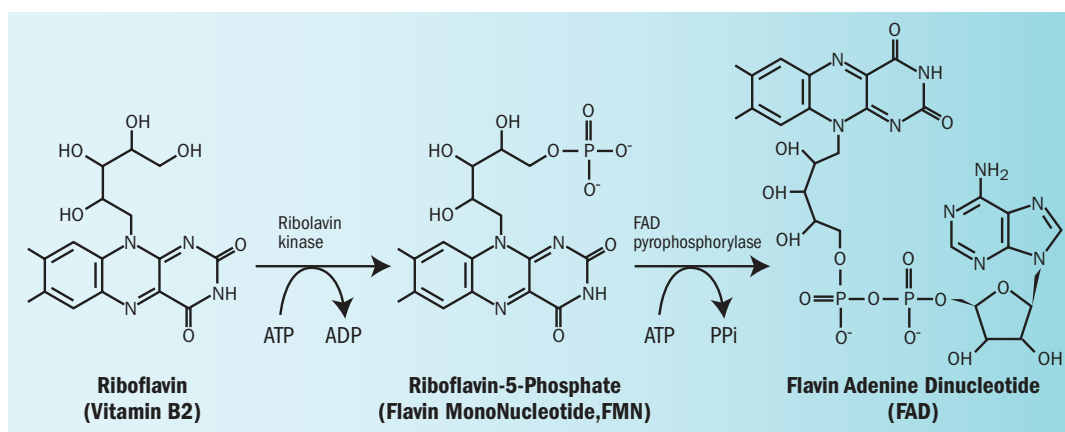


# Riboflavin

Figure 1. Conversion of Riboflavin to the Active Coenzyme Forms



## Introduction

Riboflavin was originally recognized as a growth factor in 1879 and named vitamin B<sub>2</sub> according to the British nomenclature system. It was first isolated from egg whites in 1934 and synthesized in 1935.<sup>1,2</sup> Riboflavin fluoresces yellow-orange and gives the yellow-white hue to egg whites and milk. Riboflavin has two active coenzyme forms, riboflavin 5'-phosphate (R5P; flavin mononucleotide [FMN]) and flavin adenine dinucleotide (FAD). Dietary sources include milk, eggs, meats, yogurt, broccoli, almonds, cheese, soy, fortified grains, and dark green vegetables, in descending order of concentration.<sup>3</sup> Normal colonic bacteria synthesize riboflavin, contributing to a soluble pool of the vitamin that can be utilized in addition to dietary intake.<sup>2</sup>

As one of the family of B vitamins, riboflavin contributes to cellular growth, enzyme function, and energy production. FAD is a cofactor in many reactions of intermediary metabolism, such as carbohydrate, fat, and amino acid synthesis; FAD and R5P are also necessary for the activation of other vitamins and enzyme systems. Folate and pyridoxine are vitamins that rely on riboflavin for activation. Clinically, riboflavin has several applications due to its ubiquitous nature in metabolism. Research supports the use of riboflavin in anemia, cataracts, hyperhomocysteinemia, migraine prophylaxis, and alcoholism. A riboflavin deficiency can result in angular stomatitis, seborrhea, glossitis, neuropathy, and anemia.

## Biochemistry

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) consists of a conjugated isoalloxazine ring (flavin) and a five-carbon carbohydrate, ribitol. It is weakly soluble in water and acid, and is heat stable.<sup>1,4</sup> Alkalinity and photo-oxidation cause destruction of riboflavin, while improper storage of milk, eggs, and cooked vegetables can result in loss of riboflavin from food.<sup>1,3</sup>

Table 1. Common Flavoprotein Reactions

Enzyme	Cofactor	Function
Dihydrolipoyl dehydrogenase	FAD	Energy metabolism
Fatty acyl-CoA dehydrogenase	FAD	Fatty acid oxidation
Succinate dehydrogenase	FAD	Krebs cycle
NADH dehydrogenase	FMN	Respiratory chain
Xanthine dehydrogenase	FAD	Purine catabolism
Glutathione reductase	FAD	Reduction of GSSG to 2GSH
Methylene-H <sub>4</sub> folate reductase	FAD	5-Methyl-ethyl-tetrahydrofolate
Sphinganine oxidase	FAD	Sphingosine synthesis
Pyridoxine phosphate oxidase	FMN	Vitamin B6 metabolism
Monoamine oxidase	FAD	Metabolism of neurotransmitters

