficult and messy animal studies). By 1932, Szent-Györgyi's group had discovered that paprika peppers, a common spice in the Hungarian diet, was a rich source of hexuronic acid, the antiscorbutic factor, by then named ascorbic acid, in honor of its activity against scurvy. Ascorbic acid turned out *not* to be an amine, or even to contain any nitrogen.

For his accomplishment, Szent-Györgyi was alone awarded the 1937 Nobel Prize in Medicine "for his discoveries in connection with the biological combustion processes, with special reference to vitamin C and the catalysis of fumaric acid".

Between 1933 and 1934, the British chemists Sir Walter Norman Haworth and Sir Edmund Hirst and, independently, the Polish chemist Tadeus Reichstein, succeeded in

synthesizing the vitamin, making it the first to be artificially produced. This made possible the cheap mass-production of what was by then known as vitamin C. Only Haworth was awarded the 1937 Nobel Prize in Chemistry for this work, but the Reichstein process, a combined chemical and bacterial fermentation sequence still used today to produce vitamin C, retained Reichstein's name. In 1934 Hoffmann–La Roche, which bought the Reichstein process patent, became the first pharmaceutical company to mass produce and market synthetic vitamin C, under the brand name of Redoxon.

In 1957, the American J.J. Burns showed that the reason some mammals are susceptible to scurvy is the inability of their liver to produce the active enzyme L-gulonolactone oxidase, which is the last of the chain of four enzymes that synthesize vitamin C. American biochemist Irwin Stone was the first to exploit vitamin C for its food preservative properties. He later developed the theory that humans possess a mutated form of the L-gulonolactone oxidase coding gene.

In 2008, researchers at the University of Montpellier discovered that, in humans and other primates, the red blood cells have evolved a mechanism to more efficiently utilize the vitamin C present in the body by recycling oxidized L-dehydroascorbic acid (DHA) back into ascorbic acid, which can be reused by the body. The mechanism was not found to be present in mammals that synthesize their own vitamin C.

Physiological function in mammals

In humans, vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress; a substrate for ascorbate peroxidase in plants (APX is plant specific enzyme); and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C acts as an electron donor for important enzymes:

Collagen, carnitine, and tyrosine synthesis, and microsomal metabolism

Ascorbic acid performs numerous physiological functions in the human body. These functions include the synthesis of collagen, carnitine, and neurotransmitters; the synthesis and catabolism of tyrosine; and the metabolism of microsome. During biosynthesis ascorbate acts as a reducing agent, donating electrons and preventing oxidation to keep iron and copper atoms in their reduced states.

Vitamin C acts as an electron donor for eight different enzymes:

- Three enzymes participate in collagen hydroxylation. These reactions add hydroxyl groups to the amino acids proline or lysine in the collagen molecule via prolyl hydroxylase and lysyl hydroxylase, both requiring vitamin C as a cofactor. Hydroxylation allows the collagen molecule to assume its triple helix structure and making vitamin C essential to the development and maintenance of scar tissue, blood vessels, and cartilage.

- Two enzymes are necessary for synthesis of carnitine. Carnitine is essential for the transport of fatty acids into mitochondria for ATP generation.

- The remaining three enzymes have the following functions in common, but have other functions as well:
    - dopamine beta hydroxylase participates in the biosynthesis of norepinephrine from dopamine.
    - another enzyme adds amide groups to peptide hormones, greatly increasing their stability.
    - one modulates tyrosine metabolism.

Antioxidant

Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. When there are more free radicals (reactive oxygen species, ROS) in the human body than antioxidants, the condition is called oxidative stress, and has an impact on cardiovascular disease, hypertension, chronic inflammatory diseases, diabetes as well as on critically ill patients and individuals with severe burns. Individuals experiencing oxidative stress have ascorbate blood levels lower than 45 μmol/L, compared to healthy individual who range between 61.4-80 μmol/L.

It is not yet certain whether vitamin C and antioxidants in general prevent oxidative stress-related diseases and promote health. Clinical studies regarding the effects of vitamin C supplementation on lipoproteins and cholesterol have found that vitamin C supplementation does not improve disease markers in the blood. Vitamin C may contribute to decreased risk of cardiovascular disease and strokes through a small reduction in systolic blood pressure, and was also found to both increase ascorbic acid levels and reduce levels of resistin serum, another likely determinant of oxidative stress and cardiovascular risk. However, so far there is no consensus that vitamin C intake has an impact on cardiovascular risks in general, and an array of studies found negative results. Meta-analysis of a large number of studies on antioxidants, including vitamin C supplementation, found no relationship between vitamin C and mortality. Thus vitamin C does not appear to help people live longer.

Pro-oxidant

Ascorbic acid behaves not only as an antioxidant but also as a pro-oxidant. Ascorbic acid has been shown to reduce transition metals, such as cupric ions (Cu^{2+}), to cuprous (Cu^{1+}), and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) during conversion from ascorbate to dehydroascorbate *in vitro*. This reaction can generate superoxide and other ROS. However, in the body, free transition elements are unlikely to be present while iron and copper are bound to diverse proteins and the intravenous use of vitamin C does not appear to increase pro-oxidant activity. Thus, ascorbate as a pro-oxidant is unlikely to convert metals to create ROS *in vivo*. However, vitamin C supplementation has been associated with increased DNA damage in the lymphocytes of healthy volunteers.

Immune system

Some advertisements claim that Vitamin C "supports" or is "important" for immune system function. Vitamin C deficiency is detrimental to immune function, resulting in reduced resistance to some pathogens. Routine supplementation is not indicated in the general population, though there is some evidence that it reduces symptom severity but not incidence of the common cold. Effects are most pronounced in cases of physical strain or insufficient dietary intake.

Antihistamine

Vitamin C is a natural antihistamine. It both prevents histamine release and increases the detoxification of histamine. A 1992 study found that taking 2 grams vitamin C daily lowered blood histamine levels 38 percent in healthy adults in just one week. It has also been noted that low concentrations of serum vitamin C has been correlated with increased serum histamine levels.

Physiologic function in plants

Ascorbic acid is associated with chloroplasts and apparently plays a role in ameliorating the oxidative stress of photosynthesis. In addition, it has a number of other roles in cell division and protein modification. Plants appear to be able to make ascorbate by at least one other biochemical route that is different from the major route in animals, although precise details remain unknown.

Daily requirements

The North American Dietary Reference Intake recommends 90 milligrams per day and no more than 2 grams (2,000 milligrams) per day. Other related species sharing the same inability to produce vitamin C and requiring exogenous vitamin C consume 20 to 80 times this reference intake. There is continuing debate within the scientific community over the best dose schedule (the amount and frequency of intake) of vitamin C for maintaining optimal health in humans. It is generally agreed that a balanced diet without supplementation contains enough vitamin C to prevent scurvy in an average healthy adult, while those who are pregnant, smoke tobacco, or are under stress require slightly more.

High doses (thousands of milligrams) may result in diarrhea in healthy adults, as a result of the osmotic water-retaining effect of the unabsorbed portion in the gastrointestinal tract (similar to cathartic osmotic laxatives). Proponents of orthomolecular medicine claim the onset of diarrhea to be an indication of where the body’s true vitamin C requirement lies, though this has not been clinically verified.

United States vitamin C recommendations	
Recommended Dietary Allowance (adult male)	90 mg per day
Recommended Dietary Allowance (adult female)	75 mg per day
Tolerable Upper Intake Level (adult male)	2,000 mg per day
Tolerable Upper Intake Level (adult female)	2,000 mg per day

Government recommended intakes

Recommendations for vitamin C intake have been set by various national agencies:

- 40 milligrams per day: the United Kingdom's Food Standards Agency
- 45 milligrams per day: the World Health Organization
- 90 mg/day (males) and 75 mg/day (females): Health Canada 2007
- 60–95 milligrams per day: United States' National Academy of Sciences.

The United States defined Tolerable Upper Intake Level for a 25-year-old male is 2,000 milligrams per day.

Therapeutic uses

Vitamin C functions as an antioxidant and is necessary for the treatment and prevention of scurvy, though in nearly all cases dietary intake is adequate to prevent deficiency and supplementation is not necessary. Though vitamin C has been promoted as useful in the treatment of a variety of conditions, most of these uses are poorly supported by the evidence and sometimes contraindicated.

Vitamin C may also be useful in lowering serum uric acid levels resulting in a correspondingly lower incidence of gout, and an oxidized version that can cross the blood-brain barrier may reduce neurological deficits and mortality following a stroke. There is suggestive evidence vitamin C may be useful in the treatment of pneumonia.

Vitamin C's effect on the common cold has been extensively researched and has not been shown effective in prevention or treatment of the common (viral) cold, except in a limited number of cases (specifically, individuals exercising in cold environments). Routine vitamin C supplementation does not reduce the incidence of the common cold in the general population. However, it is effective at preventing cold when consumed regularly by athletes exposed to periods of training in subarctic conditions (i.e.marathon runners, skiers and soldiers on subarctic exercises). In one study vitamin C supplementation did significantly reduce the *frequency* of the common cold but without apparent effect on the duration or severity (however the authors of this research pointed out that the findings should be interpreted with caution, since they are the opposite of those found in larger analyses). Recently, result of a meta-analysis of 29 trials involving 11,306 participants found that regular vitamin C supplementation (oral doses of 200 mg per day or more) reduced the duration of viral colds both in adults and children by 8%-13%, respectively. The authors of the meta-analysis do not believe routine megadosing with vitamin C to

prevent or treat colds is justified. Thus, consumption of vitamin C supplements may reduce the duration of cold symptoms by 8 to 13%, but do not appear to significantly impact the incidence or severity of colds.

Vitamin C megadosage

Several individuals and organizations advocate large doses of vitamin C, in the form of oral or intravenous therapy. Large, randomized clinical trials on the effects of high doses on the general population have never taken place. Arguments for megadosage are based on the diets of closely related apes and the hypothesized diet of prehistoric humans, and that most mammals synthesize vitamin C rather than relying on dietary intake. Linus Pauling spent much of his life advocating for the use of megadose vitamin C and believed the established RDA was sufficient to prevent scurvy, but not necessarily the dosage for optimal health.

A 2010 review of 33 years of research on vitamin C to treat cancer stated "we have to conclude that we still do not know whether Vitamin C has any clinically significant antitumor activity. Nor do we know which histological types of cancers, if any, are susceptible to this agent. Finally, we don't know what the recommended dose of Vitamin C is, if there is indeed such a dose, that can produce an anti-tumor response." Vitamin C has also been shown to reduce the effectiveness of some chemotherapeutic agents *in vitro*, and the Cochrane Collaboration has published literature reviews arguing that vitamin C supplementation should not be be used to attempt to prevent lung and gastrointestinal cancer, with some evidence of increased mortality for the latter with some vitamin supplements. Clinical trials investigating the use of vitamin C in the prevention of coronary disease or strokes have produced equivocal results, with positive, negative and neutral outcomes, and were generally methodologically flawed.

Testing for ascorbate levels in the body

Simple tests use dichlorophenolindophenol, a redox indicator, to measure the levels of vitamin C in the urine and in serum or blood plasma. However these reflect recent dietary intake rather than the level of vitamin C in body stores. Reverse phase high performance liquid chromatography is used for determining the storage levels of vitamin C within lymphocytes and tissue. It has been observed that while serum or blood plasma levels follow the circadian rhythm or short term dietary changes, those within tissues themselves are more stable and give a better view of the availability of ascorbate within the organism. However, very few hospital laboratories are adequately equipped and trained to carry out such detailed analyses, and require samples to be analyzed in specialized laboratories.

