

EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF RIBOFLAVIN

The attached review of riboflavin is a revised version of the paper presented to the Expert Group on Vitamins and Minerals at the meeting in October 2001.

The following annexes are also included:

- Annex 1 Intakes of riboflavin from food and supplements
- Annex 2 Summary table of selected nutrition related information and existing guidance on regulations

Expert Group on Vitamins and Minerals Secretariat
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RIBOFLAVIN

(Flavin Adenine Dinucleotide: FAD)

1. Riboflavin is a water-soluble vitamin of the B group (vitamin B₂) that was named from the Latin word *flavius* (yellow) to denote the deep yellow colour of crystals formed from the pure vitamin and the deep yellow colour it gives to urine.

Chemistry

CAS no.	83-88-5
Chemical formula	C ₁₇ H ₂₀ N ₄ O ₆
Chemical name	7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxy-pentyl)isoalloxazine
Proprietary names	Riboflavine, vitamin B ₂ , Belflavit, Beflavin, Flavin, Ribipea, Beflavina, Berivine
Molecular weight	376.36
Dissociation constants	pK _a : 10.2; pK _b : 1.7
pH	Neutral to litmus; saturated aq soln: about 6
Form	Precipitated as fine orange-yellow needles from 2 N acetic acid, alcohol, water, or pyridine
Taste	Bitter
Octanol/water partition coefficient	log K _{ow} = -1.46
Solubilities	In alcohol: <1 in 10,000 In water: 1 in 3000 to 1 in 20,000 Insoluble in lipid solvents

(Furia 1972, Hawley 1977, Sax 1979; Weast 1979, Nahum and Horvath 1980, Budavari 1989, 1996, Yalkowsky and Dannenfelser 1992).

2. Riboflavin has three crystalline forms, which have different rates of dissolution in water, owing to differences in the crystalline structure. Riboflavin is stable to mineral acids in the dark at 27°C. Decomposition of acidic solutions buffered to maintain pH 5 was observed to take place at a rate of 1.2% per month. When dry, riboflavin is quite stable, but in alkaline solution it deteriorates quite rapidly, the deterioration being accelerated by light (Budavari 1989). The photodegradation of riboflavin appears to produce the reactive oxygen species ¹O₂ (Joshi 1985). Riboflavin is more stable to heat in acid solution, particularly from pH 1.0 to 6.5, but upon irradiation

forms lumichrome. Riboflavin is readily adsorbed from acid or neutral solution on such agents as frankonite, Fuller's earth, and certain zeolites, and eluted with acetone or pyridine solutions (Chase *et al* 1970).

Natural occurrence

3. Riboflavin is widely distributed in nature, in plants and animals, as an essential constituent of all living cells and is therefore, widely distributed in small amounts in foods (Osol and Hoover 1975).

4. Common sources of riboflavin are almonds, yeast, cheese, eggs, chicken, beef, kidney, liver, and wheat germ (Budavari 1989). In the UK the main sources of riboflavin in the diets of 15-18 year olds are milk and milk products (32%), cereal and cereal products (31%) and meat and meat products (14%) (Gregory and Lowe 2000). Breads and cereals are often fortified with riboflavin. Because riboflavin is degraded by light, if foods rich in riboflavin are left out in sunlight, or any UV light, loss of riboflavin content will occur. Riboflavin is stable when heated but will leach into cooking water. The pasteurization process causes milk to lose about 20% of its riboflavin content. Alkalis, such as baking soda, also destroy riboflavin.

5. A study by Roughead and McCormick (1990a) found that riboflavin and flavin adenine dinucleotide (FAD) were the predominant flavins in all milk samples; 10-(2'-hydroxyethyl)flavin was next most common. The latter is potentially an antivitamin that may exert its effect at either the absorption or utilization level, and should be considered when evaluating the riboflavin efficiency of milk.

