

Riboflavin^{1,2}

Riboflavin (vitamin B-2) was first isolated from milk whey in the late 1870s as the water-soluble, yellowish pigment called lactochrome. By the 1930s, lactochrome was structurally established as riboflavin, a heterocyclic isoalloxazine derivative with a ribitol side chain and the parent precursor to the coenzymes FMN or riboflavin 5'-phosphate and FAD. The majority of flavoproteins are capable of conducting oxidation and reduction reactions (redox) while ~10% of them participate in transferase, lyase, isomerase, and ligase reactions. The nature of the flavin nucleotides to undergo redox reactions governs its utility to participate in an eclectic array of cellular processes involving electron transport, metabolism of lipids, drugs, and xenobiotics, as well as cell signaling and protein folding. Further information on studies mentioned in this review is available in the references provided (1, 2).

Efflux of riboflavin into extracellular fluids is controlled by an ATP-binding cassette G2 transporter (ABCG2)³. This efflux protein was initially identified as the breast cancer resistance protein and belongs to a family of multidrug resistance proteins. In addition to its direct role in extruding drugs and xenobiotics, ABCG2 secretes riboflavin into breast milk and other extracellular fluids such as semen, cerebral spinal fluid, and bile. Expression of the ABCG2 protein is strongly induced during lactation, and expression is abated during weaning periods. Mature breast milk contains ~650 µg total flavins/L, with the majority being FAD (54%) and riboflavin (39%).

Activation of riboflavin into its physiologically important coenzymes requires an initial phosphorylation by flavokinase (ATP:riboflavin 5-phosphotransferase) to form FMN and a subsequent pyrophosphorylation with AMP catalyzed by FAD synthetase (ATP:FMN adenyl transferase). Both flavin biosynthetic enzymes are up-regulated by thyroid hormones in most mammalian cells.

In consideration of the major metabolic pathways that flavoenzymes influence, riboflavin deficiencies impact primarily on lipid metabolism. Flavin coenzymes function with transferases, dehydrogenases, oxidoreductases, monooxygenases, hydroxylases, and oxidases for reactions that desaturate essential fatty acids, form phospholipids and ether lipids, and synthesize sphingosine, cholesterol, and steroid hormones. Individuals with marginal degrees of riboflavin deficiency experience skin dyscrasias characteristic of those observed during essential fatty acid deficiencies. Hepatic mitochondrial fatty acid oxidation is markedly compromised in riboflavin deficiency (1).

Riboflavin plays a central role in controlling the 2-electron acceptor/donor and 1-electron acceptor/donor complexes within the electron transport chain. Embedded within the

inner membrane of the mitochondria are 4 complexes (numbered I–IV) that consist of several different electron carriers. Complexes I and II contain flavoprotein reductases (dehydrogenases) and electron transferring flavoproteins, whereas complexes III and IV are composed of a ubiquinone (coenzyme Q₁₀) component and a variety of cytochromes, respectively. Studies show that abnormalities within complex I correlate highly with mitochondrial disorders manifesting predominantly as human neurodegenerative diseases (3).

Riboflavin deficiency compromises oxidant defense mechanisms by interfering with the maintenance of reduced glutathione (GSH), the master antioxidant within cells. When exposed to oxidants, GSH is subsequently oxidized to its disulfide form. The intracellular ratio of GSH:oxidized glutathione (≥100:1) is maintained by an FAD-dependent glutathione reductase. Diminished glutathione reductase activity diminishes GSH that serves as a substrate for glutathione peroxidase and glutathione S-transferase. Thus, riboflavin nutritional status impacts directly on maintaining lipid metabolism, energy metabolism, redox balance, and metabolizing drugs and xenobiotic substances (2).

Deficiencies

Dietary intake information derived originally from the NHANES and the second NHANES 2003–2006 indicated little-to-no concern for inadequate riboflavin consumption (4). Later studies corroborated these findings, showing that 0% of 2- to 8-y-old children, 5% of 14- to 18-y-old girls, and 2% of adults ≥19 y had dietary intakes of riboflavin marginally below their estimated average requirement.

Interference with intestinal transport that includes digestive and absorptive disorders and bowel resection can lead to the development of suboptimal status or frank deficiency and eventual clinical abnormalities. Persistent riboflavin deficiency is observed in patients with Brown-Vialetto-Van Laere (BVVL) and Fazio-Londe syndromes. The phenotype of BVVL syndrome is characterized by hearing loss and sensory ataxia followed by progressive upper limb weakness, optic atrophy, bulbar weakness, and respiratory failure. Both BVVL and Fazio-Londe syndromes are rare congenital defects of the riboflavin transporter gene and exhibit overlapping clinical features except that sensorineural hearing loss is not observed in Fazio-Londe syndrome.