Adverse effects

Common side-effects

Relatively large doses of ascorbic acid may cause indigestion, particularly when taken on an empty stomach. However, taking vitamin C in the form of sodium ascorbate and calcium ascorbate may minimize this effect. When taken in large doses, ascorbic acid causes diarrhea in healthy subjects. In one trial in 1936, doses up to 6 grams of ascorbic acid were given to 29 infants, 93 children of preschool and school age, and 20 adults for more than 1400 days. With the higher doses, toxic manifestations were observed in five adults and four infants. The signs and symptoms in adults were nausea, vomiting, diarrhea, flushing of the face, headache, fatigue and disturbed sleep. The main toxic reactions in the infants were skin rashes.

Possible side-effects

As vitamin C enhances iron absorption, iron poisoning can become an issue to people with rare iron overload disorders, such as haemochromatosis. A genetic condition that results in inadequate levels of the enzyme glucose-6-phosphate dehydrogenase (G6PD) can cause sufferers to develop hemolytic anemia after ingesting specific oxidizing substances, such as very large dosages of vitamin C.

There is a longstanding belief among the mainstream medical community that vitamin C causes kidney stones, which is based on little science. Although recent studies have found a relationship, a clear link between excess ascorbic acid intake and kidney stone formation has not been generally established. Some case reports exist for a link between patients with oxalate deposits and a history of high-dose vitamin C usage.

In a study conducted on rats, during the first month of pregnancy, high doses of vitamin C may suppress the production of progesterone from the corpus luteum. Progesterone, necessary for the maintenance of a pregnancy, is produced by the corpus luteum for the first few weeks, until the placenta is developed enough to produce its own source. By blocking this function of the corpus luteum, high doses of vitamin C (1000+ mg) are theorized to induce an early miscarriage. In a group of spontaneously aborting women at the end of the first trimester, the mean values of vitamin C were significantly higher in the aborting group. However, the authors do state: 'This could not be interpreted as an evidence of casual association.' However, in a previous study of 79 women with threatened, previous spontaneous, or habitual abortion, Javert and Stander (1943) had 91% success with 33 patients who received vitamin C together with bioflavonoids and vitamin K (only three abortions), whereas all of the 46 patients who did not receive the vitamins aborted.

A study in rats and humans suggested that adding Vitamin C supplements to an exercise training program lowered the expected effect of training on VO2Max. Although the results in humans were not statistically significant, this study is often cited as evidence that high doses of Vitamin C have an adverse effect on exercise performance. In rats, it

was shown that the additional Vitamin C resulted in lowered mitochondria production. Since rats are able to produce all of their needed Vitamin C, however, it is questionable whether they offer a relevant model of human physiological processes in this regard.

A cancer-causing mechanism of hexavalent chromium may be triggered by vitamin C.

Chance of overdose

Vitamin C is water soluble, with dietary excesses not absorbed, and excesses in the blood rapidly excreted in the urine. It exhibits remarkably low toxicity. The LD_{50} (the dose that will kill 50% of a population) in rats is generally accepted to be 11.9 grams per kilogram of body weight when given by forced gavage (orally). The mechanism of death from such doses (1.2% of body weight, or 1.8 lbs for a 150 lb human) is unknown, but may be more mechanical than chemical. The LD_{50} in humans remains unknown, given lack of any accidental or intentional poisoning death data. However, as with all substances tested in this way, the rat LD_{50} is taken as a guide to its toxicity in humans.

Natural and synthetic dietary sources

Rose hips are a particularly rich source of vitamin C

The richest natural sources are fruits and vegetables, and of those, the Kakadu plum and the camu camu fruit contain the highest concentration of the vitamin. It is also present in some cuts of meat, especially liver. Vitamin C is the most widely taken nutritional supplement and is available in a variety of forms, including tablets, drink mixes, crystals in capsules or naked crystals.

Vitamin C is absorbed by the intestines using a sodium-ion dependent channel. It is transported through the intestine via both glucose-sensitive and glucose-insensitive mechanisms. The presence of large quantities of sugar either in the intestines or in the blood can slow absorption.

Plant sources

While plants are generally a good source of vitamin C, the amount in foods of plant origin depends on the precise variety of the plant, soil condition, climate where it grew, length of time since it was picked, storage conditions, and method of preparation.

The following table is approximate and shows the relative abundance in different raw plant sources. As some plants were analyzed fresh while others were dried (thus, artifactually increasing concentration of individual constituents like vitamin C), the data are subject to potential variation and difficulties for comparison. The amount is given in milligrams per 100 grams of fruit or vegetable and is a rounded average from multiple authoritative sources:

Plant source	Amount (mg / 100g)
Kakadu plum	3100
Camu Camu	2800
Rose hip	2000
Acerola	1600
Seabuckthorn	695
Jujube	500
Indian gooseberry	445
Baobab	400
chili pepper, green	244
Guava (common, raw)	228.3
Blackcurrant	200
Red pepper	190
chili pepper, red	144
Parsley	130
Kiwifruit	90
Broccoli	90
Loganberry	80
Redcurrant	80
Brussels sprouts	80
Wolfberry (Goji)	73 †
Lychee	70
Persimmon (native, raw)	66.0
Cloudberry	60
Elderberry	60

† average of 3 sources; dried

Plant source	Amount (mg / 100g)
Papaya	60
Strawberry	60
Orange	50
Kale	41
Lemon	40
Melon, cantaloupe	40
Cauliflower	40
Garlic	31
Grapefruit	30
Raspberry	30
Tangerine	30
Mandarin orange	30
Passion fruit	30
Spinach	30
Cabbage raw green	30
Lime	30
Mango	28
Blackberry	21
Potato	20
Melon, honeydew	20
Cranberry	13
Tomato	10
Blueberry	10
Pineapple	10
Pawpaw	10

Plant source	Amount (mg / 100g)
Grape	10
Apricot	10
Plum	10
Watermelon	10
Banana	9
Carrot	9
Avocado	8
Crabapple	8
Persimmon (Japanese, fresh)	7.5

Cherry	7
Peach	7
Apple	6
Asparagus	6
Horned melon	5.3
Beetroot	5
Chokecherry	5
Pear	4
Lettuce	4
Cucumber	3
Eggplant	2
Raisin	2
Fig	2
Bilberry	1
Medlar	0.3

Plant sources notes

- United States Department of Agriculture Research Service (2010), *USDA National Nutrient Database for Standard Reference, Release 23*, Nutrient Data Laboratory,

1. ^ USDA *Guava, common, raw*
2. ^ USDA *Persimmons, native, raw*
3. ^ USDA *Persimmon, japanese, raw*
4. ^ USDA *Horned melon*

Animal sources

Goats, like almost all animals, make their own vitamin C. An adult goat, weighing approx. 70 kg, will manufacture more than 13,000 mg of vitamin C per day in normal health, and levels manyfold higher when faced with stress.

The overwhelming majority of species of animals and plants synthesise their own vitamin C. Therefore, some animal products can be used as sources of dietary vitamin C.

Vitamin C is most present in the liver and least present in the muscle. Since muscle provides the majority of meat consumed in the western human diet, animal products are not a reliable source of the vitamin. Vitamin C is present in mother's milk but, not present in raw cow's milk. All excess vitamin C is disposed of through the urinary system.

The following table shows the relative abundance of vitamin C in various foods of animal origin, given in milligram of vitamin C per 100 grams of food:

Animal Source	Amount (mg / 100g)
Calf liver (raw)	36
Beef liver (raw)	31
Oysters (raw)	30
Cod roe (fried)	26

Pork liver (raw)	23
Lamb brain (boiled)	17
Chicken liver (fried)	13

Animal Source	Amount (mg / 100g)
Lamb liver (fried)	12
Calf adrenals (raw)	11
Lamb heart (roast)	11
Lamb tongue (stewed)	6
Human milk (fresh)	4
Goat milk (fresh)	2
Camel milk (fresh)	5
Cow milk (fresh)	2

Food preparation

Vitamin C chemically decomposes under certain conditions, many of which may occur during the cooking of food. Vitamin C concentrations in various food substances decrease with time in proportion to the temperature they are stored at and cooking can reduce the Vitamin C content of vegetables by around 60% possibly partly due to increased enzymatic destruction as it may be more significant at sub-boiling temperatures. Longer cooking times also add to this effect, as will copper food vessels, which catalyse the decomposition.

Another cause of vitamin C being lost from food is leaching, where the water-soluble vitamin dissolves into the cooking water, which is later poured away and not consumed. However, vitamin C does not leach in all vegetables at the same rate; research shows broccoli seems to retain more than any other. Research has also shown that fresh-cut fruits do not lose significant nutrients when stored in the refrigerator for a few days.

Vitamin C supplements

Vitamin C is widely available in the form of tablets and powders. The Redoxon brand, launched in 1934 by Hoffmann-La Roche, was the first mass-produced synthetic vitamin C.

Vitamin C is the most widely taken dietary supplement. It is available in caplets, tablets, capsules, drink mix packets, in multi-vitamin formulations, in multiple antioxidant formulations, and crystalline powder. Timed release versions are available, as are formulations containing bioflavonoids such as quercetin, hesperidin and rutin. Tablet and capsule sizes range from 25 mg to 1500 mg. Vitamin C (as ascorbic acid) crystals are typically available in bottles containing 300 g to 1 kg of powder (a teaspoon of vitamin C crystals equals 5,000 mg).

Industrial synthesis

Vitamin C is produced from glucose by two main routes. The Reichstein process, developed in the 1930s, uses a single pre-fermentation followed by a purely chemical route. The modern two-step fermentation process, originally developed in China in the 1960s, uses additional fermentation to replace part of the later chemical stages. Both processes yield approximately 60% vitamin C from the glucose feed.

Research is underway at the Scottish Crop Research Institute in the interest of creating a strain of yeast that can synthesise vitamin C in a single fermentation step from galactose, a technology expected to reduce manufacturing costs considerably.

