Riboflavin (vitamin B-2) and health[1,2]

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ABSTRACT

Riboflavin is unique among the water-soluble vitamins in that milk and dairy products make the greatest contribution to its intake in Western diets. Meat and fish are also good sources of riboflavin, and certain fruit and vegetables, especially dark-green vegetables, contain reasonably high concentrations. Biochemical signs of depletion arise within only a few days of dietary deprivation. Poor riboflavin status in Western countries seems to be of most concern for the elderly and adolescents, despite the diversity of riboflavin-rich foods available. However, discrepancies between dietary intake data and biochemical data suggest either that requirements are higher than hitherto thought or that biochemical thresholds for deficiency are inappropriate. This article reviews current evidence that diets low in riboflavin present specific health risks. There is reasonably good evidence that poor riboflavin status interferes with iron handling and contributes to the etiology of anemia when iron intakes are low. Various mechanisms for this have been proposed, including effects on the gastrointestinal tract that might compromise the handling of other nutrients. Riboflavin deficiency has been implicated as a risk factor for cancer, although this has not been satisfactorily established in humans. Current interest is focused on the role that riboflavin plays in determining circulating concentrations of homocysteine, a risk factor for cardiovascular disease. Other mechanisms have been proposed for a protective role of riboflavin in ischemia reperfusion injury; this requires further study. Riboflavin deficiency may exert some of its effects by reducing the metabolism of other B vitamins, notably folate and vitamin B-6. *Am J Clin Nutr* 2003;77:1352–60.

KEY WORDS Riboflavin, dairy products, iron handling, homocysteine

INTRODUCTION

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a water-soluble vitamin present in a wide variety of foods. It was initially isolated, although not purified, from milk whey in 1879 and given the name lactochrome. It can be crystallized as orange-yellow crystals and in its pure form is poorly soluble in water. Its most important biologically active forms, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), participate in a range of redox reactions, some of which are absolutely key to the function of aerobic cells. Despite this and the facts that riboflavin deficiency is endemic in many regions of the world and that certain sections of populations in affluent societies have low intakes, studies of effects of inadequate riboflavin intakes have attracted limited interest. In light of the recent interest in the putative role of riboflavin in protecting against cancer and cardiovascular disease, it is appropriate to reevaluate the metabolic roles of this vitamin and the public health relevance of low intakes.

RIBOFLAVIN IN FOOD, ABSORPTION, AND TRANSPORT

Food sources of riboflavin

Milk and dairy products make the greatest contribution to riboflavin intake in Western diets, making riboflavin exceptional among the water-soluble vitamins. National dietary surveys in the United Kingdom report that, on average, milk and dairy products contribute 51% of intake in preschool children, 35% in schoolchildren, 27% in adults, and 36% in the elderly. Cereals, meats (especially offal), and fatty fish are also good sources of riboflavin, and certain fruit and vegetables, especially dark-green vegetables, contain reasonably high concentrations.

Riboflavin deficiency is endemic in populations who exist on diets lacking dairy products and meat (1–5). In Guatemala, the riboflavin status of elderly persons was highly correlated with the frequency of consumption of fresh or reconstituted milk (2). The National Diet and Nutrition Survey of young people aged 4–18 y (6) reported a high prevalence of poor riboflavin status, determined biochemically, among adolescent girls in the United Kingdom. A clear age-related decrease in the habitual consumption of whole milk was reported for both girls and boys. The most recent National Food Consumption Survey in the United Kingdom (7) confirmed a continuing trend toward lower household consumption of liquid whole milk (47% decrease since 1990). This is partly offset by an increase in the household consumption of semi-skim and other skimmed milks, although not fully skimmed milk. Grain products contain low natural amounts of riboflavin, but fortification practices have led to certain breads and cereals being very good sources of riboflavin. Cereals now contribute > 20% to the household consumption of riboflavin in the United Kingdom. Daily consumption of breakfast cereal with milk would be expected to maintain an adequate intake of riboflavin. Thus, it is not surprising that various studies from different countries have shown a higher riboflavin intake or better riboflavin status among those who consume cereal at breakfast than among those who do not, irrespective of age (8–10).

