

Introduction

Riboflavin is one of the most widely distributed water-soluble vitamins. The above synonyms, lactoflavin and oboflavin, as well as the terms heptoflavin, verdoflavin and uroflavin, indicate the source from which the vitamin was originally isolated, i.e. milk, eggs, liver, plants and urine. The term “flavin” originates from the latin word “flavus” referring to the yellow colour of this vitamin. The fluorescent riboflavin is also part of the vitamin B-complex. In the body, riboflavin occurs primarily as an integral component of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Functions

Flavin coenzymes are essential for energy production via the respiratory chain, as they act as catalysts in the transfer of electrons in numerous essential oxidation-reduction reactions (redox reactions). They participate in many metabolic reactions of carbohydrates, fats and proteins. Riboflavin coenzymes are also essential for the conversion of pyridoxine (vitamin B₆) and folic acid into their coenzyme forms and for the transformation of tryptophan to niacin. Vitamin B₂ also promotes normal growth and assists in the synthesis of steroids, red blood cells, and glycogen. Furthermore, it helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system, and is involved in the production of adrenaline by the adrenal glands. Riboflavin is also important for the antioxidant status within cell systems, both by itself and as part of the glutathione reductase and xanthine oxidase system. This defence system may also help defend against bacterial infections and tumour cells.

Main functions in a nutshell:

- Oxidation-reduction reactions
- Energy production
- Antioxidant functions
- Conversion of pyridoxine (vitamin B₆) and folic acid into their active coenzyme forms
- Growth and reproduction
- Growth of skin, hair, and nails

Dietary sources

Riboflavin is present as an essential constituent of all living cells, and is therefore widely distributed. However, there are very few rich sources in food. Yeast and liver have the highest concentrations, but they do not have much relevance in today's human nutrition. The most important and common dietary sources are milk and milk products, lean meat, eggs and green leafy vegetables. Cereal grains, although poor sources of riboflavin, are important for those who rely on cereals as their main dietary component. Fortified cereals and bakery-products supply large amounts. Animal sources of riboflavin are better absorbed than vegetable

sources. In milk from cows, sheep and goats, at least 90% of the riboflavin is in the free form; in most other sources, it occurs bound to proteins.

Absorption and body stores

Most dietary riboflavin is consumed as a food protein with FMN and FAD. These are released in the stomach by acidification and absorbed in the upper part of the small intestine by an active, rapid, saturable transport mechanism. The rate of absorption is proportional to intake and increases when riboflavin is ingested along with other foods. So approximately 15% is absorbed if taken alone versus 60% absorption when taken with food. Passive diffusion plays only a minor role at the physiological doses ingested in the diet.

In the mucosal cells of the intestine, riboflavin is converted to the coenzyme form flavin mononucleotide (FMN). In the portal system it is bound to plasma albumin or to other proteins, mainly immunoglobulins, and transported to the liver, where it is converted to the other coenzyme form, FAD, and bound to specific proteins as flavoproteins.

Riboflavin, mainly as FAD, is distributed in all tissues, but concentrations are low and little is stored. The liver and retinal tissues are the main storage places. Riboflavin is excreted mainly in the urine where it contributes to the yellow colour. Small amounts are also excreted in sweat and bile. During lactation, about 10% of absorbed riboflavin passes into the milk.

Vitamin B₂ content of foods

Food	Vitamin B ₂ (mg/100g)
Brewer's yeast	3.7
Pork liver	3.2
Chicken breast	0.9
Wheat germ	0.7
Camembert/Parmesan	0.6
White mushrooms	0.4
Egg	0.3
Spinach	0.23
Milk/Yoghurt	0.18

(Souci, Fachmann, Kraut)

Measurement

Body status can be determined by direct and indirect methods. Direct methods include the determination of FAD and FMN in whole blood by HPLC (High Performance Liquid Chromatography). Usually, whole blood concentrations (FAD) of 175 - 475 nmol/L are measured. Another possibility for riboflavin status assessment is the monitoring of urinary excretion. Values < 27 µg/g creatinine point to deficiency, 27 - 79 µg/g creatinine are considered marginal, and values > 80 µg/g creatinine are considered normal. Urinary excretion rises sharply after tissue saturation is reached.

Indirect methods include determining the activity of the FAD dependent erythrocyte glutathion reductase (EGR). This biochemical method gives a valid indication of riboflavin status. During riboflavin deficiency EGR is no longer saturated with FAD, so enzyme activity increases when FAD is added in vitro. The difference in activity in erythrocytes

with and without added FAD is called the activity coefficient (EGRAC). An EGRAC >1.30 is indicative of biochemical riboflavin deficiency.

Stability

Because riboflavin is degraded by light, loss may be up to 50% if foods are left out in sunlight or any UV light. Because of this light sensitivity, riboflavin will rapidly disappear from milk kept in glass bottles exposed to the sun or bright daylight (85% within 2 hours).

Riboflavin is stable when heated and so is not easily destroyed in the ordinary processes of cooking, but it will leach into cooking water. The pasteurisation process causes milk to lose about 20% of its riboflavin content. Alkalis such as baking soda also destroy riboflavin. Sterilisation of foods by irradiation or treatment with ethylene oxide may also cause destruction of riboflavin.

Interactions

Positive interactions

Thyroxine and triiodothyroxine stimulate the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in mammalian systems. Anticholinergic drugs increase the absorption of riboflavin by allowing it to stay longer at absorption sites.

