Vitamin B2 (Riboflavin)

Riboflavin, also known as vitamin B2, is a vitamin found in food and used as a dietary supplement. As a supplement it is used to prevent and treat riboflavin deficiency and prevent migraines. It may be given by mouth or injection. It is nearly always well tolerated. Normal doses are safe during pregnancy. Riboflavin is in the vitamin B group. It is required by the body for cellular respiration. Food sources include eggs, green vegetables, milk, and meat. Riboflavin was discovered in 1920, isolated in 1933, and first made in 1935.[4] It is on the World Health Organization’s List of Essential Medicines, the most effective and safe medicines needed in a health system.[5] Riboflavin is available as a generic medication and over the counter. In the United States a month of supplements costs less than 25 USD.[6] Some countries require its addition to grains.[3]

Medical uses

A solution of riboflavin.
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.

One clinical trial found that high-dose riboflavin appears to be useful alone or along with beta-blockers in the prevention of migraine.[7][8] A dose of 400 mg daily has been used effectively 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.[9] However, two other clinical studies have failed to find any significant results for the effectiveness of B2 as a treatment for migraine.[10][11]

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.[12][13][14] 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.[14][15] 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 (i.e. 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.[16]

Corneal ectasia is a progressive thinning of the cornea; the most common form of this condition is keratoconus. Collagen cross-linking is a non-surgical treatment intended to slow progression of corneal ectasia by strengthening corneal tissue. The standard protocol calls for application directly to the eye of a 0.1% riboflavin solution for 30 minutes followed by 30 minutes of ultraviolet-A irradiation with a wavelength of 370 nm and power of 3 mW/cm².[17]

### Side effects

In humans, there is no evidence for riboflavin toxicity produced by excessive intakes, in part because it has lower water solubility than other B vitamins, because absorption becomes less efficient as doses increase, and because what excess is absorbed is excreted via the kidneys into urine. Even when 400 mg of riboflavin per day 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.[18][19][20] Although toxic doses can be administered by injection,[19] any excess at nutritionally relevant doses is excreted in the urine,[21] imparting a bright yellow color when in large quantities.

The Food and Nutrition Board of the U.S. Institute of Medicine sets Tolerable Upper Intake Levels (known as ULs) for vitamins and minerals when evidence is sufficient. In the case of riboflavin there is no UL, as there is no human data for adverse effects from high doses. The European Food Safety Authority reviewed the same safety question and also reached the conclusion that there was not sufficient evidence to set a UL for riboflavin.[22]
Function

Riboflavin functions as a coenzyme, meaning that it is required for enzymes (proteins) to perform normal physiological actions. Specifically, the active forms of riboflavin flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) function as cofactors for a variety of flavoproteine enzyme reactions:

- Flavoproteins of electron transport chain, including FMN in Complex I and FAD in Complex II
- FAD is required for the production of pyridoxic acid from pyridoxal (vitamin B₆) by pyridoxine 5'-phosphate oxidase
- The primary coenzyme form of vitamin B₆ (pyridoxal phosphate) is FMN dependent
- Oxidation of pyruvate, α-ketoglutarate, and branched-chain amino acids requires FAD in the shared E₃ portion of their respective dehydrogenase complexes
- Fatty acyl CoA dehydrogenase requires FAD in fatty acid oxidation
- FAD is required to convert retinol (vitamin A) to retinoic acid via cytosolic retinal dehydrogenase
- Synthesis of an active form of folate (5-methyltetrahydrofolate) from 5,10-methylenetetrahydrofolate by Methylene tetrahydrofolate reductase is FADH₂ dependent
- FAD is required to convert tryptophan to niacin (vitamin B₃)
- Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) by Glutathione reductase is FAD dependent

For the molecular mechanism of action see main articles Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)

Food sources

Food and beverages that provide riboflavin without fortification are milk, cheese, eggs, leaf vegetables, liver, kidneys, legumes, mushrooms, and almonds.[23]

The milling of cereals results in considerable loss (up to 60%) of vitamin B₂, so white flour is enriched in some countries such as US by addition of the vitamin. The enrichment of bread and ready-to-eat breakfast cereals contributes significantly to the dietary supply of vitamin B₂. 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 flavin content of whole brown rice is retained if the rice is steamed (parboiled) 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.[24] 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.

Riboflavin is added to baby foods, breakfast cereals, pastas and vitamin-enriched meal replacement products. It is difficult to incorporate riboflavin into liquid products because it has poor solubility in water, hence the requirement for riboflavin-5'-phosphate (E101a), a more soluble form of riboflavin. Riboflavin is also used as a food coloring and as such is designated in Europe as the E number E101.[25]
Dietary reference intake

The Food and Nutrition Board of the U.S. Institute of Medicine updated Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) in 1998. The current EARs for riboflavin for women and men ages 14 and up are 0.9 mg/day and 1.1 mg/day, respectively; the RDAs are 1.1 and 1.3 mg/day. RDAs are higher than EARs so as to identify amounts that will cover people with higher than average requirements. RDA for pregnancy equals 1.4 mg/day. RDA for lactation equals 1.6 mg/day. For infants up to 12 months the Adequate Intake (AI) is 0.3-0.4 mg/day and for children ages 1–13 years the RDA increases with age from 0.5 to 0.9 mg/day. Collectively the EARs, RDAs and ULs (see Toxicity) are referred to as Dietary Reference Intakes.[18][26]

For U.S. food and dietary supplement labeling purposes the amount in a serving is expressed as a percent of Daily Value (%DV). For riboflavin labeling purposes 100% of the Daily Value was 1.7 mg, but as of May 2016 it has been revised to 1.3 mg. A table of the pre-change adult Daily Values is provided at Reference Daily Intake. Food and supplement companies have until July 2018 to comply with the change.