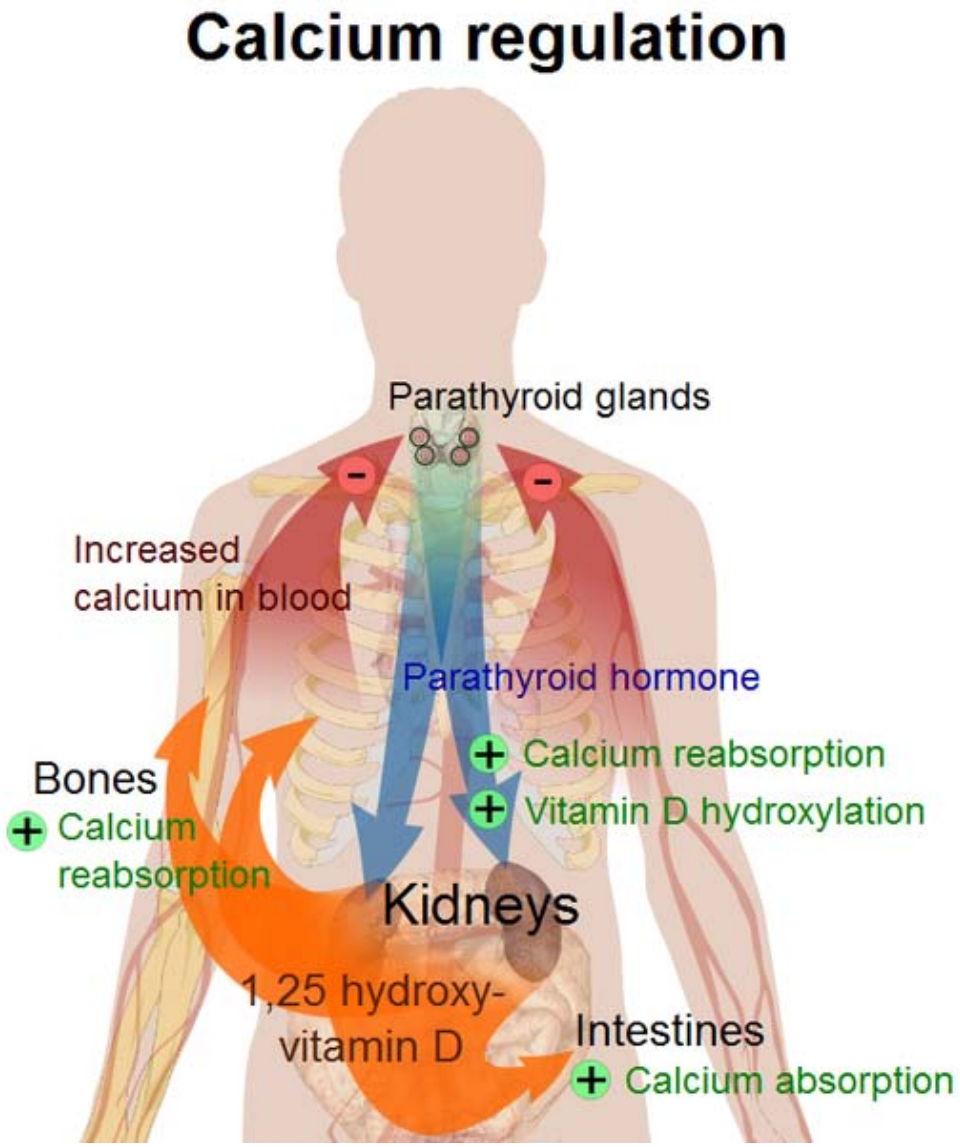
World production of synthesised vitamin C is currently estimated at approximately 110,000 tonnes annually. Main producers have been BASF/Takeda, DSM, Merck and the China Pharmaceutical Group Ltd. of the People's Republic of China. China is slowly becoming the major world supplier as its prices undercut those of the US and European manufacturers. By 2008 only the DSM plant in Scotland remained operational outside the strong price competition from China. The world price of vitamin C rose sharply in 2008 partly as a result of rises in basic food prices but also in anticipation of a stoppage of the two Chinese plants, situated at Shijiazhuang near Beijing, as part of a general shutdown of polluting industry in China over the period of the Olympic games. Five Chinese manufacturers met in 2010, among them Northeast Pharmaceutical Group and North China Pharmaceutical Group, and agreed to temporarily stop production in order to maintain prices.

Food fortification

In 2005, Health Canada evaluated the effect of fortification of foods with ascorbate in the guidance document, *Addition of Vitamins and Minerals to Food*. Abscorbate was categorized as a ‘Risk Category A nutrients’, meaning it is a nutrient for which an upper limit for intake is set but allows a wide margin of intake that has a narrow margin of safety but non-serious critical adverse effects. Health Canada recommended a minimum of 3 mg or 5% of RDI for the food to claim to be a source of Vitamin C, and maximum fortification of 12 mg (20% of RDI) to claim "Excellent Source".

Chapter 12

Vitamin D



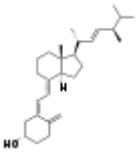
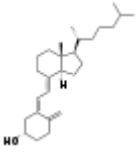
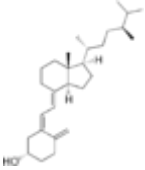
Calcium regulation in the human body. The role of vitamin D is shown in orange.

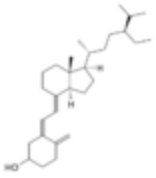
Vitamin D is a group of fat-soluble secosteroids, the two major physiologically relevant forms of which are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D without a subscript refers to either D_2 or D_3 or both. Vitamin D_3 is produced in the skin of vertebrates after exposure to ultraviolet B light from the sun or artificial sources, and occurs naturally in a small range of foods. In some countries, staple foods such as milk, flour and margarine are artificially fortified with vitamin D, and it is also available as a supplement in pill form. Food sources such as fatty fish, eggs, and meat are rich in vitamin D and are often recommended for consumption to those suffering vitamin D deficiency. Light-exposed mushrooms may provide up to 100% of the recommended Daily Value of vitamin D.

Vitamin D is carried in the bloodstream to the liver, where it is converted into the prohormone calcidiol. Circulating calcidiol may then be converted into calcitriol, the biologically active form of vitamin D, either in the kidneys or by monocyte-macrophages in the immune system. When synthesized by monocyte-macrophages, calcitriol acts locally as a cytokine, defending the body against microbial invaders.

When synthesized in the kidneys, calcitriol circulates as a hormone, regulating, among other things, the concentration of calcium and phosphate in the bloodstream, promoting the healthy mineralization, growth and remodeling of bone, and the prevention of hypocalcemic tetany. Vitamin D insufficiency can result in thin, brittle, or misshapen bones, while sufficiency prevents rickets in children and osteomalacia in adults, and, together with calcium, helps to protect older adults from osteoporosis. Vitamin D also modulates neuromuscular function, reduces inflammation, and influences the action of many genes that regulate the proliferation, differentiation and apoptosis of cells.

Forms

Name	Chemical composition	Structure
Vitamin D_1	molecular compound of ergocalciferol with lumisterol, 1:1	
Vitamin D_2	ergocalciferol (made from ergosterol)	
Vitamin D_3	cholecalciferol (made from 7-dehydrocholesterol in the skin).	
Vitamin D_4	22-dihydroergocalciferol	

Vitamin D_5	sitocalciferol (made from 7-dehydrositosterol)	

Several forms (vitamers) of vitamin D exist. The two major forms are vitamin D_2 or ergocalciferol, and vitamin D_3 or cholecalciferol. These are known collectively as **calciferol**. Vitamin D_2 was chemically characterized in 1932. In 1936 the chemical structure of vitamin D_3 was established and resulted from the ultraviolet irradiation of 7-dehydrocholesterol.

Chemically, the various forms of vitamin D are secosteroids; i.e., steroids in which one of the bonds in the steroid rings is broken. The structural difference between vitamin D_2 and vitamin D_3 is in their side chains. The side chain of D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24.

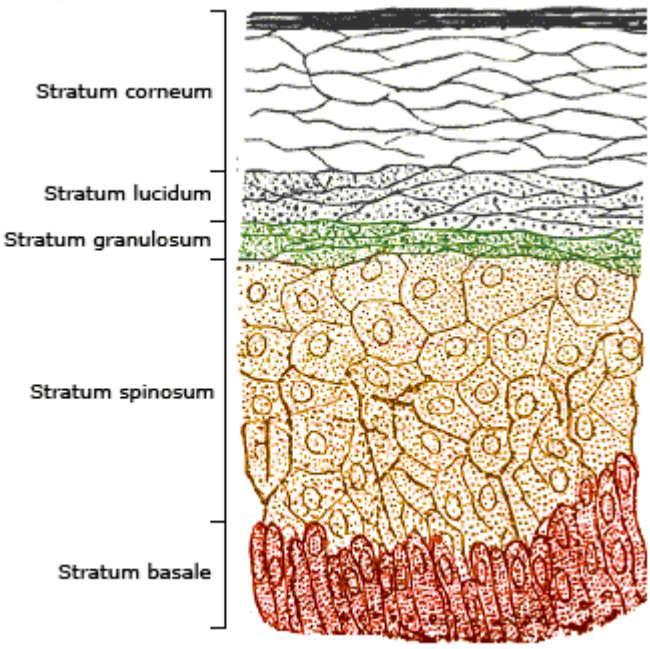
Vitamin D_2 is a derivative of ergosterol, a membrane sterol, and is produced by some organisms of phytoplankton, invertebrates, and fungi in response to UV irradiation; D_2 is not produced by land plants or vertebrates. The biological role of vitamin D_2 in invertebrate species is unknown, and some controversy exists over whether or not D_2 can fully substitute for vitamin D_3 in the human diet.

Evolution

The photosynthesis of vitamin D evolved over 750 million years ago; the phytoplankton coccolithophor *Emeliani huxleii* is an early example. Vitamin D played a critical role in the maintenance of a calcified skeleton in vertebrates as they left their calcium-rich ocean environment for land over 350 million years ago.

"Because vitamin D can only be synthesized via a photochemical process, early vertebrates that ventured onto land either had to ingest foods that contained vitamin D or had to be exposed to sunlight to photosynthesize vitamin D in their skin to satisfy their body's vitamin D requirement.

Production in the skin



In the epidermal strata of the skin, production is greatest in the stratum basale (colored red in the illustration) and stratum spinosum (colored light brown).

Vitamin D_3 is made in the skin when 7-dehydrocholesterol reacts with ultraviolet light (UVB) at wavelengths between 270 and 300 nm, with peak synthesis occurring between 295 and 297 nm. These wavelengths are present in sunlight when the UV index is greater than three and also in the light emitted by the UV lamps in tanning beds. Tanning lamps produce ultraviolet primarily in the UVA spectrum, but typically produce 4% to 10% of the total UV emissions as UVB. At this solar elevation, which occurs daily within the tropics, daily during the spring and summer seasons in temperate regions, and almost never within the arctic circles, vitamin D_3 can be made in the skin. Depending on the intensity of UVB rays and the minutes of exposure, an equilibrium can develop in the skin, and vitamin D degrades as fast as it is generated.

The skin consists of two primary layers: the inner layer called the dermis, composed largely of connective tissue, and the outer, thinner epidermis. Thick epidermis in the soles and palms consists of five *strata*; from outer to inner they are: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. Vitamin D is produced in the two innermost strata, the stratum basale and stratum spinosum.

Cholecalciferol is produced photochemically in the skin from 7-dehydrocholesterol; 7-dehydrocholesterol is produced in relatively large quantities in the skin of most vertebrate animals, including humans. The naked mole rat appears to be naturally cholecalciferol deficient, as serum 25-OH vitamin D levels are undetectable. Interestingly, the naked

mole rat is resistant to aging, maintains healthy vascular function and is the longest lived of all rodents.

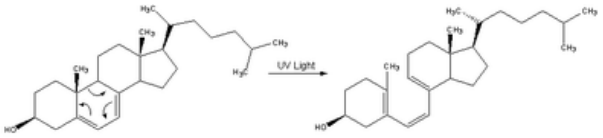
In some animals, the presence of fur or feathers blocks the UV rays from reaching the skin. In birds and fur-bearing mammals, vitamin D is generated from the oily secretions of the skin deposited onto the fur and obtained orally during grooming.

In 1923, it was established that when 7-dehydrocholesterol is irradiated with light, a form of a fat-soluble vitamin is produced. Alfred Fabian Hess showed "light equals vitamin D." Adolf Windaus, at the University of Göttingen in Germany, received the Nobel Prize in Chemistry in 1928, for his work on the constitution of sterols and their connection with vitamins. In the 1930s he clarified further the chemical structure of vitamin D.

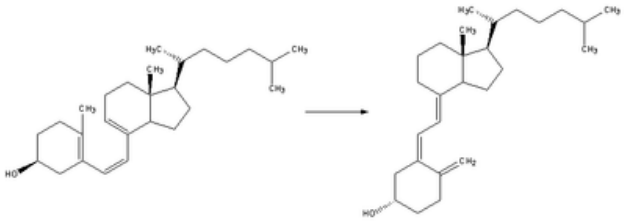
In 1923, Harry Steenbock at the University of Wisconsin demonstrated irradiation by ultraviolet light increased the vitamin D content of foods and other organic materials. After irradiating rodent food, Steenbock discovered the rodents were cured of rickets. A vitamin D deficiency is a known cause of rickets. Using $300 of his own money, Steenbock patented his invention. His irradiation technique was used for foodstuffs, most memorably for milk. By the expiration of his patent in 1945, rickets had all but been eliminated in the US.