The active forms of riboflavin are synthesized in the mitochondria, forming R5P/FMN (referred to as FMN in the remainder of the monograph) and FAD through phosphorylation (Figure 1). Riboflavin is phosphorylated to form FMN by flavokinase and ATP. The conversion of FMN to FAD is catalyzed by FAD pyrophosphorylase and ATP.

FMN and FAD coenzymes are structurally different, but participate in similar oxidative-phosphorylation reactions at the cellular level. A unique feature of FAD and FMN is their function as prosthetic groups in many enzyme systems and as catalysts of oxidation-reduction reactions. The function of a prosthetic group is to prevent reoxidation of the apoenzyme. The reduced forms of FMN and FAD are FMNH<sub>2</sub> and FADH<sub>2</sub>, which oxidize rapidly in solutions that contain oxygen. Unlike other coenzymes, they carry out one- or two-electron transfers. The oxidation-reduction versatility of the flavins is metabolically significant in reactions involving carbohydrates, lipids, amino acids, and the electron transport chain. Table 1 summarizes the common reactions involving FAD and FMN.

## Pharmacokinetics

Absorption of riboflavin occurs primarily in the proximal small intestine. Recent studies show it is necessary for FAD and FMN to be converted to riboflavin by brush border phosphatases.<sup>5,6</sup> Absorption of riboflavin also occurs in the large intestine as a result of bacterial synthesis. An *in vitro* study by Said et al revealed a carrier-mediated mechanism regulated by a calcium/calmodulin pathway within the large intestine.<sup>5,7</sup> Absorption of dietary riboflavin occurs through a saturable, intestinal transport mechanism that proceeds through Michaelis-Menten type reaction kinetics.<sup>8</sup> Dosages over 20 mg have been shown to exceed the carrier-mediated process, with half-life absorption rates of 1.1 hours.<sup>7,9</sup> Absorption is increased with food and delayed on an empty stomach. A decrease in absorption can be seen with obstructive biliary disease, hepatitis, and cirrhosis. The colonic uptake of riboflavin is an adaptive absorptive mechanism dependent on the concentration within the lumen and the number of receptors in brush border enterocytes, and is regulated by an energy-dependent transport system.<sup>5</sup>

Excretion proceeds through glomerular filtration and net kidney tubular secretion rates that increase linearly with increasing doses.<sup>10</sup> Studies show dependence on glomerular filtration rates with no differences

between men and women.<sup>7</sup> The predominant flavin excreted in the urine is riboflavin, with 30-40 percent of the remaining flavins represented by 7-alpha-hydroxy-riboflavin, 10-hydroxyethylflavin, and lumiflavin. These metabolites are produced by tissue oxidation and microbial degradation.<sup>7,10</sup>

A recent study of the pharmacokinetics of riboflavin revealed a circadian rhythm of plasma and urinary excretion that seems to be connected to the circadian rhythms of thyroxine and thyrotrophin.<sup>7,11</sup> The synthesis of flavoenzymes is dependent on thyroid hormone synthesis within tissues, but independent of hormone synthesis in the plasma, as concentrations remain fairly constant over time.

Most riboflavin in food is FAD, with a small proportion of free-form riboflavin and FMN comprising the remainder. FAD and FMN are usually found non-covalently bound to enzymes and must be dephosphorylated prior to absorption.

