8. Riboflavin

Physiology and metabolism

Riboflavin is the precursor for the synthesis of two coenzymes, riboflavin phosphate (flavin mononucleotide, FMN) and flavin adenine dinucleotide (FAD), and covalently bound flavin prosthetic groups in enzymes. These function in a variety of enzymes catalysing oxidation and reduction reactions and electron transport; riboflavin is thus involved in a wide variety of metabolic pathways, including the biosynthesis and catabolism of amino acids, fatty acids and carbohydrates.

Apart from milk and eggs, which contain a relatively large amount of riboflavin bound to specific binding proteins, most of the riboflavin in foods is as riboflavin phosphate and FAD bound to enzymes. After release by digestion of the enzyme proteins, the coenzymes are hydrolysed in the intestinal lumen by phosphatases. The resultant free riboflavin is absorbed in the upper small intestine by an active process.

Riboflavin is transported in plasma both as the free vitamin and as coenzymes, largely bound to plasma proteins. There is rapid excretion from tissues of any riboflavin which is not bound to enzymes, and hence functionally active. Riboflavin and riboflavin phosphate which are not bound to plasma proteins are rapidly excreted by the kidneys, both by simple filtration and by active secretion into the urine. Active resorption of riboflavin from the urine is saturated at normal plasma concentrations of the vitamin, and so is mainly important in deficiency, acting to conserve the vitamin.

About 25% of the urinary excretion of riboflavin is as the unchanged vitamin; the remainder is excreted as a variety of metabolites. There is little or no storage of riboflavin in the body; any surplus intake is rapidly excreted. Once intake is adequate to meet requirements, the urinary excretion of the vitamin reflects intake until the capacity for intestinal absorption is exceeded. There is very efficient conservation of tissue riboflavin in deficiency; as the vitamin is released by protein breakdown, it is re-used in the synthesis of new enzymes. Only that relatively small proportion which is covalently bound to enzyme proteins cannot be re-utilised [1].

Deficiency and excess

Riboflavin deficiency is characterised by lesions of the margin of the lips (cheilosis) and corners of the mouth (angular stomatitis), a painful desquamation of the tongue,

so that it is red, dry and atrophic, and a seborrhoeic dermatitis, with filiform excrescences. There may also be conjunctivitis, with vascularisation of the cornea and opacity of the lens, leading to the development of cataract. On a global scale, riboflavin deficiency is common, yet never seems to be fatal, since there is very efficient conservation and reutilization of riboflavin in tissues when the dietary intake is inadequate.

Riboflavin deficiency can also result in secondary deficiency of iron, leading to anaemia; iron absorption is impaired in deficiency, and the utilisation of iron reserves also requires riboflavin. Similarly, riboflavin deficiency can result in impaired formation of the active metabolite of vitamin B_6, and can thus lead to secondary vitamin B_6 deficiency, and can also impair the metabolism of tryptophan, so leading to development of the tryptophan-niacin deficiency disease pellagra [2].

Inadequate riboflavin intake can be demonstrated biochemically by measuring the erythrocyte glutathione reductase (EGR) activation coefficient. EGR is an enzyme which has FAD as a coenzyme; addition of FAD *in vitro* increases its activity. The size of the activation coefficient is inversely related to riboflavin status.

Riboflavin has a low solubility in water, and there is only a limited capacity for absorption. There is also rapid excretion of any riboflavin not bound to enzymes. This means that there is little or no accumulation or storage of the vitamin in the body, and there is no evidence of any toxicity of riboflavin taken by mouth. There is some concern about the safety of high doses of riboflavin given to infants undergoing phototherapy for neonatal hyperbilirubinaemia.

Requirements

Adult males

A number of studies of subjects maintained on controlled intakes of riboflavin over several months, conducted in the 1940s and 1950s, defined the requirements of male adults.

Long-term studies of subjects maintained on controlled intakes of riboflavin show that 0.55 mg/d is inadequate to prevent signs of deficiency. Intakes of 0.7 mg/d do not result in deficiency signs over 41 weeks, while in 22 subjects maintained on 0.75-0.85 mg/d, deficiency signs were seen in only one. Epidemiological studies show that clinical signs of deficiency are apparent in subjects whose habitual intake is between 0.5-0.8 mg/d, but not at higher intakes [3,4].