Synthesis mechanism (form 3)

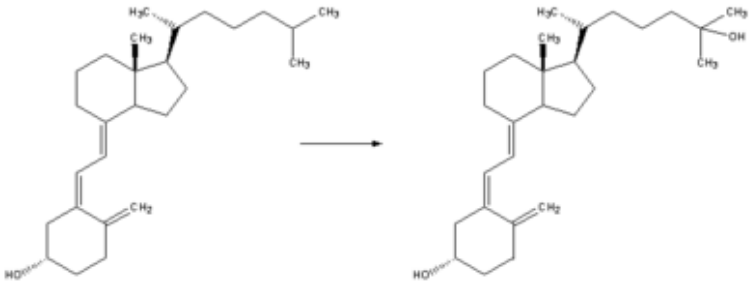
In the skin, 7-dehydrocholesterol, a derivative of cholesterol, is photolyzed by ultraviolet light in a 6-electron conrotatory electrocyclic reaction. The product is previtamin D_3.



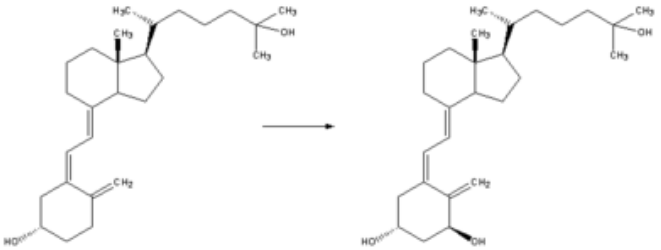
Previtamin D_3 spontaneously isomerizes to vitamin D_3 (cholecalciferol) in a antarafacial sigmatropic [1,7] hydride shift. At room temperature, the transformation of previtamin D_3 to vitamin D_3 takes about 12 days to complete.



Whether it is made in the skin or ingested, cholecalciferol is hydroxylated in the liver at position 25 (upper right of the molecule) to form 25-hydroxycholecalciferol (calcidiol). This reaction is catalyzed by the microsomal enzyme vitamin D 25-hydroxylase, which is produced by hepatocytes. Once made, the product is stored in the hepatocytes until it is needed and then can be released into the plasma, where it will be bound to an α-globulin.



Calcidiol is transported to the proximal tubules of the kidneys, where it is hydroxylated at the 1-α position (lower right of the molecule) to form calcitriol. This product is a potent ligand of the vitamin D receptor (VDR), which mediates most of the physiological actions of the vitamin. The conversion of calcidiol to calcitriol is catalyzed by the enzyme 25-hydroxyvitamin D3 1-alpha-hydroxylase, the levels of which are increased by parathyroid hormone (and additionally by low calcium or phosphate).



Mechanism of action

Following the final converting step in the kidney, calcitriol (the physiologically active form of vitamin D) is released into the circulation. By binding to vitamin D-binding protein (VDBP), a carrier protein in the plasma, calcitriol is transported to various target organs.

Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells. The binding of calcitriol to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins (such as TRPV6 and calbindin), which are involved in calcium absorption in the intestine.

The vitamin D receptor belongs to the nuclear receptor superfamily of steroid/thyroid hormone receptors, and VDRs are expressed by cells in most organs, including the brain, heart, skin, gonads, prostate, and breast. VDR activation in the intestine, bone, kidney, and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and to the maintenance of bone content.

Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. It also is involved in the biosynthesis of neurotrophic factors, synthesis of nitric oxide synthase, and increased glutathione levels

The VDR is known to be involved in cell proliferation and differentiation. Vitamin D also affects the immune system, and VDRs are expressed in several white blood cells, including monocytes and activated T and B cells.

Apart from VDR activation, various alternative mechanisms of action are known. An important one of these is its role as a natural inhibitor of signal transduction by hedgehog (a hormone involved in morphogenesis).

History

American researchers Elmer McCollum and Marguerite Davis in 1913 discovered a substance in cod liver oil which later came to be called "vitamin A." British doctor Edward Mellanby noticed dogs that were fed cod liver oil did not develop rickets and concluded vitamin A, or a closely associated factor, could prevent the disease. In 1921, Elmer McCollum tested modified cod liver oil in which the vitamin A had been destroyed. The modified oil cured the sick dogs, so McCollum concluded the factor in cod liver oil which cured rickets was distinct from vitamin A. He called it vitamin D because it was the fourth vitamin to be named. Unlike other vitamins, vitamin D can be synthesised by humans and it is therefore not a vitamin (vital food substance) except for people who lack sufficient UV light exposure.

Nutrition

In some countries, milk and cereal grains are fortified with vitamin D.

Dietary reference intakes

USA

A new Dietary Reference Intake was made for vitamin D on November 30, 2010 by the Institute of Medicine. The previous recommendation was an Adequate Intake (AI). The reference intake is based on more evidence than the previous AI. The recommendations were formed assuming the individual has little to no sun exposure. The reference intake includes intake from diet (food and beverages) and supplements.

The new reference intakes for vitamin D are:

- 1–70 years of age: 600 IU/day (200 IU is 5 μg equivalent)
- 71+ years of age: 800 IU/day
- Pregnant/lactating: 600 IU/day

An AI remains for infants:

- 0–12 months: 400 IU/day

The upper level intakes for vitamin D are:

- 0–6 months of age: 1,000 IU
- 6–12 months of age: 1,500 IU
- 1–3 years of age: 2,500 IU
- 4–8 years of age: 3,000 IU
- 9-71+ years of age: 4,000 IU
- Pregnant/lactating: 4,000 IU

European Union

The Recommended Daily Amount (RDA) for nutrition labelling of food products in the EU for vitamin D is 5 µg.

Australia and New Zealand

Australia and New Zealand have established Average Intakes for vitamin D, as follows:

Average intakes for vitamin D

Children

- 5.0 µg /day

Men

- 19–30 yr 5.0 µg /day
- 31–50 yr 5.0 µg /day
- 51–70 yr 10.0 µg /day
- >70 yr 15.0 µg /day

Women

- 19–30 yr 5.0 µg /day
- 31–50 yr 5.0 µg /day
- 51–70 yr 10.0 µg /day
- >70 yr 15.0 µg /day

Australian studies into vitamin D deficiency have yielded tables of recommended sunlight intake based on the country's major cities.

Natural sources

Fatty fish such as salmon are natural sources of vitamin D

Natural sources of vitamin D include:

- Fatty fish species, such as:
  - Catfish, 85 g (3 oz) provides 425 IU (5 IU/g)
  - Salmon, cooked, 100 g (3.5 oz) provides 360 IU (3.6 IU/g)
  - Mackerel, cooked, 100 g (3.5 oz), 345 IU (3.45 IU/g)
  - Sardines, canned in oil, drained, 50 g (1.75 oz), 250 IU (5 IU/g)
  - Tuna, canned in oil, 100 g (3.5 oz), 235 IU (2.35 IU/g)
  - Eel, cooked, 100 g (3.5 oz), 200 IU (2.00 IU/g)
- A whole egg provides 20 IU if egg weighs 60 g (0.33 IU/g)
- Beef liver, cooked, 100 g (3.5 oz), provides 15 IU (0.15 IU/g)
- Fish liver oils, such as cod liver oil, 1 Tbs. (15 ml) provides 1360 IU (90.6 IU/ml)
- UV-irradiated mushrooms and UV-irradiated yeast are the only vegan sources of vitamin D from food stuffs. A 100g portion provides: (regular) 14 IU (0.14 IU/g), (exposed to UV) 500 IU (5 IU/g) Both yeast and mushroom materials, when irradiated with UV, produce vitamin D_2, but it is not known whether the D_2 is biologically fully equivalent to the D_3 vitamin in humans.

Nutrition Facts labels on food products in the US are not required to list vitamin D content unless a food has been fortified with this nutrient.

Industrial production

Vitamin D3 (cholecalciferol) is produced industrially by exposing 7-dehydrocholesterol from wool fat to UVB light, followed by purification. Vitamin D2 (ergocalciferol) is produced in a similar way using ergosterol from yeast as a starting material.

Deficiency

Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun. Deficiency results in impaired bone mineralization, and leads to bone softening diseases including:

- Rickets, a childhood disease characterized by impeded growth, and deformity, of the long bones which can be caused by calcium or phosphorus deficiency as well as a lack of vitamin D; today it is largely found in low income countries in Africa, Asia or the Middle East and in those with genetic disorders such as pseudovitamin D deficiency rickets. Rickets was first described in 1650 by Francis Glisson who said it had first appeared about 30 years previously in the counties of Dorset and Somerset. In 1857 John Snow suggested the rickets then widespread in Britain was being caused by the adulteration of bakers bread with alum. The role of diet in the development of rickets was determined by Edward Mellanby between 1918–1920. Nutritional rickets exists in countries with intense year round sunlight such as Nigeria and can occur without vitamin D deficiency. Although rickets and osteomalacia are now rare in Britain there have been outbreaks in some immigrant communities in which osteomalacia sufferers included women with seemingly adequate daylight outdoor exposure wearing Western clothing. Having darker skin and reduced exposure to sunshine did not produce rickets unless the diet deviated from a Western omnivore pattern characterized by high intakes of meat, fish and eggs, and low intakes of high-extraction cereals. The dietary risk factors for rickets include abstaining from animal foods. Vitamin D deficiency remains the main cause of rickets among young infants in most countries, because breast milk is low in vitamin D and social customs and climatic conditions can prevent adequate UVB exposure. In sunny countries such as Nigeria, South Africa, and Bangladesh where the disease occurs among older toddlers and children it has been attributed to low dietary calcium intakes, which are characteristic of cereal-based diets with limited access to dairy products. Rickets was formerly a major public health problem among the US population; in Denver where ultraviolet rays are approximately 20% stronger than at sea level on the same latitude almost two thirds of 500 children had mild rickets in the late 1920s. An increase in the proportion of animal protein in the 20th century American diet coupled with increased consumption of milk fortified with relatively small quantities of vitamin D coincided with a dramatic decline in the number of rickets cases.

- Osteomalacia, a bone-thinning disorder that occurs exclusively in adults and is characterized by proximal muscle weakness and bone fragility. The effects of osteomalacia are thought to contribute to chronic musculoskeletal pain, there is no persuasive evidence of lower vitamin D status in chronic pain sufferers.

Adequate vitamin D may also be associated with healthy hair follicle growth cycles. There are also associations between low 25(OH)D levels and peripheral vascular disease, certain cancers, multiple sclerosis, rheumatoid arthritis, juvenile diabetes, Parkinson's and Alzheimer's disease. However these associations were found in observational studies and vitamin D vitamin supplements have not been demonstrated to reduce the risks of these diseases.

Research shows that dark-skinned people living in temperate climates have lower vitamin D levels. It has been suggested that dark-skinned people are less efficient at making vitamin D because melanin in the skin hinders vitamin D synthesis, however a recent study has found novel evidence that low vitamin D levels among Africans may be due to other reasons. Recent evidence implicates parathyroid hormone in adverse cardiovascular outcomes, black women have an increase in serum PTH at a lower 25(OH)D level than white women. A large scale association study of the genetic determinants of vitamin D insufficiency in Caucasians found no links to pigmentation.

The Director General of Research and Development and Chief Scientific Adviser for the Department of Health and NHS said that children aged six months to five years should be given vitamin D supplements—particularly during the winter. However, people who get enough vitamin D from their diet and from sunlight are not recommended for vitamin D supplements.

Measuring vitamin D status

The serum concentration of 25-hydroxy-vitamin D is typically used to determine vitamin D status. It reflects vitamin D produced in the skin as well as that acquired from the diet, and has a fairly long circulating half-life of 15 days. It does not, however, reveal the amount of vitamin D stored in other body tissues. The level of serum 1,25-dihydroxy-vitamin D is not usually used to determine vitamin D status because it has a short half-life of 15 hours and is tightly regulated by parathyroid hormone, calcium, and phosphate, such that it does not decrease significantly until vitamin D deficiency is already well advanced.