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Vegetarians with access to a diversity of fruit and vegetables can avoid deficiency, although intakes in vegetarians may be lower than in omnivores (11), and elderly vegetarians may be at higher risk (12). Although relatively heat-stable, riboflavin is readily degraded by light. Milk kept in glass bottles and delivered to the doorstep might be particularly susceptible to loss through this route, which is also associated with flavor changes, because the oxidative products of photolysis can damage milk lipids. This light sensitivity of riboflavin has led to loss of riboflavin from banked breast milk used in the parenteral nutrition of newborns (13).

Bioavailability

A small amount of riboflavin is present in foods as free riboflavin, which is an isoalloxazine ring bound to a ribitol side chain; most is present as the derivative FAD, and a smaller amount occurs as the monophosphorylated form, FMN. FAD and FMN occur predominantly in a non-covalently-bound form to enzymes; flavins that are covalently bound do not appear to be available for absorption (14). In contrast with most foodstuffs, milk and eggs contain appreciable quantities of free riboflavin bound to specific binding proteins (15). A prerequisite for the absorption of dietary riboflavin is the hydrolysis of FAD and FMN to riboflavin, catalyzed by nonspecific phosphatases in the brush border membranes of enterocytes. Absorption takes place predominantly in the proximal small intestine through an active, carrier-mediated, saturable transport process (16) that is reported to be linear up to ≈30 mg riboflavin given in a meal (17). There is little additional absorption of riboflavin in amounts greater than this (18). Urinary excretion increases linearly with increasing intakes in riboflavin-replete subjects, with an absorption half-life of 1.1 h (18). Initially, free riboflavin is taken up into enterocytes and undergoes ATP-dependent phosphorylation catalyzed by cytosolic flavokinase (EC 2.7.1.26) to form FMN; most of this is further converted to FAD by the FAD-dependent FAD synthetase (EC 2.7.7.2). Nonspecific phosphatases act on intracellular flavins to permit transport across the basolateral membrane. Riboflavin may enter the plasma from the small intestine as the free form or as FMN.

Research has indicated that carrier-mediated absorption of riboflavin in the colon might be more important than previously thought (19). Riboflavin synthesized by bacterial metabolism in the colon might therefore be a more important source of this vitamin than previously recognized.

Little information is available regarding the relative bioavailability of riboflavin from different food sources. However, no reports have suggested that the efficiency of absorption of dietary riboflavin is a limiting factor in determining riboflavin status. The upper limit of the uptake process greatly exceeds usual daily intakes (*see* the section "Dietary requirements for riboflavin").

Transport and metabolism

Free riboflavin is transported in the plasma bound both to albumin and to certain immunoglobulins, which will also bind flavin coenzymes (20). Other riboflavin binding proteins are specific to pregnancy. Riboflavin binding proteins expressed in fetuses of different species are evidently essential to normal fetal development. Early classic studies identified a riboflavin binding protein in chicken egg white that is induced by estrogen and is essential to fetal survival (21). Further studies in various other species confirmed the presence of similar riboflavin binding proteins in the circulation, which have been ascribed various functions, including placental transport (22). Elevated plasma binding of riboflavin has been reported in patients with malignancies, attributable to an elevation in specific immunoglobulins, which may contribute to riboflavin retention in such patients (23).

Almost all riboflavin in tissues is enzyme bound, such as FAD covalently bound to succinic dehydrogenase (EC 1.3.5.1) (24). Unbound flavins are relatively labile and are rapidly hydrolyzed to free riboflavin, which diffuses from cells and is excreted. The intracellular phosphorylation of riboflavin is therefore a form of metabolic trapping key to riboflavin homeostasis (25).

Intakes of riboflavin in excess of tissue requirements are excreted in the urine as riboflavin or other metabolites, such as 7-hydroxymethylriboflavin (7-α-hydroxyriboflavin) and lumiflavin. Some urinary metabolites reflect bacterial activity in the gastrointestinal tract as well (26).