Absorption studies demonstrate riboflavin bioavailability is not affected by different forms of dietary riboflavin.<sup>9</sup> Dainty et al designed a study to determine bioavailability based on the appearance of stable radioisotopes in the urine and plasma of healthy subjects. Results from this experiment demonstrate that free-form riboflavin (found in high amounts in milk and eggs) is more rapidly absorbed than FMN from spinach, due to conversion time of FMN to riboflavin in the gut wall; however, the slower absorption rate did not impact bioavailability.<sup>9</sup>

## Mechanism of Action

Riboflavin and its active coenzymes function as hydrogen carriers in oxidation-reduction reactions. FMN and FAD are tightly bound as prosthetic groups in flavoproteins that mediate electron transfers at multiple points of intermediary metabolism.<sup>2</sup> Flavoenzymes, flavoproteins, and metalloproteins are examples of substrates that use FAD as a cofactor to drive reactions. Examples include amino acid oxidases, xanthine oxidases, beta oxidation of lipids, and dehydrogenase reactions in the citric acid cycle. Riboflavin plays a role in erythropoiesis, epinephrine and norepinephrine catabolism, gluconeogenesis, activation of pyridoxine, conversion of folate to 5-methyltetrahydrofolate (5-MTHF), and conversion of tryptophan to niacin.<sup>1,2,4,7</sup>

## Deficiency States

A riboflavin deficiency is defined as ariboflavinosis. The signs associated with a deficiency include cheilosis, angular stomatitis, glossitis, scrotal and vulvar dermatitis, seborrheic dermatitis, keratitis, and ocular changes. Severe deficiency states are associated with normochromic, normocytic anemia and neuropathy. Clinical signs and symptoms of deficiency develop after inadequate dietary riboflavin for 3-8 months. Riboflavin deficiency, noted worldwide, is prevalent in underdeveloped countries. In the United States, riboflavin deficiency is common in pregnancy, infancy, and the elderly.<sup>6</sup> Rates of deficiency correlate with decreased dietary intake of meat and dairy products.

## Clinical Indications

### Anemia

Riboflavin plays a role in erythropoiesis, improves iron absorption, and aids the mobilization of ferritin from tissues.<sup>6,12</sup> Early clinical studies of anemia showed a positive response to riboflavin supplementation.<sup>13,14</sup>

A more recent study by Fairweather-Tait et al determined that riboflavin deficiency interferes with iron utilization, but not absorption.<sup>15</sup> In this study, 37 men were selected at random from three villages in Gambia; the group had a riboflavin deficiency and hemoglobin levels less than 11.5 g/dL. The treatment group received 10 mg riboflavin six days per week, for four weeks, while a second group received no riboflavin; all subjects received a low dose of 3.38 mg iron in a 100-mL cola drink. Riboflavin status was determined by measuring the enzymatic activity of glutathione reductase in erythrocytes (EGRAC). Riboflavin supplementation resulted in decreased EGRAC and increased hemoglobin concentrations, with no differences in packed cell volume or ferritin levels. The authors concluded that a riboflavin deficiency interferes with iron utilization and the observed anemia was not due to a lack of iron but to impairment of hemoglobin synthesis. The study revealed that, as hemoglobin concentration increased with riboflavin supplementation, there was no subsequent change in iron absorption.

Animal studies correlate riboflavin deficiency with decreased iron absorption and increased iron loss, and conflict with current human studies.<sup>16</sup> Further human studies are needed to determine iron absorption and iron loss associated with riboflavin deficiency.

## Alcoholism

Early nutritional studies revealed an association between chronic alcoholism and nutrient deficiencies, including protein, essential fatty acids, B vitamins, and antioxidants.<sup>17</sup> Clinically, however, overt deficiencies are uncommon due to B-vitamin enrichment of bread and flour. Improved economic status and food availability probably contribute to the low number of patients in treatment facilities with frank deficiencies.<sup>18</sup>

Alcohol diminishes the bioavailability of riboflavin and impairs the transport of FAD across the epithelial layer within the small intestine. *In vitro* studies demonstrate alcohol inhibits FAD pyrophosphatase and FMN phosphatase.<sup>19</sup> Riboflavin deficiencies in alcoholics can affect skin health, decrease metabolism of xenobiotics, disturb lipid metabolism, and interfere with energy utilization.<sup>20</sup> Vitamin deficiencies in alcoholics are commonly associated with decreased food intake, decreased absorption, and disturbances in metabolism, which makes supplementation with riboflavin and other B vitamins a necessary part of the treatment protocol.