There has been variability in results of laboratory analyses of the level of 25-hydroxy-vitamin D. Falsely low or high values have been obtained depending on the particular test or laboratory used. Beginning in July 2009 a standard reference material became available which should allow laboratories to standardise their procedures.

There is some disagreement concerning the exact levels of 25-hydroxy-vitamin D needed for good health. A level lower than 10 ng/mL (25 nmol/L) is associated with the most severe deficiency diseases: rickets in infants and children, and osteomalacia in adults. A

concentration above 15 ng/ml (37.5 nmol/L) is generally considered adequate for those in good health. Levels above 30 ng/ml (75 nmol/L) are proposed by some as desirable for achieving optimum health, but there is not yet enough evidence to support this.

Levels of 25-hydroxy-vitamin D that are consistently above 200 ng/mL (500 nmol/L) are thought to be potentially toxic, although data from humans is sparse. In animal studies levels up to 400 ng/mL (1,000 nmol/L) were not associated with toxicity. Vitamin D toxicity usually results from taking supplements in excess. Hypercalcemia is typically the cause of symptoms, and levels of 25-hydroxy-vitamin D above 150 ng/mL (375 nmol/L) are usually found, although in some cases 25-hydroxy-vitamin D levels may appear to be normal. It is recommended to periodically measure serum calcium in individuals receiving large doses of vitamin D.

In overweight persons increased fat mass is inversely associated with 25(OH)D levels. This association may confound the reported relationships between low vitamin D status and conditions which occur more commonly in obesity as the circulating 25(OH)D underestimates their total body stores.

A study of highly sun exposed (tanned) healthy young skateboarders and surfers in Hawaii found levels below the proposed higher minimum of 30 ng/ml in 51% of the subjects. The highest 25(OH)D concentration was around 60 ng/ml (150nmol/L). A similar <using the same data> study in Hawaii found a range of (11–71 ng/mL) in a population with prolonged extensive skin exposure while as part of the same study Wisconsin breastfeeding mothers were given supplements. The range of circulating 25(OH)D levels in women in the supplementated group was from 12–77 ng/mL. It is noteworthy that the levels in the supplemented population in Wisconsin were higher than the sun exposed group in Hawaii (which again included surfers because it was the same data set).

Overdose by ingestion

In healthy adults, sustained intake of 1250 micrograms/day (50,000 IU) can produce overt toxicity after several months; those with certain medical conditions are far more sensitive to vitamin D and develop hypercalcemia in response to any increase in vitamin D nutrition, while maternal hypercalcemia during pregnancy may increase fetal sensitivity to effects of vitamin D and lead to a syndrome of mental retardation and facial deformities. Pregnant or breastfeeding women should consult a doctor before taking a vitamin D supplement. For infants (birth to 12 months), the tolerable Upper Limit (maximum amount that can be tolerated without harm) is set at 25 micrograms/day (1000 IU). One thousand micrograms (40,000 IU) per day in infants has produced toxicity within one month. After being commissioned by the Canadian and American governments, the Institute of Medicine (IOM) as of 30 November 2010, has increased the tolerable upper limit (UL) to 2500 IU per day for ages 1–3 years, 3000 IU per day for ages 4–8 years and 4000 IU per day for ages 9–71+ years (including pregnant or lactating women). Vitamin D overdose causes hypercalcemia, and the main symptoms of vitamin D overdose are those of hypercalcemia: anorexia, nausea, and vomiting can occur,

frequently followed by polyuria, polydipsia, weakness, nervousness, pruritus, and, ultimately, renal failure. Proteinuria, urinary casts, azotemia, and metastatic calcification (especially in the kidneys) may develop. Vitamin D toxicity is treated by discontinuing vitamin D supplementation and restricting calcium intake. Kidney damage may be irreversible.

Exposure to sunlight for extended periods of time does not normally cause vitamin D toxicity. Within about 20 minutes of ultraviolet exposure in light skinned individuals (3–6 times longer for pigmented skin), the concentrations of vitamin D precursors produced in the skin reach an equilibrium, and any further vitamin D that is produced is degraded. According to some sources, endogenous production with full body exposure to sunlight is approximately 250 μg (10,000 IU) per day. According to Holick, "the skin has a large capacity to produce cholecalciferol"; his experiments indicate

"[W]hole-body exposure to one minimal erythemal dose [a dose that would just begin to produce sunburn in a given individual] of simulated solar ultraviolet radiation is comparable with taking an oral dose of between 250 and 625 micrograms (10 000 and 25 000 IU) vitamin D."

The similar effect of supplementation and whole body exposure to one erythemal dose prompted a researcher to suggest 250 micrograms/day (10,000 IU) in healthy adults should be adopted as the tolerable upper limit. Supplements and skin synthesis have a different effect on serum 25(OH)D concentrations; endogenously synthesized vitamin D_3 travels in plasma almost exclusively on vitamin D-binding protein (VDBP), providing for a slower hepatic delivery of the vitamin D and the more sustained increase in plasma 25-hydroxycholecalciferol. Orally administered vitamin D produces swift hepatic delivery and increases in plasma 25-hydroxycholecalciferol. The richest food source of vitamin D — wild salmon — would require 35 ounces a day to provide 10,000IU. Recommending supplementation, when those supposedly in need of it are labeled healthy, has proved contentious, and doubt exists concerning long term effects of attaining and maintaining serum 25(OH)D of at least 80nmol/L by supplementation.

A Toronto study concluded, "skin pigmentation, assessed by measuring skin melanin content, showed an inverse relationship with serum 25(OH)D." The uniform occurrence of low serum 25(OH)D in Indians living in India and Chinese in China, does not support the hypothesis that the low levels seen in the more pigmented are due to lack of synthesis from the sun at higher latitudes; the leader of the study has urged dark-skinned immigrants to take vitamin D supplements nonetheless, saying, "I see no risk, no downside, there's only a potential benefit." Whether the toxicity of oral intake of vitamin D is due to that route being unnatural, as suggested by Fraser, is not known, but there is evidence to suggest dietary vitamin D may be carried by lipoprotein particles into cells of the artery wall and atherosclerotic plaque, where it may be converted to active form by monocyte-macrophages. These findings raise questions regarding the effects of vitamin D intake on atherosclerotic calcification and cardiovascular risk.

Health effects

Bone health

One of the most important roles of vitamin D is to maintain skeletal calcium balance by promoting calcium absorption in the intestines, promoting bone resorption by increasing osteoclast number, maintaining calcium and phosphate levels for bone formation, and allowing proper functioning of parathyroid hormone to maintain serum calcium levels. Vitamin D deficiency can result in lower bone mineral density and an increased risk of bone loss (osteoporosis) or bone fracture because a lack of vitamin D alters mineral metabolism in the body. Vitamin D has been studied as a potential treatment for osteoporosis, but since treatment of vitamin D deficiency is associated with an increase of mineralization of osteoid, it remains unclear whether vitamin D has any effect on osteoporotic bone. In cross-sectional studies there was a positive relationship between vitamin D and bone mineral density in the hip. Lips (2001) reported that bone mineral deficit in osteomalacia was larger than that in milder degrees of vitamin D deficiency.

There is also a relationship between low bone mineral density and sedentary life style. This is evident in frail, elderly subjects because they are often vitamin D deficient and lead an inactive lifestyle. Lips (2001) also reported that mild vitamin D deficiency was not associated with an increased risk for hip fracture. A study done in Norway consisted of 246 patients with hip fractures who were studied for risk factors. Results showed that a vitamin D intake lower than 100 IU/day was associated with an increased risk for hip fracture. Vitamin D supplements may also increase bone mineral density in other parts of the skeleton. A study showed that a supplement of 800 IU per day of vitamin D increased the bone mineral density of the lumbar spine in postmenopausal women in comparison with the control group. Persons over the age of 50 years need higher levels of vitamin D. In a study discussed in LoPiccolo et al. (2010), adults who consumed a daily supplementation with 482–770 IU of vitamin D had reduced fracture rates of 20% for non-vertebral fractures. However, there was no reported reduction in fracture risk for persons who had 400 IU or less of vitamin D daily.

Immune system

Vitamin D receptor ligands have been shown to increase the activity of natural killer cells, and enhance the phagocytic activity of macrophages. Active vitamin D hormone also increases the production of cathelicidin, an antimicrobial peptide that is produced in macrophages triggered by bacteria, viruses, and fungi. Suggestions of a link between vitamin D deficiency and the onset of multiple sclerosis posited that this is due to the immune-response suppression properties of Vitamin D and that vitamin D is required to activate a histocompatibility gene (HLA-DRB1*1501) necessary for differentiating between self and foreign proteins in a subgroup of individuals genetically predisposed to MS. Whether vitamin D supplements during pregnancy can lessen the likelihood of the child developing MS later in life is not known; however, vitamin D fortification has been suggested to have caused a pandemic of allergic disease and an association between vitamin D supplementation in infancy and an increased risk of atopy and allergic rhinitis

later in life has been found . Veteran vitamin D researcher Hector DeLuca has cast doubt on whether vitamin D affects MS.

Influenza

Lack of vitamin D synthesis is a possible explanation for high rates of influenza infection during winter; however, see flu season for the factors apart from vitamin D that are also hypothesized to influence rates of infection during winter. For viral infections, other implicated factors include low relative humidities produced by indoor heating and cold temperatures that favor virus spread during winter.

Cancer

The molecular basis for thinking vitamin D has the potential to prevent cancer lies in its role in a wide range of cellular mechanisms central to the development of cancer. These effects may be mediated through vitamin D receptors expressed in cancer cells. Polymorphisms of the vitamin D receptor (VDR) gene have been associated with an increased risk of breast cancer. Women with mutations in the VDR gene had an increased risk of breast cancer.

A 2006 study using data on over 4 million cancer patients from 13 different countries showed a marked increase in some cancer risks in countries with less sun and another metastudy found correlations between vitamin D levels and cancer. The authors suggested that intake of an additional 1,000 international units (IU) (or 25 micrograms) of vitamin D daily reduced an individual's colon cancer risk by 50%, and breast and ovarian cancer risks by 30%. Low levels of vitamin D in serum have been correlated with breast cancer disease progression and bone metastases. However, the vitamin D levels of a population do not depend on the solar irradiance to which they are exposed. Moreover, there are genetic factors involved with cancer incidence and mortality which are more common in northern latitudes.

A 2006 study found that taking the U.S. RDA of vitamin D (400 IU per day) cut the risk of pancreatic cancer by 43% in a sample of more than 120,000 people from two long-term health surveys. However, in male smokers a 3-fold increased risk for pancreatic cancer in the highest compared to lowest quintile of serum 25-hydroxyvitamin D concentration has been found.

A randomized intervention study involving 1,200 women, published in June 2007, reports that vitamin D supplementation (1,100 international units (IU)/day) resulted in a 60% reduction in cancer incidence, during a four-year clinical trial, rising to a 77% reduction for cancers diagnosed *after* the first year (and therefore excluding those cancers more likely to have originated prior to the vitamin D intervention). The study was criticized on several grounds including lack of reported data, use of statistical techniques and comparison with a self-selected (i.e. non-randomized) observational study that found long term convergence of breast cancer incidence (i.e. the cancer occurrence had merely been delayed) The author's response provided the requested data, explained their statistical

usage and commented that even if the vitamin D merely delayed the appearance of cancer (which they did not believe, based on other studies), that this was still a considerable benefit.