DIETARY REQUIREMENTS FOR RIBOFLAVIN

Balance studies in humans show a clear increase in the urinary excretion of riboflavin as riboflavin intakes increase, with a sharp and continuous rise in excretion at intakes above ≈1 mg/d (27). Elderly subjects consuming a riboflavin supplement of 1.7 mg above their habitual intake of 1.8 mg showed a urinary excretion of riboflavin that was twice that of unsupplemented subjects consuming 1.8 mg from the diet alone (28). The inflection of the urinary excretion curve is considered to reflect tissue saturation. Urinary excretion of riboflavin is, however, not a sensitive marker of very low riboflavin intakes, and the preferred method for assessing riboflavin status is stimulation of the FAD-dependent erythrocyte glutathione reductase (EC 1.6.4.2) in vitro. The results are expressed as an activation coefficient (EGRAC), such that the poorer the riboflavin status the higher the activation coefficient. Numerous studies have shown the sensitivity of this measurement to riboflavin intakes, especially at daily intakes ≤ 1.0 mg (2, 5). Such studies have also highlighted the speed with which tissue riboflavin depletion and repletion occur. Although in experimental riboflavin deficiency FAD is conserved at the expense of free riboflavin (29), there is no store of riboflavin or its metabolites (ie, no site from which riboflavin can be mobilized in times of low dietary intake). There is only a small difference between intakes associated with biochemical deficiency (< 0.5 mg) and those associated with tissue saturation (> 1.0 mg) in adults (30). Current recommended nutrient intakes in the United Kingdom range from 0.4 mg/d in infancy to 1.3 mg/d in adult females. An increment has been set of 0.3 mg in pregnancy and 0.5 mg during lactation to cover increased tissue synthesis for fetal and maternal development and riboflavin secretion in milk. These values are similar to recommendations made by the World Health Organization in 1974 (31), the European population reference intake (32), and the US recommended dietary allowance (33).

GROUPS AT RISK OF LOW INTAKES

The adequacy of riboflavin intakes by population groups can be evaluated in terms of daily dietary intake or with the use of biomarkers of status.

Pregnant women, lactating women, and infants

Most studies of riboflavin status among pregnant or lactating women have been conducted in communities where riboflavin intakes are low. Under these circumstances, a progressive fall in

riboflavin status occurs during the third trimester, and clinical signs of deficiency are most evident around parturition (34–37). Riboflavin depletion during gestation in rats and mice leads to fetal resorption (38). There are reports from as early as the 1940s of various congenital malformations associated with riboflavin deficiency in rats and mice (38–40). The relevance of these effects to humans is unclear, but a recent report implicated riboflavin deficiency in the etiology of recurrent cleft lip and palate in siblings (41), although the subjects were probably also deficient in vitamin A and folic acid.

If maternal status is poor during gestation, the infant is likely to be born riboflavin deficient (5). Riboflavin status characteristically improves transiently in the neonatal period, even when maternal riboflavin status is poor, but predictably deteriorates around the time of weaning. Breast-milk riboflavin concentrations are fairly sensitive to maternal riboflavin intake, and can be moderately increased by riboflavin supplementation of the mother when natural intake is low (5, 42, 43). Even in well-nourished communities, concentrations of riboflavin in breast milk are considerably lower than in cow milk. Infants receiving banked breast milk through nasogastric tubing may be at risk of developing transient riboflavin deficiency because of losses in the milk during collection, storage, and administration (13). Phototherapy used to treat hyperbilirubinemia in neonates is also associated with transient deterioration in riboflavin status (44). Transient riboflavin deficiency has been documented in infants born prematurely, although no functional deficits have been described (45, 46).