Aside from these rare genetic defects and malabsorptive conditions, isolated deficiencies of riboflavin are not widely prevalent in the general population. Although textbooks label the physical and clinical symptoms as unique features of riboflavin

³Abbreviations used: ABCG2, ATP-binding cassette G2 family member transporter; BVVL, Brown-Vialetto-Van Laere; GSH, reduced glutathione.

deficiency, in actuality none is pathognomonic of riboflavin deprivation. The classic glossitis, angular stomatitis, cheilosis, and dermatitis observed in advanced cases of riboflavin deficiency may be due to other vitamin deficiencies as well. In fact, when a deficiency of riboflavin does occur, it is almost invariably in association with multiple nutrient deficits.

Primary deficiencies and diminished intestinal transport are not the only causes of riboflavin deficiency. Endocrine abnormalities (aldosterone and thyroid hormone insufficiency), specific drugs (tricyclic antidepressants and tetracyclic antibiotics), and ethanol abuse may interfere significantly with riboflavin utilization (1).

Diet Recommendations

The estimated average requirement and RDA for riboflavin that cover men and women between the ages of 19 and 70 y are 0.9–1.1 and 1.1–1.3 mg/d, respectively (5). These values are based on observing clinical evidence of deficiency with intakes of <0.6 mg/d and measuring normal erythrocyte glutathione reductase activation coefficient values with dietary intakes of ~1 mg/d. RDAs for riboflavin in children ages 1–18 y are based on extrapolated data from adult values after compensating for growth and metabolic differences. Accordingly, RDAs for riboflavin in children 1–9 y and adolescents 10–18 y range from 0.5 to 0.6 mg/d and from 0.9 to 1.3 mg/d, respectively. Adequate Intakes of riboflavin for infants 0–12 mo of age are based on mean volume (0.78 L/d) of milk consumed. Thus, Adequate Intake for infants 0–12 mo is 0.3–0.4 mg/d.

Food Sources

Primary dietary forms of riboflavin from natural sources are FMN and FAD. Rich sources of total riboflavin include plant foods as well as animal sources, namely organ meats, poultry, fish, and eggs; dairy products (milk and cheese) offer a rich source of the parent compound, riboflavin, which contributes significantly to the RDA for children and adult populations. Plant sources, such as cereals, grain products, and breads provide nearly the entire dietary riboflavin intake of some developing countries. Green vegetables, such as broccoli, collard greens, and turnips, are moderately good sources of riboflavin. Natural grain products tend to be relatively low in riboflavin, but when they are fortified or enriched, these food items increase riboflavin bioavailability.

High quality, protein-rich foods are excellent sources not only of riboflavin but B vitamins in general. Flavoenzymes catalyze a diverse number of reactions that interact metabolically with other B vitamin-dependent enzymes present in plant and animal food sources. Thus, it is not surprising that if an individual's diet is inadequate in riboflavin, it is very likely to be inadequate in other vitamins as well. Thus, a primary deficiency of dietary riboflavin has wide implications for efficacy of other vitamins, because flavoenzymes are directly linked to metabolism of both fat- and water-soluble vitamins, namely, vitamin B-12 (cobalamin), folic acid, niacin, pyridoxine, vitamin K, and vitamin D.

The bioavailability of riboflavin can vary with methods of food processing. Thus, blanching, milling, fermenting, and extruding can result in physical removal of the vitamin. Large amounts of riboflavin are lost during sun-drying of fruits and vegetables because riboflavin photo-oxidizes in the presence of UV light. The precise magnitude of loss varies with the duration and intensity of exposure. In a similar fashion, prolonged storage of milk in clear bottles can result in riboflavin degradation. Opaque plastic or cardboard containers provide modest protection of milk that is stored on a grocery shelf exposed to continuous fluorescent lighting. Thus, milk and milk products should be protected against UV and fluorescent lighting; otherwise significant amounts of riboflavin as well as vitamin A (retinol), which is also susceptible to UV light, will be lost, and food quality will deteriorate (6).

By contrast to the degradation of riboflavin in the presence of UV light, the extent of degradation in irradiated foods varies considerably. Riboflavin in foods is relatively stable to thermal processing, microwaving, or exposure to infrared radiation. Such heating methods do not impact as much as stovetop cooking, because heating is performed more evenly and for shorter periods of time. Foods cooked at elevated temperatures and/or under slightly acidic conditions experience increased dissociation of flavin complexes. In addition, the practice of adding sodium bicarbonate (baking soda) to accentuate the green color of vegetables can result in accelerated photo-degradation of riboflavin. When flavins are associated with proteins, intramolecular associations with aromatic amino acids help stabilize the isoalloxazine ring to make it less susceptible to photolysis.