In 2007 the Canadian Cancer Society recommended that adults living in Canada should consider taking Vitamin D supplementation of 1,000 international units (IU) a day during the fall and winter. A US National Cancer Institute study analyzed data from the third national Health and Nutrition Examination Survey to examine the relationship between levels of circulating vitamin D in the blood and cancer mortality in a group of 16,818 participants aged 17 and older. It found no support for an association between 25(OH)D and total cancer mortality. However, the study did find that "[c]olorectal cancer mortality was inversely related to serum 25(OH)D level, with levels 80 nmol/L or higher associated with a 72% risk reduction (95% confidence interval = 32% to 89%) compared with lower than 50 nmol/L, $P_{trend} = .02$." Unlike other studies, this one was carried out prospectively — meaning that participants were followed looking forward — and the researchers used actual blood tests to measure the amount of vitamin D in blood, rather than trying to infer vitamin D levels from potentially inaccurate predictive models.

Cardiovascular disease

A report from the National Health and Nutrition Examination Survey (NHANES) involving nearly 5,000 participants found that low levels of vitamin D were associated with an increased risk of peripheral artery disease (PAD). The incidence of PAD was 80% higher in participants with the lowest vitamin D levels (<17.8 ng/mL). Cholesterol levels were found to be reduced in gardeners in the UK during the summer months. Low levels of vitamin D are associated with an increase in high blood pressure and cardiovascular risk. Numerous observational studies show this link, but of two systemic reviews one found only weak evidence of benefit from supplements and the other found no evidence of a beneficial effect whatsoever.

There is a certain amount of evidence to suggest that dietary vitamin D may be carried by lipoprotein particles into cells of the artery wall and atherosclerotic plaque, where it may be converted to active form by monocyte-macrophages. These findings raise questions regarding the effects of vitamin D intake on atherosclerotic calcification and cardiovascular risk. Calcifediol (25-hydroxy-vitamin D) is implicated in the etiology of atherosclerosis, especially in non-Caucasians. Freedman et al. (2010) found that serum vitamin D correlates in African Americans, but not in Euro-Americans, with calcified atheroscleratic plaque. "Higher levels of 25-hydroxyvitamin D seem to be positively correlated with aorta and carotid CP in African Americans but not with coronary CP. These results contradict what is observed in individuals of European descent." One study found an elevated risk of ischaemic heart disease in Southern India in individuals whose vitamin D levels were above 89 ng/mL. A review of vitamin D status in India concluded that studies uniformly point to low 25(OH)D levels in Indians despite abundant sunshine, and suggested a public health need to fortify Indian foods with vitamin D might exist. However the levels found in India are consistent with many other studies of tropical populations which have found that even an extreme amount of sun exposure, such as

incurred by rural Indians, does not raise 25(OH)D levels to the levels typically found in Europeans,

Mortality

Using information from the National Health and Nutrition Examination Survey a large scale study concluded that having low levels of vitamin D (<17.8 ng/ml) was independently associated with an increase in all-cause mortality in the general population. However it has been pointed out that increased mortality was also found in those with higher concentrations, (above 50 ng/ml). A sophisticated August 2010 study of plasma vitamin D and mortality in older men concluded that both high (>39 ng/ml) and low (<18 ng/ml)) concentrations of plasma 25(OH)D are associated with elevated risks of overall and cancer mortality compared with intermediate concentrations. These boundaries were less than suggested by the Melamed et al. study of National Health and Nutrition Examination Survey data but the immunoassay used by National Health and Nutrition Examination Survey tended to overestimate vitamin D values.

Overall, excess or deficiency in the calciferol system appear to cause abnormal functioning and premature aging.

Complex regulatory mechanisms control metabolism and recent epidemiological evidence suggests that there is a narrow range of vitamin D blood levels in which metabolic functions are optimized. Levels above or below this natural homeostasis of vitamin D are associated with increased mortality.

Chapter 13

Vitamin E

Vitamin E is a generic term for tocopherols which taken from the Greek words *tokos*,meaning offspring, and *phero*, meaning to bear, and tocotrienols. Vitamin E is a family of α-, β-, γ-, and δ- (respectively: alpha, beta, gamma, and delta) tocopherols and corresponding four tocotrienols. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation.

α-Tocopherol

It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidised α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. However, the importance of the antioxidant properties of this molecule at the concentrations present in the body are not clear and it is possible that the reason why vitamin E is required in the diet is unrelated to its ability to act as an antioxidant. Other forms of vitamin E have their own unique properties. For example, γ-tocopherol (also written as gamma-tocopherol) is a nucleophile that can react with electrophilic mutagens.

However, the roles and importance of all of the various forms of vitamin E are presently unclear, and it has even been suggested that the most important function of vitamin E is as a signaling molecule, and that it has no significant role in antioxidant metabolism.

So far, most studies about vitamin E have supplemented using only alpha-tocopherol, but doing so leads to reduced serum gamma- and delta-tocopherol concentrations. Moreover, a 2007 clinical study involving alpha-tocopherol concluded that supplementation did not reduce the risk of major cardiovascular events in middle aged and older men. For more information, read article tocopherol.

Tocotrienols

Compared with tocopherols, tocotrienols are sparsely studied. Less than 1% of PubMed papers on vitamin E relate to tocotrienols. Current research direction is starting to give more prominence to the tocotrienols, the lesser known but more potent antioxidants in the vitamin E family. Some studies have suggested that tocotrienols have specialized roles in protecting neurons from damage and cholesterol reduction by inhibiting the activity of HMG-CoA reductase[16-1];δ-tocotrienol blocks processing of sterol regulatory element‐binding proteins (SREBPs)[16-1].

Oral consumption of tocotrienols is also thought to protect against stroke-associated brain damage in vivo. Until further research has been carried out on the other forms of vitamin E, conclusions relating to the other forms of vitamin E, based on trials studying only the efficacy of α-tocopherol, may be premature.

Recommended daily intake

The Food and Nutrition Board at the Institute of Medicine report the following dietary reference intakes for vitamin E:

Infants

- 0 to 6 months: 4 mg/day
- 7 to 12 months: 5 mg/day

Children

- 1 to 3 years: 6 mg/day
- 4 to 8 years: 7 mg/day
- 9 to 13 years: 11 mg/day

Adolescents and Adults

- 14 and older: 15 mg/day

One IU of Vitamin E is 0.67 mg of d-alpha-tocopherol, or 1 mg of dl-alpha-tocopherol acetate (either 0.91 mg or 0.45 mg of dl-alpha-tocopherol).

Dietary sources and supplements

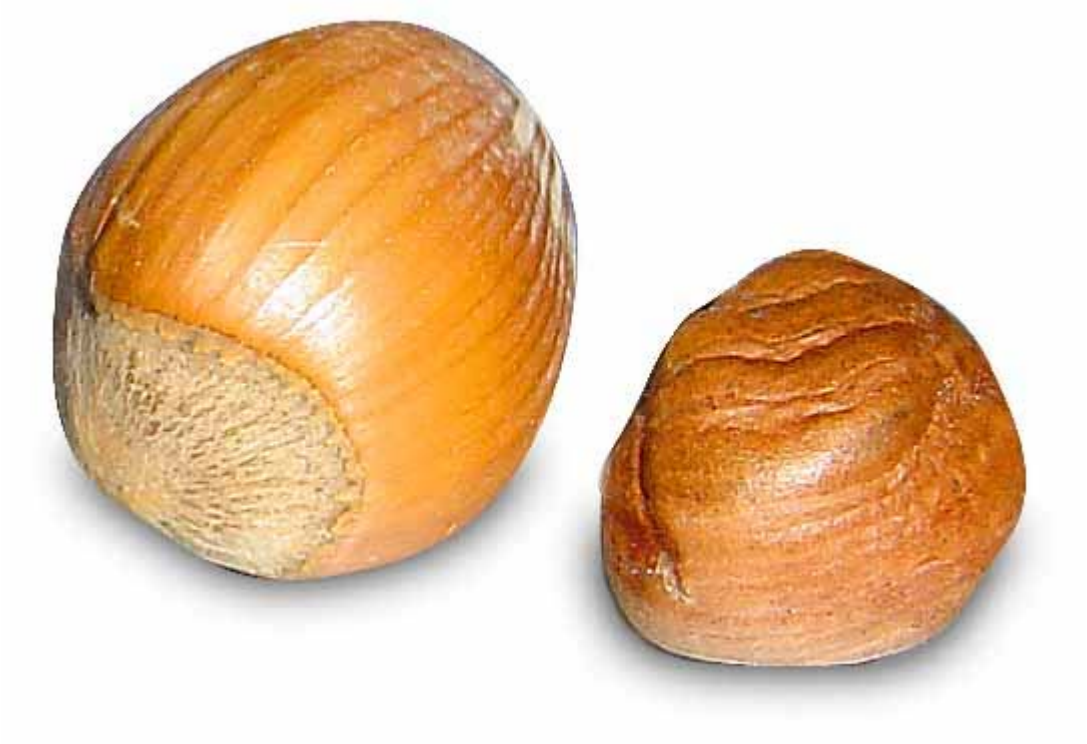
The following foods are rich in vitamin E:

- Fortified cereals
- Seeds and seed oils, like sunflower
- Nuts and nut oils, like almonds and hazelnuts
- Green leafy vegetables, like spinach, turnip, beet, collard, and dandelion greens

- Tomato products
- Pumpkin
- Sweet potato (0.26 mg/100g)
- Blue crab
- Rockfish
- Mangoes
- Asparagus
- Broccoli
- Papayas
- Olives
- Avocados