Schoolchildren

Riboflavin deficiency among schoolchildren has been documented in many regions of the world where the intake of milk products and meat is limiting (1, 4, 47). Riboflavin deficiency among children in the West seems to be largely confined to adolescents, especially girls. The National Diet and Nutrition Survey of young people aged 4–18 y in the United Kingdom collected dietary intake and riboflavin status data from a representative sample of 2127 schoolchildren (6). The proportion of boys with biochemical values indicative of poor riboflavin status rose from 59% among 4–6-y-olds to 78% among 7–10-y-olds. Ninety-five percent of 15–18-y-old girls had evidence of low riboflavin status. Riboflavin status, expressed as EGRAC, was significantly correlated with estimates of dietary intake. Mean riboflavin intakes showed a progressive increase with age among boys, but this was not evident among girls. Importantly, there was a marked decline in milk consumption with increasing age in both boys and girls, and in 15–18-y-olds, milk contributed only 10% of the daily riboflavin intake, compared with 25% among 4–6-y-olds. Compared with riboflavin intake data collected in the 1983 Diets of British Schoolchildren survey (in children aged between 10 and 15 y; 48), current mean and median intakes show a trend to be lower for both girls and boys. Data from other European countries confirm an age-related decline in milk consumption among children (49, 50). The functional significance of poor riboflavin status among adolescents is not yet known, but there may be implications for the handling of dietary iron, which would be important for the 50% of 15–18-y-old girls who have iron intakes less than the lower reference nutrient intake.

A correlation between milk consumption and riboflavin status among adolescents in New York City was reported in 1980 (51). Groups consuming ≥3 cups milk/d (≈720 mL/d) had a mean EGRAC of 1.09 compared with 1.37 among those who consumed <1 cup/wk (<240 mL/wk).

The elderly

The results of the 1994–1995 National Diet and Nutrition Survey of people aged ≥65 y provide the most up-to date data for this age group in the United Kingdom. The sampling methods ensure that the data are representative of this age group in the United Kingdom. The study recruited 2172 free-living subjects and 454 subjects from institutions. Dietary intake data gave little cause for concern regarding riboflavin, with <10% from either group having intakes less than the lower reference nutrient intake. The biochemical data gave a somewhat different picture, however. Forty-one percent of the free-living subjects and 35% of the institutionalized subjects had evidence of biochemical deficiency, expressed as EGRAC, the most commonly used marker of riboflavin status (52). EGRAC was highly correlated with estimates of intake. The apparent discrepancy between the dietary intake data and the status data may reflect increased requirements for riboflavin with increasing age as the result of reduced efficiency of absorption, although studies to date do not generally support such an effect (2, 53). Two recent studies of elderly people in the United Kingdom drew similar conclusions regarding the adequacy of intake relative to current dietary reference values and, by using a less conservative threshold for deficiency, reported suboptimal status in 49% and 78% of subjects, respectively (54, 55).

Large surveys in the United States reported riboflavin deficiency among the elderly to be between 10% (56) and 27% (57) on the basis of biochemical and dietary intake criteria, respectively. Estimates of the prevalence of riboflavin deficiency in various European countries range between 7% and 20% (58, 59), but there is a lack of standardization for the deficiency threshold for EGRAC.

Athletes

Despite the anticipated effect of riboflavin deficiency on physical work performance, relatively few studies have shown a relation. Multimicronutrient supplements that included riboflavin had beneficial effects on work performance in both Yugoslavian schoolchildren (60) and Gambian schoolchildren (61). These multisupplement studies were carried out in populations where riboflavin status was poor. There is no evidence that in generally well-nourished communities the riboflavin status of elite athletes is different from that of nonathletic control subjects (62, 63). Similarly, no published studies have shown that riboflavin deficiency specifically impairs work performance or that riboflavin supplements increase performance in healthy individuals. On the other hand, some studies report that vigorous exercise may deplete riboflavin (64, 65).

FUNCTIONS OF RIBOFLAVIN AND CONSEQUENCES OF LOW INTAKES

Riboflavin in intermediary metabolism

It is well established that riboflavin participates in a diversity of redox reactions central to human metabolism, through the cofactors FMN and FAD, which act as electron carriers (66). Most flavoproteins use FAD as a cofactor. Inadequate intake of riboflavin would therefore be expected to lead to disturbances in steps in intermediary metabolism, with functional implications. In fact, it is sometimes difficult to trace physiologic and clinical effects of riboflavin deficiency to specific metabolic "blocks."