It is thus apparent that a riboflavin intake of 0.55 mg/d is inadequate, and intakes between 0.55-0.8 mg/d are marginally adequate. In subjects maintained on graded intakes of riboflavin from 0.55-3.55 mg/d, there is a clear inflection in the relationship between intake and excretion, with a considerable increase in the excretion of the vitamin as intake is increased from 1.1 to 1.6 mg/d. At intakes of 1.1 mg/d and below, only 2-7 % of a test dose is excreted over 4h, and basal excretion is below 100 µg/24h. At intakes above 1.6 mg/d, 23-37 % of the test dose is recovered in the urine over 4h, and basal excretion begins to increase with intake [4].

There is no information on the excretion of riboflavin at intakes between 1.1 and 1.6 mg/d. Nevertheless, it is clear that intakes below 1.1 mg/d may be adequate to prevent the development of deficiency signs, but do not fill tissue reserves, while intakes above 1.6 mg/d are more than is required, so the excess is excreted. By interpolation, the 'critical intake' at which excretion increases sharply is 1.3 mg/d, and this is taken as the average requirement for adult males [5].

There have been no detailed studies of riboflavin requirements of adults in which the EGR activation coefficient has been used as an index of status. In one report an intake of 0.53 mg/d resulted in a significant elevation of the activation coefficient in 6 weeks, showing that this level of intake is inadequate to prevent the depletion of body reserves and the development of biochemical deficiency [5].

Because of its central role in energy metabolism, it has been conventional to express riboflavin requirements on the basis of energy intake. However, flavoproteins are also involved in a large number of other reactions, so riboflavin requirements are not related only to energy expenditure. The recommendations here are therefore not being given in terms of energy.

The average requirement of adult males is being taken as 1.3 mg/d, as mentioned above. In the urinary excretion studies 1.6 mg/d appeared to be adequate for all adult males, and this is given as the PRI.

Deficiency is highly probable on intakes of less than 0.6 mg/d, and this is taken as the Lowest Threshold Intake.

Adult females

Although not conclusive, there is a fair amount of indirect evidence that the daily amounts of riboflavin required by women are lower than for men. One would expect this *a priori*, and other reviewing bodies have reached this conclusion. The recommendations for adult women are therefore reduced in amounts per day below those of men, roughly in line with body weight. The Average Requirement for women is therefore given as 1.1 mg/d, with a Population Reference Intake of 1.3 mg/d. The

Lowest Threshold Intake is not reduced below 0.6 mg/d, in the absence of any information that it would be safe to do so in women.

Children

These are no good data on the riboflavin requirements of children. The PRIs given are derived from those of young adults on the basis of energy expenditure.

The PRI for infants 6-11 m is based on the finding that Gambian infants in their first 12 months receive 0.2 mg/d, and have a raised EGR activation coefficient. Increasing their intake to 0.4 mg/d restored the EGR activation coefficient satisfactorily [6].

Pregnancy and lactation

Pregnancy is associated with an increased EGR activation coefficient, and in populations where riboflavin intake is marginal clinical signs of deficiency are seen in pregnant women as parturition approaches. *Post partum* the deficiency resolves, despite continued low intake of the vitamin, and secretion of considerable amounts into the milk. While modest supplements during pregnancy prevent the development of deficiency signs, relatively large amounts (about 2.5 mg /d) are required to maintain the EGR activation coefficient within the range seen in non-pregnant women [2]. EGR activation coefficient data are therefore not being used in making a recommendation for pregnancy.

The demand for increased tissue synthesis by the fetus and the mother is estimated as 0.3 mg/d, making the PRI for pregnancy 1.6 mg/d.

The riboflavin content of breast milk varies considerably, being strongly influenced by the mother's recent intake. An increment of 0.4 mg/d is proposed during lactation, to meet the increased metabolic burden and provide an adequate amount in the milk, giving a PRI of 1.7 mg/d.

The elderly

There is no evidence that the riboflavin requirements of the elderly are greater than for younger people.

Summary

(amounts as mg/d)

Adults	*Males*	*Females*
Average Requirement	1.3	1.1
Population Reference Intake	1.6	1.3
Lowest Threshold Intake	0.6	0.6

Population Reference Intakes for other groups

	Age Group	PRI (mg/d)
	6 - 11 m	0.4
	1 - 3 y	0.8
	4 - 6 y	1.0
	7 - 10 y	1.2
Males	11-14 y	1.4
	15-17 y	1.6
Females	11-14 y	1.2
	15-17 y	1.3
	Pregnancy	1.6
	Lactation	1.7

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