Negative interactions

Certain drugs have a negative influence on the absorption or metabolism of riboflavin:

- Ouabain (treatment of congestive heart failure), theophylline (muscle relaxant, diuretic, central nervous stimulant), and penicillin displace riboflavin from its binding protein, thus inhibiting transport to the central nervous system.
- Probenecid (anti-gout remedy) inhibits gastrointestinal absorption and renal tubular secretion of riboflavin.
- Chlorpromazine (anti-psychotic drug), barbiturates and possibly tricyclic antidepressants prevent the incorporation of riboflavin into FAD.
- Antibiotics: Riboflavin impairs the antibiotic activity of streptomycin, erythromycin, tyrothricin, carbomycin and tetracyclines, but no inactivation occurs with chloramphenicol, penicillin or neomycin.

Deficiency

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



B-complex vitamins. Along with other B-vitamins, low vitamin B₂ status has been associated with unfavourably increased plasma homocysteine levels. The absorption of iron, zinc and calcium is impaired in riboflavin deficiency.

Clinically, vitamin B₂-deficiency affects many organs and tissues. Most prominent are the effects on the skin, mucosa and eyes:

- glossitis (magenta tongue, geographical tongue)
- cheilosis, angular stomatitis (fissures at the corners of the mouth)
- sore throat
- burning of the lips, mouth, and tongue
- inflamed mucous membranes
- pruritus (itching)
- seborrheic dermatitis (moist scaly skin inflammation)
- corneal vascularisation associated with sensitivity to bright light, impaired vision, itching and a feeling of grittiness in the eyes

In severe long-term deficiency, damage to nerve tissue can cause depression and hysteria.

Other symptoms are normocytic and normochromic anaemia, and peripheral neuropathy of the extremities (tingling, coldness and pain). Low intracellular levels of flavin coenzymes could effect mitochondrial function, oxidative stress and blood vessel dilation, which have been associated with pre-eclampsia during pregnancy.

Groups at risk of deficiency

Individuals who have inadequate food intake are at risk of deficiency, particularly children from low socioeconomic backgrounds in developing countries, elderly people with poor diets, chronic 'dieters', and people who exclude milk products from their diet (vegans). Riboflavin deficiency may also occur as a result of:

- trauma, including burns and surgery
- chronic disorders (e.g. rheumatic fever, tuberculosis, subacute bacterial endocarditis, diabetes, hypothyroidism, liver cirrhosis)
- intestinal malabsorption, e.g. morbus crohn, sprue, lactose intolerance
- chronic medication (tranquillisers, oral-contraceptives, thyroid hormones, fibre-based laxatives, antibiotics)
- high physical activity
- phototherapy for newborns during icterus

The consequences of a low riboflavin intake may be aggravated

by chronic alcoholism and chronic stress. During pregnancy and lactation riboflavin requirement is increased.

Disease prevention and therapeutic use

Eye-related diseases

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

Current recommendations in the USA

RDA*

Infants	< 6 months	0.3mg (Adequate Intake, AI)
Infants	7-12 months	0.4mg (AI)
Children	1-3 years	0.5mg
Children	4-8 years	0.6mg
Children	9-13 years	0.9mg
Males	> 14 years	1.3mg
Females	14-18 years	1.0mg
Females	> 19 years	1.1mg
Pregnancy		1.4mg
Lactation		1.6mg

*The Dietary Reference Intakes (DRIs) are actually a set of four reference values: Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels, (UL) that have replaced the 1989 Recommended Dietary

Allowances (RDAs). The RDA was established as a nutritional norm for planning and assessing dietary intake, and represents intake levels of essential nutrients considered to meet adequately the known needs of practically all healthy people

Migraines

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

Prevention of deficiencies in high-risk patients

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

Recommended Dietary Allowance (RDA)

Dietary recommendations for riboflavin exist in many countries, where mean values for adult males vary between 1.2 and 2.2 mg daily. The recommendations of the Food and Nutrition Board of the US

National Research Council are based on feeding studies conducted in the 1940s, which showed that a riboflavin intake of 0.55 mg or less per day results in clinical signs of deficiency after about 90 days. These data have led to the assumption that an intake of 0.6 mg per 1000 kcal should supply the needs for essentially all healthy people.

Safety

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

Supplements and food fortification

Riboflavin is available as oral preparations, alone or most commonly in multivitamin and vitamin B-complex

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

Industrial production

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



History

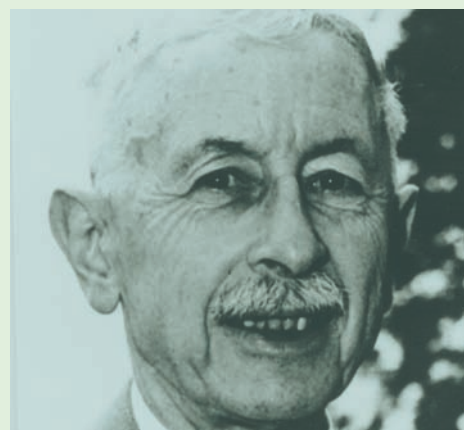
- 1879** Blyth isolates lactochrome – a water-soluble, yellow fluorescent material – from whey.
- 1932** Warburg and Christian extract a yellow enzyme from brewer's yeast and suggest that it plays an important part in cell respiration.
- 1933** Kuhn and coworkers obtain a crystalline yellow pigment with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



Otto Heinrich Warburg



Richard Kuhn



Paul Karrer