Riboflavin deficiency in rats was associated with a dose-response, tissue-specific reduction in succinate oxidoreductase

(EC 1.3.99.1; succinate dehydrogenase) activity (67, 68). Such an effect may have implications for energy production via oxidative phosphorylation of the electron transport chain.

Steps in the cyclical β oxidation of fatty acids are also dependent on flavins as electron acceptors. An effect on the β oxidation of fatty acids is thought to be responsible for the altered fatty acid profile in hepatic lipids in severely riboflavin-deficient rats (69, 70), which seems to be independent of the dietary source of lipid. The most marked effect was an increase in 18:2n−6 and a lowering of 20:4n−6. Similar but less striking differences were observed in plasma, erythrocyte membranes, and kidney. The influence of riboflavin deficiency on fatty acid profiles may reflect an overall reduction in the β oxidation of fatty acids, while essential fatty acids present in the diet accumulate. Weanling rats fed a riboflavin-deficient diet rapidly showed impaired oxidation of palmitoyl CoA and stearic, oleic, and linoleic acids (71, 72). Associated with this is the excretion of various dicarboxylic acids, resulting from microsomal and peroxisomal handling of the fatty acids (73–75). This scenario has its counterpart in humans with inborn errors of lipid metabolism leading to organic aciduria that is responsive to pharmacologic doses of riboflavin (76). Transient riboflavin depletion associated with phototherapy in full-term neonates was not associated with any measurable change in long-chain fatty acid β oxidation (77). An elegant stable-isotope approach to measuring fatty acid oxidation in premature infants with riboflavin deficiency also failed to detect any effects of riboflavin supplementation (46). It is unknown whether riboflavin deficiency in other human groups is associated with impaired fatty acid oxidation.

Riboflavin deficiency and developmental abnormalities

Early studies of riboflavin deficiency in pregnant animals documented abnormal fetal development with a variety of characteristics. Diverse skeletal and soft tissue abnormalities are well described in the offspring of rats and mice fed riboflavin-deficient diets (78). The importance of riboflavin carrier protein to fetal development has been documented in mice (79) and chickens (21). Riboflavin deficiency, along with deficiency of other vitamins, has been implicated in the etiology of cleft lip-palate abnormalities in 2 infants born to a woman with malabsorption syndrome (41), although no measurement of riboflavin status was made, so the association remains unconfirmed. The role of riboflavin in gastrointestinal development is discussed in the section "Riboflavin and gastrointestinal development."

Riboflavin and hematologic status

Very early studies of riboflavin deficiency in human populations (in which it almost certainly coexisted with other deficiencies) and animals indicated effects of riboflavin on aspects of the hemopoietic system. Riboflavin-responsive anemia in humans was described by Foy and Kondi (80, 81) in the 1950s, the characteristic features being erythroid hypoplasia and reticulocytopenia. Further studies in subhuman primates fed a riboflavin-deficient diet showed marked disturbances in the production of red blood cells in the bone marrow and in the kinetics of iron handling (82, 83). Some of the effects of riboflavin deficiency on the activity of the bone marrow may be mediated by the adrenal cortex, which is both structurally and functionally impaired by riboflavin deficiency (84). More recent work, however, suggests other mechanisms whereby riboflavin deficiency might interfere with iron handling and thereby influence hematologic status.

Ferritin iron mobilization

The mobilization of iron from the intracellular protein ferritin is a reducing process. Reduced flavins can evidently reduce and thereby mobilize ferritin iron in a variety of tissues, at a rate that is physiologically relevant (85, 86). We and others have shown that tissues from rats fed riboflavin-deficient diets are less efficient at mobilizing ferritin iron than are tissues from control animals (87–89). In our experience, the most profound effect is in mucosal preparations from the gastrointestinal tract, suggesting a relevance to iron absorption (90).