Breakfast cereals

Sunflower oil



Nuts

Spinach

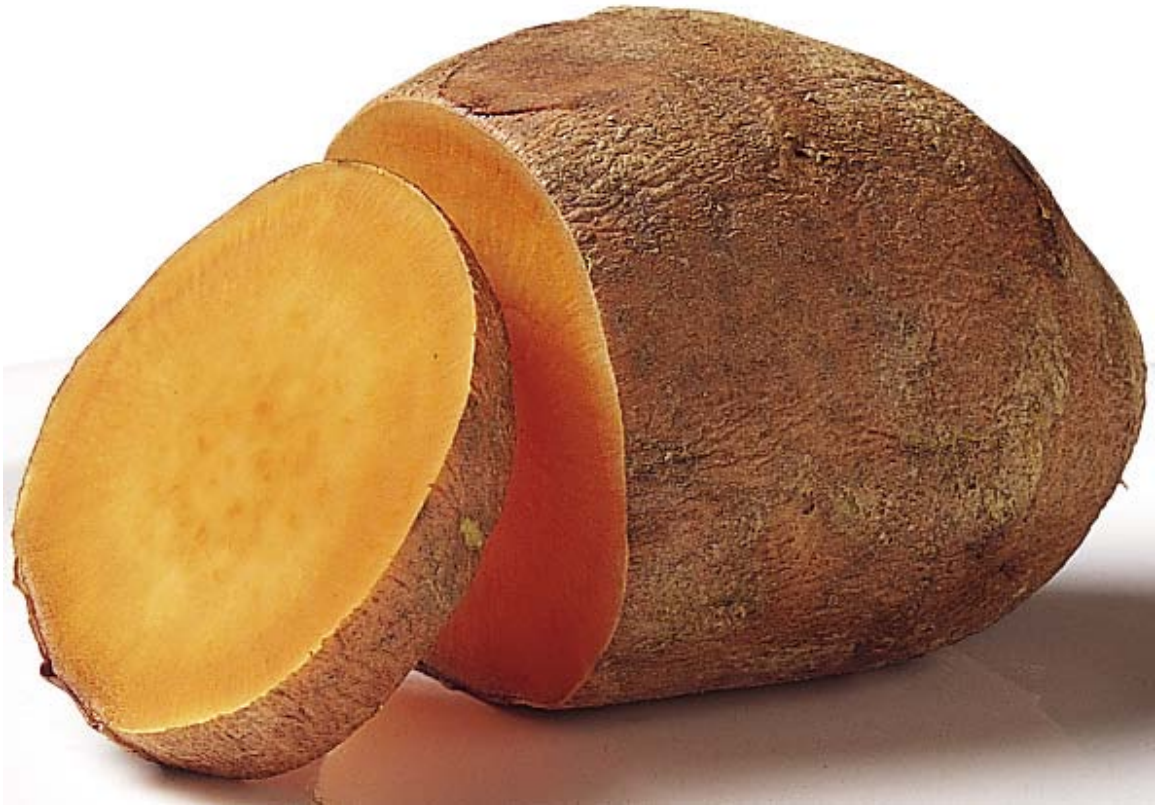
Turnip

Beet

Collard

Tomato

Pumpkin



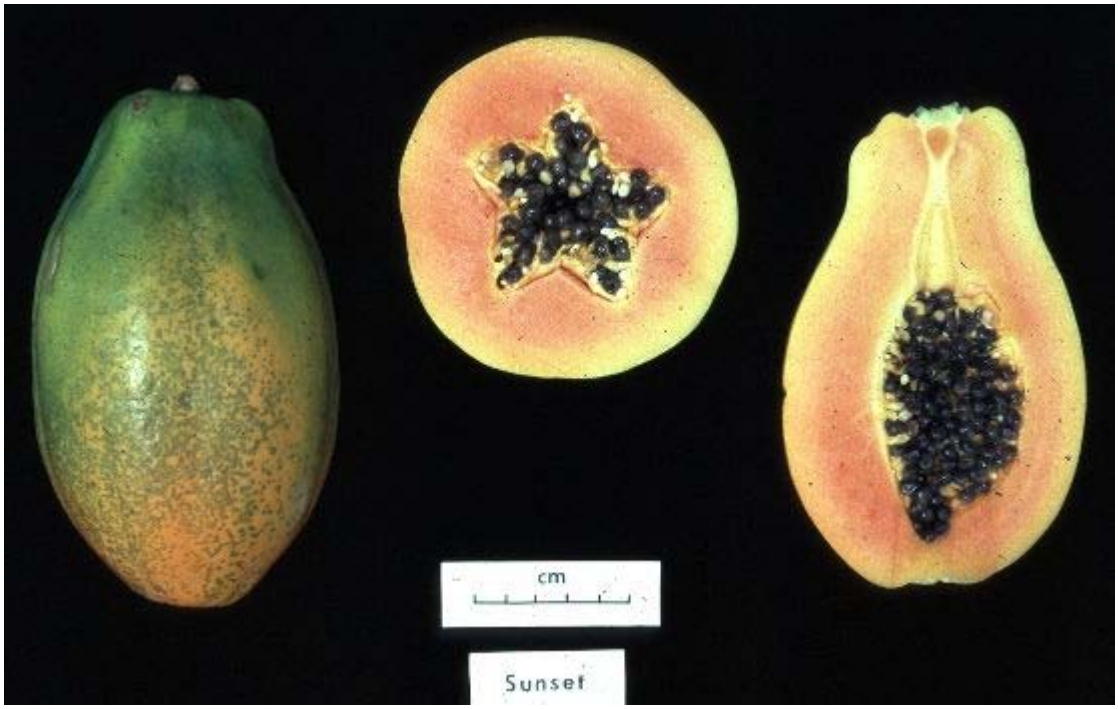
Sweet potato

Blue crab

Mango

Asparagus

Broccoli



Papaya

Olive

Health effects

The consensus in the medical community is that there is no good evidence to support health benefits from vitamin E supplementation in the short term, yet there is some evidence that taking more than 400 IU of vitamin E per day for extended periods increases the risk of death , although this has been questioned.

Chapter 14

Vitamin K

Vitamin K is a group of fat soluble vitamins that are needed for the posttranslational modification of certain proteins, mostly required for blood coagulation but also involved in metabolism pathways in bone and other tissue. They are 2-methyl-1,4-naphthoquinone derivatives.

Vitamin K_1 is also known as phylloquinone or phytomenadione (also called phytonadione). Vitamin K_2 (menaquinone, menatetrenone) is normally produced by bacteria in the large intestine, and dietary deficiency is extremely rare unless the intestines are heavily damaged, are unable to absorb the molecule, or are subject to decreased production by normal flora, as seen in broad spectrum antibiotic use.

There are three synthetic forms of vitamin K, vitamins K_3, K_4, and K_5, which are used in many areas including the pet food industry (vitamin K_3) and to inhibit fungal growth (vitamin K_5).

Chemical structure

All members of the vitamin K group of vitamins share a methylated naphthoquinone ring structure, and vary in the aliphatic side chain attached at the 3-position. Phylloquinone (also known as vitamin K_1) invariably contains in its side chain four isoprenoid residues, one of which is unsaturated.

Menaquinones have side chains composed of a variable number of unsaturated isoprenoid residues; generally they are designated as MK-n, where n specifies the number of isoprenoids.

It is generally accepted that the naphthoquinone is the functional group, so that the mechanism of action is similar for all K-vitamins. Substantial differences may be expected, however, with respect to intestinal absorption, transport, tissue distribution, and bio-availability. These differences are caused by the different lipophilicity of the various side chains, and by the different food matrices in which they occur.

Physiology

Vitamin K is involved in the carboxylation of certain glutamate residues in proteins to form gamma-carboxyglutamate residues (abbreviated Gla-residues). The modified residues are often (but not always) situated within specific protein domains called Gla domains. Gla-residues are usually involved in binding calcium. The Gla-residues are essential for the biological activity of all known Gla-proteins.

At this time 15 human proteins with Gla domains have been discovered, and they play key roles in the regulation of three physiological processes:

- Blood coagulation: (prothrombin (factor II), factors VII, IX, X, protein C, protein S, and protein Z).
- Bone metabolism: osteocalcin, also called bone Gla-protein (BGP), matrix gla protein (MGP), and periostin.
- Vascular biology: growth arrest-specific protein 6 (Gas6)
- unknown function: proline-rich g-carboxy glutamyl proteins (PRGPs) 1 and 2, and transmembrane g-carboxy glutamyl proteins (TMGs) 3 and 4.

Like other liposoluble vitamins (A, D, E), vitamin K is stored in the fat tissue of the human body.

Recommended amounts

The U.S. Dietary Reference Intake (DRI) for an Adequate Intake (AI) of vitamin K for a 25-year old male is 120 micrograms/day. The Adequate Intake (AI) for adult women is 90 micrograms/day, for infants is 10–20 micrograms/day, for children and adolescents 15–100 micrograms/day. In 2002 it was found that to get maximum carboxylation of osteocalcin, one may have to take up to 1000 μg of vitamin K_1.

Toxicity

Although allergic reaction from supplementation is possible, there is no known toxicity associated with high doses of the phylloquinone (vitamin K_1) or menaquinone (vitamin K_2) forms of vitamin K and therefore no tolerable upper intake level (UL) has been set.

However, a synthetic form of vitamin K, vitamin K_3 (menadione), is demonstrably toxic. In fact, the FDA has banned this synthetic form of the vitamin from over-the-counter supplements because large doses have been shown to cause allergic reactions, hemolytic anemia, and cytotoxicity in liver cells.

Drug interactions

Phylloquinone (K_1) or menaquinone (K_2) are capable of blocking the blood thinning action of anticoagulants like warfarin, which work by interfering with the action of

vitamin K. They also reverse the tendency of these drugs to cause arterial calcification in the long term.

Sources

Vitamin K_1 is found chiefly in leafy green vegetables such as spinach, swiss chard, and *Brassica* (*e.g.* cabbage, kale, cauliflower, broccoli, and brussels sprouts); some fruits such as avocado and kiwifruit are also high in vitamin K. By way of reference, two tablespoons of parsley contain 153% of the recommended daily amount of vitamin K. Some vegetable oils, notably soybean, contain vitamin K, but at levels that would require relatively large calorific consumption to meet the USDA recommended levels. Colonic bacteria synthesize a significant portion of humans' vitamin K needs- this is one of the reasons why newborns often receive a vitamin K shot at birth- in order to tide them over until day 5-7 when their colon becomes colonized.

It is believed that phylloquinone's tight binding to the thylakoid membranes in the chloroplasts is the reason behind the poor bioavailability of vitamin K in green plants. For example, cooked spinach has a 5 percent bioavailability of phylloquinone. However when one adds fat to the spinach, the bioavailability increases to 13 percent due to the increased solubility of vitamin K in fat.

Menaquinone-4 and menaquinone-7 (vitamin K_2) are found in meat, eggs, dairy, and natto. MK-4 is synthesized by animal tissues, the rest (mainly MK-7) are synthesized by bacteria during fermentation. In natto 0% of vitamin K is from MK-4 and in cheese 2–7%.

Deficiency

Average diets are usually not lacking in vitamin K and primary vitamin K deficiency is rare in healthy adults. As previously mentioned, newborn infants are at an increased risk of deficiency. Other populations with an increased prevalence of vitamin K deficiency include individuals who suffer from liver damage or disease (e.g. alcoholics), people with cystic fibrosis, inflammatory bowel diseases or those who have recently had abdominal surgeries. Groups that may suffer from secondary vitamin K deficiency include bulimics, those on stringent diets, and those taking anticoagulants. Other drugs that have been associated with vitamin K deficiency include salicylates, barbiturates, and cefamandole, although the mechanism is still unknown. There is no difference between the sexes as both males and females are affected equally. Symptoms of deficiency include heavy menstrual bleeding in women, anemia, bruising, and bleeding of the gums or nose. They could also have disorders such as coagulopathy.

Osteoporosis and coronary heart disease are strongly associated with lower levels of K_2 (menaquinone). Menaquinone is not inhibited by salicylates as happens with K_1, so menaquinone supplementation can alleviate the chronic vitamin K deficiency caused by long term aspirin use.

Biochemistry

Discovery

In 1929, Danish scientist Henrik Dam investigated the role of cholesterol by feeding chickens a cholesterol-depleted diet. After several weeks, the animals developed hemorrhages and started bleeding. These defects could not be restored by adding purified cholesterol to the diet. It appeared that—together with the cholesterol—a second compound had been extracted from the food, and this compound was called the coagulation vitamin. The new vitamin received the letter K because the initial discoveries were reported in a German journal, in which it was designated as *Koagulationsvitamin*. Edward Adelbert Doisy of Saint Louis University did much of the research that led to the discovery of the structure and chemical nature of vitamin K. Dam and Doisy shared the 1943 Nobel Prize for medicine for their work on vitamin K (K_1 and K_2) published in 1939. Several laboratories synthesized the compound(s) in 1939.

For several decades the vitamin K-deficient chick model was the only method of quantifying vitamin K in various foods: the chicks were made vitamin K-deficient and subsequently fed with known amounts of vitamin K-containing food. The extent to which blood coagulation was restored by the diet was taken as a measure for its vitamin K content. Three groups of physicians independently found this: Biochemical Institute, University of Copenhagen (Dam and Johannes Glavind), University of Iowa Department of Pathology (Emory Warner, Kenneth Brinkhous, and Harry Pratt Smith), and the Mayo Clinic (Hugh Butt, Albert Snell, and Arnold Osterberg).

The first published report of successful treatment with vitamin K of life-threatening hemorrhage in a jaundiced patient with prothrombin deficiency was made in 1938 by Smith, Warner, and Brinkhous.

Function

The function of vitamin K in the cell is to convert glutamate in proteins to gamma-carboxyglutamate (gla).

Within the cell, vitamin K undergoes electron reduction to a reduced form of vitamin K (called vitamin K hydroquinone) by the enzyme vitamin K epoxide reductase (or VKOR). Another enzyme then oxidizes vitamin K hydroquinone to allow carboxylation of Glu to Gla; this enzyme is called the gamma-glutamyl carboxylase or the vitamin K-dependent carboxylase. The carboxylation reaction will only proceed if the carboxylase enzyme is able to oxidize vitamin K hydroquinone to vitamin K epoxide at the same time; the carboxylation and epoxidation reactions are said to be coupled reactions. Vitamin K epoxide is then re-converted to vitamin K by vitamin K epoxide reductase. These two enzymes comprise the so-called vitamin K cycle. One of the reasons humans are rarely deficient in vitamin K is that vitamin K is continually recycled in our cells.