6. The same authors (Roughead and McCormick 1990b) found that flavin adenine dinucleotide (FAD) and riboflavin were also the predominant flavins in human milk, followed by 10-(2'-hydroxyethyl)-flavin. In addition, traces of 7-alpha- and 8-alpha-hydroxyriboflavins (7-hydroxymethylriboflavin and 8-hydroxymethylriboflavin, respectively) were detected. This finding may have implications for dietary recommendations concerning both lactating women and infants. In practical terms, the types and amounts of flavins in human milk are very similar to those reported for cows' milk. Table 1 lists the riboflavin content of various foods listed in US composition tables. A summary of the contribution of different food groups to riboflavin to intake in the UK is given in Annex 1, table 3

Table 1. Riboflavin content of foods

Food	Riboflavin content (mg)
Lambs liver, fried 100 g	4.03 mg
Almonds, blanched ½ cup	0.98 mg
Scallops, fried 6 pieces	0.85 mg
Pink salmon, canned 1 can	0.84 mg
Malted milk powder 4-5 tsp	0.75 mg
All Bran ½ cup	0.42 mg
Spinach, cooked 1 cup	0.42 mg
Milk, whole and skim 1 cup	0.40 mg
Mackerel, cooked 1 fillet	0.36 mg
Veal, cooked 100 g	0.35 mg
Wheatgerm ½ cup	0.29 mg
Lamb, cooked 100 g	0.27 mg
Pork sausages, grilled 100 g	0.25 mg
Eggs, boiled 1 medium	0.25 mg
Milk chocolate 100 g	0.24 mg
Fruit yoghurt 1 tub	0.23 mg
Feta cheese 1 cup	0.23 mg
Oats 1 cup	0.21 mg
Beef steak, grilled 100 g	0.21 mg
Green peas 1 cup	0.19 mg
Soy milk 1 cup	0.17 mg
Pork, cooked 100 g	0.11 mg
Cheddar cheese 1 slice	0.11 mg
Brazil nuts ¼ cup	0.17 mg

Uses

- Riboflavin is used as a yellow colouring agent in pharmaceuticals and as an ingredient in enriched flours. (Rossoff 1974, Lewis 1993). It is a permitted food colour and an ADI of 0-0.5 mg/kg bw has been established (JECFA 1999).

Licensed medicinal products for oral use

- Riboflavin and riboflavin sodium phosphate are regulated under the Medicines (General Sales List) Order 1984 which lists substances which may be included in products which may be sold in supermarkets and other retail outlets without the supervision of a pharmacist.
- Thirty-six medicinal products containing riboflavin are authorised for general availability, all of which are multi-constituent products, which are indicated for the prevention or treatment of nutrient deficiencies. For the prevention of deficiencies the dosing recommendations generally provide 0.5-10 mg riboflavin per day, whilst for the treatment of deficiencies the recommendations generally provide 5-25 mg/day with a maximum dose of 45 mg. A further 34 products may only be sold in a pharmacy, usually because one or more of the other constituents

cannot be sold without the supervision of a pharmacist. The indications and doses for these are generally similar to those for the general sales products.

Reported beneficial effects of riboflavin supplementation

Anaemia

10. Riboflavin supplements may be of benefit in the treatment of sickle cell anaemia and may also enhance the effectiveness of iron supplements when these are used to treat anaemia (see paragraphs 52-56).

Migraine

11. A deficit in mitochondrial function resulting in impaired oxygen metabolism may play a role in migraine pathogenesis. High-dose riboflavin was shown to be effective in migraine prophylaxis (Schoenen *et al* 1994). The study of Schoenen *et al* (1998) compared riboflavin (400 mg) and placebo in 55 patients with migraine in a randomized trial of 3 months duration. Using an intention-to-treat analysis, riboflavin was superior to placebo in reducing attack frequency ($P = 0.005$) and headache days ($P = 0.012$). Regarding the latter, the proportion of patients who improved by at least 50%, i.e. responders, was 15% for placebo and 59% for riboflavin ($P = 0.002$) and the number-needed-to-treat for effectiveness was 2.3. No serious side-effects occurred. The researchers felt that because of its effectiveness, excellent tolerability, and low cost, riboflavin could be of value in migraine prevention.

