Riboflavin

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Importance of riboflavin for health

Riboflavin (vitamin B2) is the key building block for its co-enzymatic forms flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which serve as electron carriers in various redox reactions in energy production and metabolic pathways (1, 2):

- Carbohydrate, lipids, and protein metabolism
- Electron transport (respiratory) chain
- Metabolism of drugs and toxins (in conjunction with cytochrome P-450)
- Antioxidant functions (glutathione reductase, glutathione peroxidase, xanthine oxidase)

Humans are neither capable of *in vivo* riboflavin synthesis nor efficient in riboflavin storage, requiring a steady supply through dietary intake (3).

Absorption is mainly occurring in the proximal small intestine by an active, carrier-mediated, saturable transport process (2). Riboflavin metabolism is closely regulated by riboflavin status of the individual. Conversion of riboflavin to its co-enzymatic forms occurs within the cellular cytoplasm of many tissues, but mostly in the small intestine, liver, heart, and kidney (4). An ATP-dependent phosphorylation transform riboflavin into FMN, which subsequently is complexed with certain apoenzymes to various flavoproteins. However, most FMN is phosphorylated to FAD, which is

therefore the main form of vitamin B2 in body tissue (2, 4). In plasma, riboflavin, FMN, and FAD are found, all associated with plasma proteins such as albumin (5).

Milk, dairy products, eggs, and (organ) meat are a major source of dietary riboflavin, but most plant- and animal derived foods contain at least small amounts of this vitamin (1, 6). Approximately 95% of riboflavin in foods is present as FAD and FMN. Its bioavailability (for all vitamers) is estimated to a maximum of 27mg per meal or dose (6). Being a water-soluble vitamin, riboflavin losses are about twice as high when foods are boiled in water compared to other food processing such as steaming or microwaving (6).

Nutrient Interactions

Vitamin B2 as flavoproteins are involved in the metabolism of several other vitamins, such as niacin, vitamin B6, and folate, but also other nutrients (1, 2):

Nutrient	**Function of riboflavin**
Niacin/tryptophan	Kynurenine mono-oxygenase (NAD, NADP synthesis from tryptophan)
Vitamin B6	Pyridoxine 5'-phosphate oxidase (PPO; converts the most naturally available vitamin B6 into its co-enzymatic form, PLP)
Folate	MTHFR (FAD-dependent enzyme for maintaining a specific folate co-enzyme required for methionine formation from homocysteine
Vitamin B12	Methionine synthase (FAD-dependent; synthesis of methyl-cobalamin)
Iron	Riboflavin may impair iron absorption, increase intestinal loss of iron, or impair iron utilization for haemoglobin synthesis

MTHFR: 5,10-methylene-tetrahydrofolate reductase; PLP: pyridoxal 5'-phosphate;

Risks of deficiency:

Clinical riboflavin deficiency is called ariboflavinosis and is usually found in combination with other deficiencies of other water-soluble vitamins due to decreasing levels of flavin co-enzymes. Signs of deficiency include endocrine abnormalities, skin disorders, hyperaemia and oedema of the mouth and throat, angular stomatitis, cheilosis, hair loss, reproductive problems, sore throat, itchy and red eyes, and degeneration of liver and nervous system. In severe and prolonged states of deficiency, anaemia and cataracts can occur. Riboflavin deficiency has also been associated with preeclampsia in pregnant women (1, 4, 6).

Groups at risk for riboflavin deficiency include vegetarian athletes due to increased stress in the riboflavin-dependent metabolic pathways, pregnant and lactation women and their infants, vegans,

alcoholics or anorexic individuals (1, 6). However, riboflavin supplementation may positively affect migraine headaches and may aid in the prevention of DNA damage caused by carcinogens (6).

Risks of excess:

There is no evidence of adverse effects of excess riboflavin intake. However, given the little data available, the Food and Nutrition Board advises to be cautious about excess riboflavin consumption since adverse effects may still occur. High-dose riboflavin therapy can intensify the yellow colour of urine, which is a harmless side-effect (1, 6).

Human biomarkers for measuring riboflavin intake and status

Riboflavin status can be assessed by different methods (4, 5, 7):

Biomarker	Analysis type	Sample	Benefits	Intricacies
EGRAC	Indirect analysis	RBC	Only a marker of B2 deficiency	Does not reflect status, fresh RBC are needed
FAD	Direct analysis	RBC (plasma)	Reflects status (reflects intake)	Does not reflect intake (status)
Urinary flavin	Direct analysis	urine	Reflects recent intake	Not a marker for low B2 levels
EPPOA	Indirect analysis	plasma	Suitable for G6PD deficient individuals	Not readily available

EGRAC: erythrocyte glutathione reductase activity coefficient; EPPOA: erythrocyte pyridoxine phosphate oxidase activity FAD: Flavin adenine dinucleotide; RBC: red blood cells

Erythrocyte glutathione reductase activity coefficient (EGRAC)

This assay has been commonly used to determine riboflavin adequacy. The enzyme activity of the erythrocyte glutathione reductase is measured before and after exposure to FAD. The results are expressed as EGRAC; an EGRAC of 1.0 indicates no stimulation by FAD due to more than adequate riboflavin status (4, 7). The IOM suggest the following interpretation of EGRAC: <1.2 is acceptable, 1.2-1.4 is low, >1.4 is deficient. This assay cannot be used for individuals with glucose 6-phosphate dehydrogenase (G6PD) deficiency due to the resulting increased avidity of EGR towards FAD (4).

Method: Erythrocyte Glutathione Reductase Activity Coefficient (EGRAC), see reference (9).

FAD analysis

Vitamin B2 vitamers (riboflavin, FMN, FAD) can be simultaneously analysed using chromatographic techniques, such as HPLC with fluorescence detection, or LC-MS. Riboflavin in plasma is accepted as general clinical analysis of status and supplementation monitoring (7).

Method: Riboflavin metabolites in plasma by HPLC-FLD, see reference (4); or by LC-MS, see reference (10).

Urinary vitamin B2

Status can be assessed by analysis of urinary excretion in a random 24h specimen, expressed as either total riboflavin excreted or in relation to creatinine excretion (5). Since little riboflavin is stored in the body, urine measurements are a good proxy for dietary intake (7).

Method: Riboflavin in urine, see reference (11).

Erythrocyte pyridoxine phosphate oxidase activity (EPPOA)

This assay has been described for plasma samples and appears to be suitable for population with high prevalence of G6PD deficiency (5, 8). However, isolation and purification of the riboflavin-apoprotein prior to analysis is required (8).

Method: Erythrocyte pyridoxine phosphate oxidase activity (EPPOA), see reference (8)

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Vitamin B2, plasma: http://ltd.aruplab.com/Tests/Pub/0081123

Alternatively, please see the OpeN-Global page on laboratory accreditation: https://open-global.kcl.ac.uk/accreditation/

Technical assistance

For questions on methods of riboflavin assessment or for technical assistance, please contact the OpeN-Global team at https://open-global.kcl.ac.uk/contact/ or write to:

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Useful links

US National Academies Press IOM Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline, 2000: http://nationalacademies.org/hmd/reports/2000/dietary-reference-intakes-for-thiamin-riboflavin-niacin-vitamin-b6-folate-vitamin-b12-pantothenic-acid-biotin-and-choline.aspx

Linus Pauling Institute Micronutrient Information Center (Oregon State University): https://lpi.oregonstate.edu/mic/vitamins/riboflavin

NIH Health Information: https://ods.od.nih.gov/factsheets/Riboflavin-HealthProfessional/#h3

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