Deficiency

Signs and symptoms

Humans

Mild deficiencies can exceed 50% of the population in third world countries and in refugee situations. Deficiency is uncommon in the United States and in other countries that have wheat flour, bread, pasta, corn meal or rice enrichment regulations. In the U.S., starting in the 1940s, flour, corn meal and rice have been fortified with B vitamins as a means of restoring some of what is lost in milling, bleaching and other processing. For adults 20 and older, average intake from food and beverages is 1.8 mg/day for women and 2.5 mg/day for men. An estimated 23% consume a riboflavin-containing dietary supplement that provides on average 10 mg. The U.S. Department of Health and Human Services conducts National Health and Nutrition Examination Survey every two years and reports food results in a series of reports referred to as "What We Eat In America." From NHANES 2011–2012, the latest for which data has been reported, estimates are that 8% of women and 3% of men consume less than the RDA. When compared to the lower Estimated Average Requirements, fewer than 3% do not achieve the EAR level. However, anyone choosing a gluten-free or low gluten diet should as a precaution take a multi-vitamin/mineral dietary supplement which provides 100% DV for riboflavin and other B vitamins.

Riboflavin deficiency (also called ariboflavinosis) results in stomatitis including painful red tongue with sore throat, chapped and fissured lips (cheilosis), and inflammation of the corners of the mouth (angular stomatitis). There can be oily scaly skin rashes on the scrotum, vulva, philtrum of the lip, or the nasolabial folds. The eyes can become itchy, watery, bloodshot and sensitive to light.[27] Due to interference with iron absorption, even mild to moderate riboflavin deficiency results in an anemia with normal cell size and normal hemoglobin content (i.e. normochromic normocytic anemia). This is distinct from anemia caused by deficiency of folic acid (B9) or cyanocobalamin (B12), which causes anemia with large
blood cells (megaloblastic anemia). Deficiency of riboflavin during pregnancy can result in birth defects including congenital heart defects and limb deformities.

The stomatitis symptoms are similar to those seen in pellagra, which is caused by niacin (B3) deficiency. Therefore, riboflavin deficiency is sometimes called "pellagra sine pellagra" (pellagra without pellagra), because it causes stomatitis but not widespread peripheral skin lesions characteristic of niacin deficiency.

Riboflavin has been noted to prolong recovery from malaria, despite preventing growth of plasmodium (the malaria parasite).

Other animals

In other 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. Riboflavin deficiency in birds results in low egg hatch rates.

Diagnosis

Overt clinical signs are rarely seen among inhabitants of the developed countries. The assessment of Riboflavin status is essential for confirming cases with unspecific symptoms where deficiency is suspected.

- Glutathione reductase is a nicotinamide adenine dinucleotide phosphate (NADPH) and FAD-dependent enzyme, and the major flavoprotein 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 Bashor reported that a decrease in the intakes of riboflavin was associated with increase in EGR AC. In the UK study of Norwich elderly, 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.

- 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.
Causes

Riboflavin is continuously excreted in the urine of healthy individuals, making deficiency relatively common when dietary intake is insufficient. Riboflavin deficiency is usually found together with other nutrient deficiencies, particularly of other water-soluble 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. Subclinical deficiency has also been observed in women taking oral contraceptives, in the elderly, in people with eating disorders, chronic alcoholism and in diseases such as HIV, inflammatory bowel disease, diabetes and chronic heart disease. The Celiac Disease Foundation points out that a gluten-free diet may be low in riboflavin (and other nutrients) as enriched wheat flour and wheat foods (bread, pasta, cereals, etc.) is a major dietary contribution to total riboflavin intake. Phototherapy to treat jaundice in infants can cause increased degradation of riboflavin, leading to deficiency if not monitored closely.

Treatment

Treatment involves a diet which includes an adequate amount of riboflavin containing foods. Multi-vitamin and mineral dietary supplements often contain 100% of the Daily Value for riboflavin, and can be used by persons concerned about an inadequate diet. Over-the-counter dietary supplements are available in the United States with doses as high as 100 mg (5882% of Daily Value), but there is no evidence that these high doses have any additional benefit for healthy people.

Chemistry

As a chemical compound, riboflavin is a yellow-orange solid substance with poor solubility in water compared to other B vitamins. Visually, it imparts color to vitamin supplements (and bright yellow color to the urine of persons taking a lot of it).

History

Vitamin B was originally considered to have two components, a heat-labile vitamin B₁ and a heat-stable vitamin B₂. In the 1920s, vitamin B₂ 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 (which is now called biotin or vitamin B7). Since both pellagra and vitamin H deficiency were associated with dermatitis, Gyorgy decided to test the effect of vitamin B₂ on vitamin H deficiency in rats. He enlisted the service of Wagner-Jauregg in Kuhn's laboratory. 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, the researchers 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 Ovolavin. 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 B₂.
Name

The name "riboflavin" (often abbreviated to Rbf or RBF) comes from "ribose" (the sugar whose reduced form, ribitol, forms part of its structure) 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 along with the oxidized form, is colorless.