## Cataracts

Riboflavin deficiency is implicated in the formation of cataracts due to the concentration of reduced glutathione in the lens and its ability to protect the tissue from oxidative damage.<sup>21</sup> Glutathione reductase, the enzyme responsible for the production of glutathione, is decreased in cataracts,<sup>22</sup> and decreased enzymatic activity of glutathione reductase is associated with riboflavin deficiency.

In a study examining the B-vitamin status of cataract patients, a comparison of 37 patients with 16 age-matched controls revealed that 80 percent of the cataract patients had a riboflavin deficiency compared to 12.5 percent of the control group.<sup>23</sup> Further reports by the Lens Opacities Case-Control Study support a link between low levels of riboflavin and cataracts, as determined by EGRAC.<sup>24</sup>

A large, randomized, double-blind, controlled trial (RCT) in China studied nutrient effects on cataract formation in 12,141 individuals, ages 45-74, given a multivitamin/mineral or placebo for 5-6 years. A statistically significant, 36-percent reduction of cataracts was noted in the 65- to 74-year age group. The second part of the study measured risk of cataract formation against vitamin/mineral combinations. Combinations

of retinol/zinc, riboflavin/niacin, ascorbic acid/molybdenum, and selenium/alpha-tocopherol/beta-carotene were given to 23,249 study participants. The most protective effect was noted in the 65- to 74-year age group taking riboflavin (3 mg)/niacin (940 mg) daily.<sup>25</sup>

Additional support for the connection between riboflavin and cataracts comes from the University of Georgia where a series of case reports reveal improvement in lens opacity with 15 mg riboflavin daily.<sup>26</sup>

In contrast, a prospective 1992 study analyzing the association between dietary intake of vitamin C, vitamin E, carotenes, and riboflavin and cataract extraction over eight years in nurses ages 45-67 concluded that only increased intakes of vitamin A and carotenes are inversely associated with cataract formation.<sup>27</sup> No dietary correlations were noted between vitamins C or E or riboflavin and cataract formation according to Hankinson et al.

Although Skalka and Prchal found no correlation between riboflavin deficiency and early cataract formation, they did report a relationship between riboflavin deficiency and late-stage cataract formation.<sup>28</sup> They conclude that riboflavin deficiency in the general public does not appear to be cataractogenic, although they did find an association between riboflavin deficiency and cataract formation in the elderly.

In summary, riboflavin does appear to play an essential role in prevention of cataract formation. Riboflavin acts as a cofactor for glutathione reductase, is linked to cataract formation by decreased glutathione levels in the lens, and cataract formation increases in the elderly with riboflavin deficiencies. Further study is warranted to determine the exact relationship between riboflavin deficiency and risk for cataract formation.

## Hyperhomocysteinemia

Several studies report a link between homocysteine levels and cardiovascular disease.<sup>29-31</sup> Vitamins B<sub>6</sub> and B<sub>12</sub>, folate, and riboflavin play an important role in homocysteine homeostasis. There are two pathways that govern homocysteine metabolism – transsulfuration and remethylation. Transsulfuration is dependent on vitamin B<sub>6</sub> and catabolizes homocysteine to cysteine, while remethylation of homocysteine to methionine is dependent on vitamin B<sub>12</sub>, folate, and riboflavin.<sup>32</sup>

The Framingham Offspring Cohort Study and Hustad et al report riboflavin modulates plasma

homocysteine concentrations in healthy adults.<sup>33,34</sup> It is proposed that the link to hyperhomocysteinemia is through FAD and methylenetetrahydrofolate reductase. FAD is the cofactor necessary to activate folate for homocysteine methylation. A study by McNulty et al reveals that high plasma concentrations of homocysteine are linked to an enzyme variant of methylenetetrahydrofolate reductase (MTHFR), which is represented in 5-30 percent of the population.<sup>35</sup> This study observed reduced activity of the thermolabile MTHFR variant with increased plasma total homocysteine and decreased riboflavin status. In a cross-sectional analysis, 286 healthy subjects, ages 19-63, were examined for EGRAC, MTHFR genotype, and homocysteine levels. The study found that higher total homocysteine concentrations are associated with homozygosity for the 677 -> T variant of MTHFR and poor riboflavin status. The authors conclude that this study supports the use of riboflavin and other vitamin fortifications in the prevention and treatment of cardiovascular disease.<sup>35</sup>