Clinical Uses

The photoreactive properties of riboflavin have been exploited for clinical use under controlled conditions such as reducing the presence of pathogens in blood products and treating progression of corneal disorders such as keratoconus.

Photosensitization and blood-borne pathogens

UV light-induced activation of riboflavin can selectively damage pathogen DNA and RNA and reduce replication of viruses, bacteria, and protozoa in blood products. Under controlled conditions, riboflavin can activate leukocytes without significantly compromising the efficacy of blood products or result in product loss. Riboflavin exhibits clinical utility in pathogen reduction technology and illustrates the need to assess the specific conditions under which phototherapy with riboflavin are efficacious in association with safety issues for its use. Thus, controlling the formation of excited states of riboflavin and channeling reactive oxygen species toward micro-organisms and viruses also holds promise in disease prevention and treatment of cancer. These and other considerations involving riboflavin and light have been extended to food safety and remain a subject in need of further investigation.

Photosensitization and treatment of corneal disorders (keratoconus)

In contrast to the use of high-energy UV wavelengths for pathogen reduction in blood products and for safety considerations, higher and less energetic wavelengths are required for direct photo-irradiation in the eye. Riboflavin administration followed by UV-A treatment slows or stops progression of the corneal disorder, keratoconus. Treatment of keratoconus with riboflavin and UV light increases corneal stiffness by increasing collagen cross-linking and, in this manner, appears to inhibit progression of the disorder.

Toxicity

The level of riboflavin consumed orally from the diet or from most multivitamin supplements rarely causes side effects or exhibits toxicity. Riboflavin that is not converted to FMN or FAD can exist as free riboflavin and be excreted by the kidney, causing yellow-colored urine, or gets secreted into extracellular fluids via the ABCG2 transporter.

Repeatedly consumed pharmacologic doses (>100 mg) have the potential to react with light, which can have adverse cellular effects. The photoreactive properties of the isoalloxazine ring cause free riboflavin to become a strong oxidizer by producing potentially toxic peroxides or other reactive oxygen species and/or forming an atypical tryptophan metabolite. The tryptophan-riboflavin adduct has been shown to exhibit hepato- and cytotoxic effects and to be particularly detrimental to lens proteins and the retina, which are permanently exposed to light.

Recent Research

Interest in the relation between riboflavin and stroke has focused on the role that riboflavin plays in determining circulating concentrations of homocysteine, a risk factor for cardiovascular disease. Acute stroke patients, evaluated immediately after infarction, were biochemically deficient of riboflavin. These studies suggest that riboflavin treatment can decrease brain infarct area induced by middle cerebral artery occlusion compared with sham-operated animals (7). Thus, riboflavin may inhibit progression of stroke and protect brain tissue against ischemic injury. Studies that make use of primary cultured neurons have shown that riboflavin administration can decrease oxygen-glucose deprivation-induced apoptosis and cell death by a mechanism that blocks expression of pro-apoptotic Bax protein. If a relation between riboflavin and stroke could be confirmed and mechanisms

clarified, riboflavin supplementation may provide a novel therapeutic strategy for stroke.

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John T Pinto*

Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY

Janos Zempleni

Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE

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*To whom correspondence should be addressed. E-mail: john_pinto@nymc.edu.

References

1. Pinto JT, Rivlin RS. Riboflavin. In: Zempleni J, Suttie JW, Gregory J III, Stover PI, editors. Handbook of vitamins. 5th ed. New York: Taylor and Francis; 2014. p. 191–265.
2. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* 2003;77:1352–60.
3. Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, Hertz-Picciotto I, Tassone F, Pessah IN. 2010. Mitochondrial dysfunction in autism. *JAMA* 304:2389–96.
4. Curtin LR, Mohadjer LK, Dohrmann SM, Montaquila JM, Kruszan-Moran D, Mirel LB, Carroll MD, Hirsch R, Schober S, Johnson CL. The National Health and Nutrition Examination Survey: Sample Design, 1999–2006. *Vital Health Stat* 2;2012:1–39.
5. Institute of Medicine. Dietary reference intakes: guiding principles for nutrition labeling and fortification. Food and Nutrition Board, Washington (DC): The National Academies Press; 2003.
6. Mestdagh F, De Meulenaer B, De Clippelaar J, Devlieghere F, Huyghebaert A. Protective influence of several packaging materials on light oxidation of milk. *J Dairy Sci* 2005;88:499–510.
7. Zou YX, Zhang XH, Su FY, Liu X. Importance of riboflavin kinase in the pathogenesis of stroke. *CNS Neurosci Ther* 2012;18(10):834–40.