Iron absorption and loss

Intervention studies in humans further support the idea that riboflavin status might influence iron handling, possibly including effects at the level of iron absorption. Correcting a riboflavin deficiency in pregnant or lactating women, adult males, and school-aged children improved the hematologic response to iron supplements (61, 91–93). Subsequently, animal studies confirmed that moderate riboflavin deficiency impairs iron absorption (94, 95), and mechanistic studies in vitro provided further evidence for such an effect (96). In addition to effects on iron absorption, riboflavin deficiency in weanling rats was shown to significantly increase the rate of gastrointestinal iron loss (95). The mechanism for this is discussed in the section "Riboflavin and gastrointestinal development." There has been a single attempt to show an effect of riboflavin status on iron absorption in humans by using a stable isotope of iron (^{58}Fe) (97). In that study, there was large variability in iron absorption between subjects, and we could find no measurable effect on iron absorption. However, the study did show an effect of riboflavin supplements on the concentration of circulating hemoglobin, suggesting that improving riboflavin status had an effect on iron absorption or iron mobilization from existing stores.

Riboflavin and gastrointestinal development

The maturation of gastrointestinal function at the time of weaning is regulated in part by changes in the composition of the diet. Animal studies have identified qualitative and quantitative changes in the gastrointestinal tract after alterations in diet at this time. Weanling rats fed a riboflavin-deficient diet from weaning showed early morphologic and cell kinetic changes in the gastrointestinal tract, some of which were not reversible with correction of the riboflavin deficiency (98–101). After only 4 d of feeding a riboflavin-deficient diet, a significant increase in the size and cellularity of the crypts was seen, with a decreased incidence of bifurcating crypts and a decreased proliferation index. Seven days of riboflavin depletion led to fewer villi per unit area of mucosa than in controls, suggesting a smaller absorptive surface area. After more prolonged depletion, villus hypertrophy was observed and may represent an adaptation response to this deficiency.

Recent work has shown that even when riboflavin is supplied to tissues intraperitoneally, the absence of riboflavin from the lumen of the gastrointestinal tract from the time of weaning leads to a disruption of normal gastrointestinal development in rats. The changes in gastrointestinal development mirror early effects of riboflavin deficiency induced by feeding a diet deplete in riboflavin from weaning (101). Duodenal crypts increased in cellularity and depth, but the proliferative index and the proportion of crypts bifurcating decreased. These results suggest that a crypt-sensing mechanism may be involved in the gastrointestinal response to dietary depletion of riboflavin. This has important

implications for the effects of early dietary inadequacy of riboflavin on gastrointestinal maturation. These effects may occur in utero if mothers are riboflavin deficient during pregnancy, which is the case in many developing countries.

Such marked effects of riboflavin deficiency on the development of the gastrointestinal tract may be important in the etiology of growth impairment associated with riboflavin deficiency, through general effects on the efficiency of nutrient absorption. This remains to be established.

Riboflavin, neurodegeneration, and peripheral neuropathy

Symptoms of neurodegeneration and peripheral neuropathy have been documented in several studies of riboflavin deficiency in different species. Young, rapidly growing chickens fed a riboflavin-depleted diet developed peripheral nerve demyelation (102, 103). Peripheral nerve demyelination has also been documented in racing pigeons (104) and riboflavin-deficient rats (105). Little information is available regarding the relevance of these observations to humans, although an interesting case of a 2.5-y-old girl with biochemical evidence of moderate riboflavin deficiency has been described. The child had a range of neurologic abnormalities, with anemia and visual impairment (106). With high-dose riboflavin supplementation, the anemia resolved quickly and the neurologic and visual abnormalities resolved over several months. Riboflavin plays a role in thyroxine metabolism, and riboflavin deficiency may contribute to the pathophysiology of some mental illness via this route (107). An early report of personality changes in riboflavin deficiency has not been substantiated (108).