Carpal tunnel syndrome (CTS)

12. Riboflavin may help to relieve the symptoms of CTS, a neurological disorder of the wrists and hands which causes pain and stiffness. Riboflavin is usually combined with vitamin B₆ to treat this disorder and treatment with both vitamins may be more effective than treatment with B₆ alone (Folkers *et al* 1984, Folkers and Ellis 1990). In the study of Folkers *et al* (1984) riboflavin for 5 months caused nearly complete disappearance of CTS and caused no change in the specific activity of erythrocyte glutamic-oxaloacetic transaminase. Combined riboflavin and pyridoxine treatment increased ($P < 0.001$) the specific activities of erythrocyte glutathione reductase and erythrocyte glutamic-oxaloacetic transaminase to normal levels with total disappearance of the CTS. Objectively, the strength of pinch of both hands increased ($P < 0.001$) on treatment with riboflavin and further increased ($P < 0.001$) on combined treatment.

Other uses

13. Skin problems such as acne, dermatitis, eczema and ulcers; eye problems such as cataracts; and muscle cramps, may be improved by treatment with riboflavin supplements. Like the other B vitamins, riboflavin has been advocated in the treatment of stress, although there is little evidence for its efficacy.

Function

14. Clinically, riboflavin promotes normal growth, is required for the breakdown of fat, and assists in the synthesis of steroids and glycogen and formation of red blood cells. FAD plays roles in oxidation-reduction reactions as well, interacting with a group of enzymes known as flavoproteins. This class of proteins uses FAD to catalyse the following of many reactions: pyridine nucleotide-dependent and -independent dehydrogenations, reactions with sulphur-containing compounds, hydroxylation, oxidative decarboxylations, deoxygenations, and reduction of O₂ to hydrogen peroxide following the donation of hydrogen from donor molecules (American Medical Association 1994, United States Pharmacopeia 1994). Donor molecules transfer their hydrogens to the two reactive nitrogen groups on the riboflavin group.
15. Riboflavin helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system. It is thought also to be necessary for the absorption of iron, since it is common for iron deficiency to accompany a deficiency in riboflavin (Butler and Topham 1993).
16. Requirements for riboflavin may be increased and/or supplementation may be necessary in the following conditions: burns, chronic fever, gastrectomy, hepatobiliary tract disease, alcoholism with cirrhosis, obstructive jaundice, hyperbilirubinaemia in neonates (phototherapy used in treatment may cause photodecomposition of riboflavin by blue light), hyperthyroidism, prolonged infection, intestinal disease (coeliac, tropical sprue, regional enteritis, persistent diarrhoea, malignancy), or prolonged stress (United States Pharmacopeia 1994). Requirements may also be increased in patients receiving phenothiazines, tricyclic antidepressants and probenecid.

Deficiency

17. The syndrome associated with riboflavin deficiency is known as ariboflavinosis. Individuals who have inadequate food intake are at risk, particularly children in developing countries. Other groups prone to riboflavin deficiency include elderly persons with poor diet, chronic dieters, patients taking tranquilisers, persons who use fibre-based laxatives regularly, hypothyroidism patients, anorexic adolescent girls and women who exercise excessively.

Animal studies

18. In the majority of mammalian species, riboflavin deficiency is associated with decreased growth rate in young animals. In calves, symptoms include anorexia, alopecia, scours, excessive salivation and lacrimation, and sensitive or bleeding gums. In horses, catarrhal conjunctivitis is followed by photophobia and lacrimation (Rossoff 1974). In lambs, increased leukocyte counts and fatty degeneration of liver have been observed, while in pigs symptoms include anorexia, alopecia, rough hair and skin, dermatitis, catarrhal conjunctivitis, sensitivity to light, cataracts, swollen front legs, intestinal ulceration, anal inflammation, diarrhoea, fatty liver and vomiting. Sows show ovarian

degeneration with poor reproductive efficiency, premature farrowing, resorbed fetuses, high litter mortality, lactation failure and nerve degeneration.