### Migraines

Studies indicate a potential role for riboflavin in migraine prophylaxis.<sup>36,37</sup> Sandor et al suggest a mitochondrial deficit in energy metabolism could play a role in the pathophysiology of migraine headaches.<sup>38</sup> They studied the effects of riboflavin and beta-blockers on the intensity dependence of auditory evoked cortical potentials. Although no improvement in cortical potentials was seen with riboflavin, an overall clinical improvement was noted. The authors conclude that beta-blockers improved the intensity dependence of auditory evoked cortical potentials, and the mechanism of action of riboflavin is distinct from that of beta-blockers in migraine prophylaxis.

Schoenen and Lenaerts conducted an RCT of riboflavin for migraine prophylaxis.<sup>39</sup> Fifty-five patients were randomized to receive 400 mg riboflavin or placebo daily for three months. Riboflavin was superior to placebo in reducing the frequency of migraines and reduced the number of headache days by 50-59 percent compared to 15 percent in the placebo group.

An RCT studied riboflavin in conjunction with magnesium and feverfew for migraine prophylaxis.<sup>40</sup> Subjects (n=120) were assigned to 300 mg magnesium, 400 mg riboflavin, and 100 mg feverfew daily or placebo for three months, following a one-month headache

diary – noting frequency, duration, and severity of headaches. The “placebo” in this study was 25 mg riboflavin, which, according to the authors, was chosen to provide color to the urine, for the purpose of blinding; the authors also believed this amount of riboflavin would not have clinical activity. An analysis was performed after 48 patients completed three months of the trial. The primary outcome was a 50-percent reduction in migraines during the third month, compared to the initial 30-day period. A 42-percent reduction in migraine frequency was seen in the active group and a 44-percent reduction in the “placebo” group. The authors concluded that 25 mg riboflavin alone and 400 mg riboflavin in combination with magnesium and feverfew resulted in comparable effects on migraine headache frequency and severity.

### Carpal Tunnel Syndrome

Although it is well established that carpal tunnel syndrome (CTS) is associated with a deficiency of vitamin B<sub>6</sub>,<sup>41</sup> a study by Folkers et al revealed a deficiency of both riboflavin and vitamin B<sub>6</sub> in CTS patients.<sup>42</sup> Several studies support the use of vitamin B<sub>6</sub> with carpal tunnel syndrome. Because riboflavin is necessary for the conversion of inactive B<sub>6</sub> (pyridoxine) to its active form, pyridoxal 5'-phosphate, supplementing riboflavin along with vitamin B<sub>6</sub> may be indicated for CTS. Although anecdotal clinical evidence supports the use of riboflavin for carpal tunnel syndrome, more research would help establish the optimum dose and necessary course of treatment.

### Drug-Nutrient Interactions

Riboflavin levels are decreased by interactions with tricyclic antidepressants, phenothiazines, oral contraceptives, and anti-malarial drugs. Probenecid decreases absorption of riboflavin in the gut.

### Nutrient-Nutrient Interactions

Several nutrients are dependent on riboflavin for synthesis and homeostasis. The conversion of folate to 5-MTHF is dependent on FAD, activation of pyridoxine to pyridoxal 5'-phosphate is dependent on FMN, and the synthesis of vitamin B<sub>12</sub> is dependent on FAD. Therefore, a riboflavin deficiency could result in deficiencies of folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>.

## Side Effects/Toxicity

No known toxicity of riboflavin has been reported.

## Dosage

Therapeutic daily dosages for riboflavin range widely, from 3-400 mg; while RDAs are 1.2-1.6 mg. Typical B-vitamin complexes or multivitamin preparations contain 10-30 mg riboflavin. According to clinical studies, 400 mg riboflavin daily can be used for migraine prophylaxis. Dosages of 30-50 mg daily are indicated for anemia and deficiency states associated with angular stomatitis, seborrheic dermatitis, and neuropathy.

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