Warfarin and other coumarin drugs block the action of the vitamin K epoxide reductase. This results in decreased concentrations of vitamin K and vitamin K hydroquinone in the tissues, such that the carboxylation reaction catalyzed by the glutamyl carboxylase is inefficient. This results in the production of clotting factors with inadequate Gla. Without Gla on the amino termini of these factors, they no longer bind stably to the blood vessel endothelium and cannot activate clotting to allow formation of a clot during tissue injury. As it is impossible to predict what dose of warfarin will give the desired degree of suppression of the clotting, warfarin treatment must be carefully monitored to avoid over-dosing.

Gamma-carboxyglutamate proteins, or Gla-proteins

At present, the following human Gla-containing proteins have been characterized to the level of primary structure: the blood coagulation factors II (prothrombin), VII, IX, and X, the anticoagulant proteins C and S, and the Factor X-targeting protein Z. The bone Gla-protein osteocalcin, the calcification inhibiting matrix gla protein (MGP), the cell growth regulating growth arrest specific gene 6 protein (Gas6), and the four transmembrane Gla proteins (TMGPs) the function of which is at present unknown. Gas6 can function as a growth factor that activates the Axl receptor tyrosine kinase and stimulates cell proliferation or prevents apoptosis in some cells. In all cases in which their function was known, the presence of the Gla-residues in these proteins turned out to be essential for functional activity.

Gla-proteins are known to occur in a wide variety of vertebrates: mammals, birds, reptiles, and fish. The venom of a number of Australian snakes acts by activating the human blood clotting system. Remarkably, in some cases activation is accomplished by snake Gla-containing enzymes that bind to the endothelium of human blood vessels and catalyze the conversion of procoagulant clotting factors into activated ones, leading to unwanted and potentially deadly clotting.

Another interesting class of invertebrate Gla-containing proteins is synthesized by the fish-hunting snail *Conus geographus*. These snails produce a venom containing hundreds of neuro-active peptides, or conotoxins, which is sufficiently toxic to kill an adult human. Several of the conotoxins contain 2–5 Gla residues.

Methods of assessment

Prothrombin time test:

Measures the time required for blood to clot
Blood sample mixed with citric acid and put in a fibrometer.
Delayed clot formation indicates a deficiency.

Unfortunately insensitive to mild deficiency as the values do not change until the concentration of prothrombin in the blood has declined by at least 50 percent

Plasma Phylloquinone:

Was found to be positively correlated with phylloquinone intake in elderly British women, but not men

However an article by Schurges et al. reported no correlation between FFQ and plasma phylloquinone

Urinary γ-carboxyglutamic acid:

Urinary Gla responds to changes in dietary vitamin K intake.
Several days are required before any change can be observed.

In a study by Booth et al. increases of phylloquinone intakes from 100 μg to between 377–417 μg for 5 days did *not* induce a significant change Response may be age-specific

Function in bacteria

Many bacteria, such as *Escherichia coli* found in the large intestine, can synthesize vitamin K_2 (menaquinone), but not vitamin K_1 (phylloquinone). In these bacteria, menaquinone will transfer two electrons between two different small molecules, in a process called anaerobic respiration. For example, a small molecule with an excess of electrons (also called an electron donor) such as lactate, formate, or NADH, with the help of an enzyme, will pass two electrons to a menaquinone. The menaquinone, with the help of another enzyme, will in turn transfer these 2 electrons to a suitable oxidant, such fumarate or nitrate (also called an electron acceptor). Adding two electrons to fumarate or nitrate will convert the molecule to succinate or nitrite + water, respectively. Some of these reactions generate a cellular energy source, ATP, in a manner similar to eukaryotic cell aerobic respiration, except that the final electron acceptor is not molecular oxygen, but say fumarate or nitrate (In aerobic respiration, the final oxidant is molecular oxygen (O_2) , which accepts four electrons from an electron donor such as NADH to be converted to water.) *Escherichia coli* can carry out aerobic respiration and menaquninone-mediated anaerobic respiration.

Vitamin K injection in newborns

The blood clotting factors of newborn babies are roughly 30 to 60 percent that of adult values; this may be due to the reduced synthesis of precursor proteins and the sterility of their guts. Human milk contains between 1 and 4 micrograms/litre of vitamin K_1, while formula derived milk can contain up to 100 micrograms/litre in supplemented formulas. Vitamin K_2 concentrations in human milk appear to be much lower than those of vitamin K_1. It is estimated that there is a 0.25 to 1.7 percent occurrence of vitamin K deficiency bleeding in the first week of the infant's life with a prevalence of 2-10 cases per 100,000 births. Premature babies have even lower levels of the vitamin and are at a higher risk from this deficiency.

USA

As a result of the occurrences of vitamin K deficiency bleeding, the Committee on Nutrition of the American Academy of Pediatrics has recommended that 0.5 to 1.0 mg vitamin K_1 be administered to all newborns shortly after birth.

UK

In the UK, vitamin K is administered to newborns as either a single injection at birth or three orally administered doses given at birth and then over the baby's first month.

Controversy

Controversy arose in the early 1990s regarding this practice when two studies were shown suggesting a relationship between parenteral administration of vitamin K and childhood cancer (14). However, poor methods and small sample sizes led to the discrediting of these studies and a review of the evidence published in 2000 by Ross and Davies found no link between the two.

Dietary vitamin K and bone health

The role vitamin K plays in bone metabolism triggered a new thinking in its use as a therapeutic agent in diseases related with bone like osteoporosis. Vit. K takes part in the post translational modification as a co‐factor in γ‐ carboxylation of Vit. K dependant proteins (VKDPs). VKDPs have glutamate residues (Glu). There is physiological and observational evidence that vitamin K plays a role in bone growth and the maintenance of bone density, but efforts to delay the onset of osteoporosis by vitamin K supplementation have proven ineffective.

Biophysical studies have indicated that supplemental vitamin K promotes osteotrophic processes and slows osteoclastic processes via calcium bonding. Study of Atkins et al.(Gerald JA, Katie JW, Asiri R W, et al. (2009) Vitamin K promotes mineralization, osteoblast‐to‐osteocyte transition, and an anticatabolic phenotype by γ‐carboxylation‐dependent and independent mechanisms. Am J Physiol Cell Physiol 297, C1358–C1367) revealed that phylloquinone (Vitamin K1), menatetrenone (Vit. K2) and manadione (Vit. K1) promote in vitro mineralisaton by human primary osteoblasts.Other studies have shown that vitamin K antagonists (usually a class of anticoagulants) lead to early calcification of the epiphysis and epiphysial line in mice and other animals, causing seriously decreased bone growth. This is due to defects in osteocalcin and matrix gla protein. Their primary function is to prevent overcalcification of the bone and cartilage. Vitamin K is important in the process of carboxylating glutamic acid (Glu) in these proteins to gamma-carboxyglutamic acid (Gla), which is necessary for their function.. Vitamin D is reported to regulate the OC transcribtion by osteoblast thereby showing that Vit. K and Vik. D works in tandem for the bone metabolism and development. Lian and his group (Lian J, Stewart C, Puchacz E,

Mackowiak S, Shalhoub V, Collart D, Zambetti G & Stein G (1989) Structure of the rat osteocalcin gene and regulation of vitamin D‐dependent expression. Proc Natl Acad Sci 86, 1143–1147) discovered two nucleotide substitution regions which they named "osteocalcin box" in the rat and human osteocalcin genes. They found a region 600 nucleotides immediately upstream from the transcription start site that support a 10‐fold stimulated transcription of the gene by 1, 25‐dihydroxy vitamin D.

Data from the 1998 Nurses Health Study, an observational study, indicated an inverse relationship between dietary vitamin K_1 and the risk of hip fracture. After being given 110 micrograms/day of vitamin K, women who consumed lettuce one or more times per day had a significantly lower risk of hip fracture than women who consumed lettuce one or fewer times per week. In addition to this, high intakes of vitamin D but low intakes of vitamin K were suggested to pose an increased risk of hip fracture.. Firmingham Heart Study (Booth SL, Tucker K, Chen H, et al. (2000) Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 71, 1201–1208.) is another study that showed the similar result. Subjects in the highest quartile of Vit. K1 intake (median K1 intake of 254 μg/ day) has 35% lower risk of hip fracture than those in the lowest quartile. Comparing with the daily recommended intake (DRI) of 90 and 120 μg/ day, both the above intakes are higher than existing DRI indicating the need for the revision of DRI taking into consideration the role it plays in bone health.

However, a large multicentre randomized placebo-controlled trial was performed to test the supplementation of vitamin K in post-menopausal women with osteopenia. Despite heavy doses of vitamin K_1, no differences were found in bone density between the supplemented and placebo groups.

In Japan, a form of vitamin K_2 is recognized as a treatment for osteoporosis. However the long term effects and benefits are unknown and it remains controversial.

Vitamin K and Alzheimer's disease

Research into the antioxidant properties of vitamin K indicates that the concentration of vitamin K is lower in the circulation of carriers of the APOE4 gene, and recent studies have shown its ability to inhibit nerve cell death due to oxidative stress. It has been hypothesized that vitamin K may reduce neuronal damage and that supplementation may hold benefits to treating Alzheimer's disease, although more research is necessary in this area.

Vitamin K used topically

Vitamin K may be applied topically, typically as a 5% cream, to diminish postoperative bruising from cosmetic surgery and injections, broken capillaries (spider veins), to treat rosacea and to aid in the fading of hyperpigmentation and dark under-eye circles.

Vitamin K and cancer

While researchers in Japan were studying the role of vitamin K_2 in the prevention of bone loss in females with liver disease, they discovered another possible effect of this phytonutrient. This two year study which involved 21 women with viral liver cirrhosis found that women in the supplement group were 90 percent less likely to develop liver cancer. A German study performed on men with prostate cancer found a significant inverse relationship between vitamin K_2 consumption and advanced prostate cancer.

Vitamin K as antidote for poisoning by 4-hydroxcoumarin drugs

Vitamin K is a true antidote for poisoning by 4-hydroxycoumarin anticoagulant drugs (sometimes loosely referred to a coumarins). These include the pharmaceutical warfarin, and also anticoagulant-mechanism poisons such as bromadiolone, which are commonly found in rodenticides. 4-hydroxycoumarin drugs possess anticoagulatory and rodenticidal properties because they inhibit vitamin K-dependent synthesis of some clotting factors by the liver. Death is usually a result of internal hemorrhage. Treatment usually consists of repeated intravenous doses of vitamin K, followed by doses in pill form for a period of at least two weeks, though possibly up to 2 months, afterwards (in the case of the more potent 4-hydoxycoumarins used as rodenticides). If caught early, prognosis is good, even when great amounts of the drug or poison are ingested.

History of discovery

The precise function of vitamin K was not discovered until 1974, when three laboratories (Stenflo *et al.*, Nelsestuen *et al.*, and Magnusson *et al.*) isolated the vitamin K-dependent coagulation factor prothrombin (Factor II) from cows that received a high dose of a vitamin K antagonist, warfarin. It was shown that while warfarin-treated cows had a form of prothrombin that contained 10 glutamate amino acid residues near the amino terminus of this protein, the normal (untreated) cows contained 10 unusual residues that were chemically identified as gamma-carboxyglutamate, or Gla. The extra carboxyl group in Gla made clear that vitamin K plays a role in a carboxylation reaction during which Glu is converted into Gla.

The biochemistry of how vitamin K is used to convert Glu to Gla has been elucidated over the past thirty years in academic laboratories throughout the world.