Riboflavin and cancer

The literature relating riboflavin with cancer is complex. Some studies indicate that riboflavin deficiency increases the risk of cancer at certain sites, whereas others point to a possible attenuating effect of riboflavin in the presence of some carcinogens and a protective effect of deficiency (109, 110). Some carcinogens are metabolized by flavin-dependent enzymes, and in these instances riboflavin may enhance or ameliorate the effects of the carcinogen (111). Studies in various animal species have shown that riboflavin deficiency can lead to disruption of the integrity of the epithelium of the esophagus, similar to precancerous lesions in humans (84). Some epidemiologic studies have identified a relation between esophageal cancer and diets low in riboflavin (112–114), although not all studies support such a relation (115). Combined daily supplements of riboflavin and niacin over 5 y were effective in reducing the incidence of esophageal cancer in Linxian, China, an area with a high prevalence of this type of cancer (116). Recent work has shown that riboflavin deficiency in rats exposed to hepatocarcinogens leads to increased DNA strand breakage. Induction of repair enzymes, which contribute to the resistance to malignant transformations, was also enhanced in the riboflavin-deficient animals (111). High-dose riboflavin supplementation reversed both effects to near-normal values. Also supportive of a protective role of riboflavin in carcinogenesis is the observation that carcinogen binding to DNA is increased in riboflavin-deficient rats (117).

Poor riboflavin status has also been implicated as a risk factor for cervical dysplasia, a precursor condition for invasive cervical cancer (118). A case-control study of 257 cases of cervical dysplasia and 133 controls showed an increased risk of cervical dysplasia at a riboflavin intake of < 1.2 mg/d, after correction for known risk factors and total energy intake. There was a significant trend effect. This study also identified lower intakes of vitamin A and folate as risk factors. It may be important that riboflavin has a role in the metabolism of folate, and low dietary riboflavin might therefore exacerbate the effects of low dietary folate in this context. This is an area that deserves further study, perhaps with the use of a more rigorous approach to estimating dietary intake and with the inclusion of a biochemical measure of riboflavin status.

Riboflavin and cardiovascular diseases

Flavin reductase and dihydroriboflavin

Dihydroriboflavin, produced from riboflavin by NADPH-dependent flavin reductase (EC 1.5.1.30), has been shown to be an efficient reducing agent for heme proteins containing ferric iron and therefore a potential antioxidant. Interesting work has emerged to indicate that riboflavin might have protective effects against the tissue damage associated with ischemia-reperfusion, probably mediated by flavin reductase and the reduction by dihydroriboflavin of oxidized heme proteins (119–121). All studies so far have been conducted in animal models. Riboflavin, administered in low concentrations in vivo or to tissues ex vivo, reduced cellular injury in 3 models: ischemia-reperfusion injury in isolated hearts, activated complement-induced lung injury, and brain edema after hypoxia-reoxygenation. Because of its nontoxicity, riboflavin is an attractive candidate as a reductant of iron in heme proteins for the protection of tissues from oxidative injury. The potential therapeutic role for this vitamin in this context should be the subject of intense investigation. Whether riboflavin status might influence recovery from oxidative injury associated with stroke, for example, remains to be established.

Riboflavin as a modulator of homocysteine concentrations

In recent years there has been much interest in the importance of plasma homocysteine as a graded risk factor for cardiovascular disease (122, 123). Homocysteine is a thiol-containing amino acid that arises as a product of the metabolism of the essential amino acid methionine. It is not incorporated into protein and therefore its concentration is regulated by the rate of its synthesis and metabolism. The main determinants of the homocysteine concentration in tissues and consequently in the circulation are genotype and diet. Homocysteine is metabolized through 2 main routes, transsulfuration, which is vitamin B-6 dependent, and remethylation to methionine, which is folate, vitamin B-12, and riboflavin dependent. Most attention has been directed toward the importance of folate, which is a strong independent predictor of plasma homocysteine and which has homocysteine-lowering activity (124). Supplementary vitamin B-12 has modest homocysteine-lowering effects under certain circumstances (124), whereas reports of the effects of supplementary vitamin B-6 are inconsistent (125, 126). Riboflavin has been largely ignored, despite the fact that FAD is a cofactor for methylenetetrahydrofolate reductase (EC 1.7.99.5), which metabolizes folate to the form used in homocysteine methylation. A common mutation of methylenetetrahydrofolate reductase, (the 677C→T thermolabile variant), for which 5–30% of different populations are reported to be homozygous, is associated with increased plasma homocysteine concentrations (127). Further evidence for a role of riboflavin in homocysteine homeostasis comes from a report of elevated homocysteine in the skin of riboflavin-deficient rats

(128). Riboflavin status was reported as being a modulator of plasma homocysteine concentrations in healthy adults, especially among subjects homozygous for the common 677C→T mutation (129). Riboflavin intake also emerged as a factor influencing plasma total homocysteine in men and women from the Framingham Offspring Cohort (130). We recently confirmed a folate-riboflavin interaction in determining plasma homocysteine that is unrelated to genotype (131).