19. In poultry, riboflavin deficiency causes reduced hatchability, stunted growth, poor development of down, pigeon toe, neuromalacia and liver degeneration.
20. In baboons, symptoms include leukocytopenia, anaemia, hyperkeratosis, dermatitis, behavioural changes, incoordination, ataxia, blindness and increased xanthuric acid excretion, with mild round cell infiltration and severe disturbances in serum parameters.
21. Rats show dermatitis, moderate hyperkeratosis, alopecia and some exudate around the eyes. Occasionally conjunctivitis, corneal vascularization and cataracts are seen, with anaemia, granulocytosis and lymphopenia. Testicular atrophy occurs in males and irreversible anoestrus occurs in female rats. The offspring of riboflavin-deficient animals show numerous developmental errors, particularly involving bone and teeth.
22. Anorexia followed by acute starvation can occur in cats, and cataracts have also been reported. Superficial vascularization and corneal opacities have been induced in riboflavin-deficient dogs.

Human studies

23. Riboflavin deficiency may occur as a result of inadequate nutrition or intestinal malabsorption but is not common in the developed world because this vitamin is plentiful in the food supply (United States Pharmacopoeia 1994). Simple nutritional deficiency of individual B vitamins is rare, since dietary inadequacy usually results in multiple deficiencies. Deficiency signs and symptoms include dry and cracked skin and eyes that are sensitive to bright light, itching, dizziness, insomnia, slow learning, weakness, sore throat, hyperaemia and oedema of the pharyngeal and oral mucous membranes, cheilosis, angular stomatitis, glossitis, seborrhoeic dermatitis, corneal vascularization and anaemia associated with pure red cell hypoplasia of the bone marrow. The anaemia that develops in riboflavin deficiency is normochromic and normocytic and is associated with reticulocytopenia; leukocytes and platelets are generally normal. Administration of riboflavin to deficient patients causes reticulocytosis, and the concentration of haemoglobin returns to normal. Anaemia in patients with riboflavin deficiency may be related, at least in part, to disturbances in folic acid metabolism (Gilman *et al* 1990).
24. Riboflavin deficiency may be associated with the development of cataracts. The New York State Lens Opacities Case-Control Study (Leske *et al* 1995) assessed the risk factors for various types of cataract among 1380 participants aged 40-79 years, in a case-control study. Vitamin E, selenium, and biochemistry profile determinations were performed on all patients; red blood cell enzymes and amino acids were measured in systematic samples of about 25% of the Lens Opacities Case-Control Study population. Laboratory test values in cases and controls were compared and expressed as odds ratios and 95% confidence intervals. The results

showed that persons with opacities were twice as likely to have high glutathione reductase activity (with flavin adenine dinucleotide), suggesting low riboflavin status (odds ratio, 2.13).

25. Another study (Mulherin *et al* 1996) assessed the links between riboflavin status and rheumatoid arthritis in patients and in those without the disease. Patients with rheumatoid arthritis were classified as active if there were features of articular inflammation which required initiation or change of disease modifying therapy, and as inactive if they had little evidence of articular inflammation. Erythrocyte glutathione reductase (EGR) was measured in patients and healthy controls by a functional assay with or without the addition of flavin adenine dinucleotide (FAD). The ratio of stimulated EGR (with FAD added) to basal EGR (no added FAD), which measures riboflavin status, is known as the EGR activation coefficient (EGRAC). An EGRAC of ≥ 1.3 represents biochemical riboflavin deficiency. Ninety-one patients with rheumatoid arthritis, including 57 with active disease, and 220 healthy controls were studied. Both basal and stimulated EGR were significantly higher in patients with rheumatoid arthritis ($P = 0.0001$) than in controls. Biochemical riboflavin deficiency was identified in 41% controls and 33% patients with active rheumatoid arthritis but was significantly less frequent (9%) in patients with inactive compared to active disease ($P = 0.02$) or healthy controls ($P = 0.0006$). Pain score, articular index, C reactive protein, and erythrocyte sedimentation rate were increased in patients with riboflavin deficiency (all $P < 0.02$). Higher basal and stimulated EGR might be expected in patients with rheumatoid arthritis in response to chronic oxidative stress due to synovial inflammation. The association of riboflavin deficiency with increased disease activity suggests that impaired EGR activity could be responsible for the continuing inflammation in these patients.
26. Riboflavin deficiency may arise during phototherapy of neonatal jaundice (Sisson 1987). The absorption maxima of bilirubin and riboflavin in the body are nearly identical, at 445-450 (447) nm. In consequence, blue visible light will cause photoisomerization of bilirubin accompanied by photodegradation of riboflavin. This results in diminished erythrocyte glutathione reductase, which indicates generalized tissue riboflavin deficiency and red cell lysis. Single- and double-strand breaks in intracellular DNA have occurred with phototherapy. Many newborns, especially if premature, have low stores of riboflavin at birth. The absorptive capacity of premature infants for enteral riboflavin is likewise reduced. Consequently, inherently low stores and low intake of riboflavin plus phototherapy for neonatal jaundice will cause a deficiency of riboflavin at a critical period for the newborn.
27. Riboflavin deficiency seldom occurs alone; it often is associated with pellagra and other vitamin B-complex deficiency states (e.g. alcoholism, malabsorption syndromes) (American Medical Association 1994). Therefore, ariboflavinosis should be treated with multivitamin B preparations.
28. The state of riboflavin deficiency, in conjunction with diet and general nutritional status, can markedly influence the activity of hepatic microsomal drug metabolizing enzymes. Riboflavin deficiency has been shown to impair NADPH