Riboflavin in vision

Corneal vascularization and corneal opacity have been described in animals fed diets low in riboflavin. Cataracts have also been described in animals fed riboflavin-deficient diets (132, 133). The importance of riboflavin deficiency in the etiology of cataracts in elderly humans is not fully understood (134). More recently, it was hypothesized that riboflavin deficiency may be associated with night blindness in some communities and that improving riboflavin status might enhance the improvement in night blindness evoked by vitamin A. Venkataswamy (135) reported riboflavin-responsive night blindness in India. Riboflavin-dependent photoreceptors (cryptochromes) identified in the retina are thought to play a role in the process of dark adaptation (136, 137). Dietary riboflavin might influence dark adaptation through these photoreceptors, through interaction with vitamin A, or independently. This is an area that deserves further attention.

INTERACTION OF RIBOFLAVIN WITH OTHER B GROUP VITAMINS

Folate

Riboflavin deficiency interferes with the metabolism of other nutrients, especially other B vitamins, through flavin coenzyme activity. Effects of acute riboflavin deficiency on fetal development have similarities with effects of folate deficiency, possibly mediated by effects of flavins on folate metabolism. Weanling rats fed a riboflavin-deficient diet showed a marked reduction in activity of hepatic methylenetetrahydrofolate reductase, referred to earlier as the source of the methyl group in the conversion of homocysteine to methionine (138). This has taken on greater significance with the interest in elevated plasma homocysteine concentrations as a risk factor for cardiovascular disease and is discussed in the section "Riboflavin and cardiovascular diseases."

Cyanocobalamin (vitamin B-12)

The enzyme methionine synthase (EC 2.1.1.13), which converts homocysteine to methionine, is dependent on 5-methyltetrahydrofolate as a methyl donor but also on vitamin B-12, as methyl cobalamin (139). The synthesis of methylcobalamin appears in turn to be dependent on flavoproteins. Despite this interrelation between riboflavin and vitamin B-12, there is no clear evidence that riboflavin deficiency leads to a functional deficiency of vitamin B-12.

Pyridoxine

Similarities exist between the clinical signs of riboflavin deficiency and those of pyridoxine (vitamin B-6) deficiency, and supplementation with both vitamins can elicit a faster and more complete recovery than can single supplements (140). In fact, the metabolism of vitamin B-6 is flavin-dependent, and studies in humans and animals have shown impaired synthesis of pyridoxal phosphate in riboflavin deficiency (141, 142). Correcting a riboflavin deficiency in humans elicited an increase in the activity of erythrocyte pyridoxamine phosphate oxidase (EC 2.6.1.54; 143), which is responsible for converting pyridoxamine phosphate and pyridoxine phosphate to pyridoxal phosphate (144).

CONCLUSIONS

Riboflavin or its derivatives are found in a wide variety of foods, although milk and milk products make a particularly important contribution to the riboflavin intakes of populations in Western countries. Riboflavin deficiency is endemic in populations consuming little milk or meat products. A decline in the consumption of milk and milk products in Western countries may contribute to the poor riboflavin status reported in sections of the population, particularly young people. Subclinical riboflavin deficiency may contribute to increased concentrations of plasma homocysteine, with an associated increased risk of cardiovascular disease. It may also be associated with impaired handling of iron and night blindness. The importance to humans of some of the effects of riboflavin deficiency observed in animal studies remains to be established. Current research of public health relevance relates to the importance of riboflavin as a factor in protecting against cardiovascular diseases and cancers and in vision.

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