cytochrome c reductase, azoreductase and benzo(a)pyrene hydroxylase levels (LaDu, Mandel and Way 1971).

Dietary intake and supplementation

29. In the UK the average intake of riboflavin from all sources for men and women was 2.29 mg and 1.84 mg respectively (see Annex 1). In the sample supplements increased average intakes above those from food by 10% for men and 17% for women.
30. The minimal requirement for riboflavin to prevent clinical signs of deficiency appears to be less than 0.35 mg/1000 kcal (Gilman *et al* 1980). Horwitt *et al* (1949 & 1950) found that subjects fed 0.55 mg/day of riboflavin for 4 months developed clinical symptoms of deficiency. From the evidence assessed by COMA they concluded that group intakes of 0.5-0.8 mg/day are necessary to meet the requirements of men and women (COMA, 1991). The Reference Nutrient Intake set by COMA for adult men and women (19-50 years) is 1.3 mg/day and 1.1 mg/day respectively (COMA 1991) (see table 2).
31. The Dietary Allowances Committee of the US National Research Council recommends a riboflavin intake of 0.6 mg/1000 kcal, which is equivalent to about 1.6 mg daily for young adult and males and 1.2 mg daily for young adult females. It is recommended that intake for elderly adults should not be less than 1.2 mg daily, even when caloric intake falls below 2000 kcal. Turnover of riboflavin appears to be related to energy expenditure, and periods of increased physical activity are associated with a modest increase in requirement (Gilman *et al* 1994).

Table 2. Reference Nutrient Intakes for riboflavin for different age groups

Age	Reference Nutrient Intake Riboflavin mg/day
Infants to 6 months	0.4
6 to 12 months	0.4
1-3 years	0.6
4 to 6 years	0.8
7 to 10 years	1.0
Males 11 to 14 years	1.2
Males 15 to 50+ years	1.3
Females 11 to 50+ years	1.1
Pregnancy	+0.3
Lactation	+0.5

32. Although riboflavin is found in many foods, the amounts in an average diet are uncertain. For this reason, it is added to white flour and breakfast cereals on a voluntary basis. In the UK cereals and cereal products provide about 31% of the total riboflavin intake for young people aged 15 to 18 years (Gregory and Lowe 2000).
33. It is the practice in Finland to feed small premature infants with human milk and with no riboflavin supplementation. In the study of Ronnholm (1986) riboflavin status was analysed in 39 premature infants, 19 with riboflavin supplementation (0.3 mg/day) and 20 without, in their mothers, and in breast-milk samples during a period of 12 weeks after delivery. The mean gestational age of the infants was 30.1 weeks and their birth weight 1183 g. Stimulation of erythrocyte glutathione reductase by FAD was used as the criterion for riboflavin status in the blood samples. At age 6 weeks, 47% of the infants without supplementation had activity coefficient values indicative of riboflavin deficiency. The riboflavin status of the infants receiving supplementation was better ($P < 0.01$). The concentration of riboflavin in the human milk samples was dependent on the amount of riboflavin supplementation of the mothers during the period from 2 to 12 weeks after delivery ($P < 0.05-0.01$).

Pregnant women

34. In the study of Vir *et al* (1981) the riboflavin status of 20 non-pregnant and 60 pregnant women was determined by the erythrocyte glutathione reductase activation test. None of the non-pregnant subjects but 26 of the pregnant subjects had an activity coefficient >1.20 , indicative of biochemical deficiency of riboflavin. The deficiency developed at any of the three stages of pregnancy under study. Follow-up of individual cases revealed no progressive deterioration in riboflavin nutrition with advancement of pregnancy. The mean intake of riboflavin was higher than the recommended intake of 1.8-2 mg/day, and revealed a significant negative correlation with activity coefficient values during the third trimester. No significant correlation of riboflavin status with the outcome of pregnancy was noted. The effect of numbers of previous pregnancies, history of oral contraceptive usage, smoking, and alcohol showed no consistent effect on the percentage incidence of deficiency at all the three periods.
35. Ajayi (1985) determined the riboflavin status of 25 primigravidae and 55 multigravidae by the erythrocyte glutathione reductase activation test. Fifteen of the primigravidae (60%) and 44% (24/55) of the multigravidae had an activity coefficient (EGRAC) >1.30 , indicative of biochemical riboflavin deficiency. The incidence of riboflavin deficiency among all women in the second trimester of pregnancy was 40% (6/15), and 51% (33/65) for all women in the third trimester of pregnancy. However, no clinical signs accompanied the biochemical deficiency of riboflavin. Riboflavin nutrition was slightly better in the multigravidae than in the primigravidae. The mean EGRAC was 1.32 for the primigravidae and 1.26 for the multigravidae.
36. The level of riboflavin intake required to correct riboflavin deficiency in seven non-pregnant and 12 pregnant Filipino women was determined by Kuizon *et al*

(1992). Increasing levels of riboflavin were given to the subjects who were rated as riboflavin-deficient based on an initial EGRAC of ≥ 1.3 in screening. The minimum riboflavin requirement, defined as the intake of riboflavin required to achieve an EGRAC of < 1.3 , was estimated from the regression of EGRAC on riboflavin intake (mg/1000 kcal). The estimates of minimum riboflavin requirement from the non-pregnant women ranged from 0.16 to 0.42 with a mean of 0.35 ± 0.09 (SD) mg/1000 kcal. For the pregnant subjects, the estimates of minimum riboflavin requirement ranged from 0.36 to 0.81 with a mean of 0.58 ± 0.18 (SD) mg/1000 kcal. Adding 30% to the mean, to cover the upper limits of 97.5% of the population, the estimated US Recommended Dietary Allowances (RDA) for non-pregnant women is 0.46/1000 kcal. For pregnant women, adding 30% to the mean minimum requirement of 0.58 mg/1000 kcal, the estimated RDA is 0.75 mg/1000 kcal or 1.75 mg/day computed at the energy allowance of 2350 kcal during pregnancy.

Lactating women

37. Requirements for all vitamins and most minerals are increased during breastfeeding. Riboflavin status and dietary riboflavin intake were measured in 156 pregnant and lactating women in the Gambia, and in 59 pregnant and lactating women in Cambridge, UK (Bates *et al* 1981). The Gambian women were studied longitudinally, for up to 19 months. In the Gambia, where the mean daily riboflavin intake was 0.5 mg, the mean EGRAC was 1.78 and there was a marked deterioration of biochemical status near parturition. In Cambridge, where the mean intake was 2.3 mg/day, the mean EGRAC was 1.19. A vitamin fortified diet supplement, given to lactating women in the Gambia for 8 months, raised their mean riboflavin intake from 0.5 to 1.5 mg/day and reduced the mean EGRAC to 1.42. It is therefore likely that an intake even greater than the current United Kingdom or United States recommended daily amounts would be needed to achieve biochemical "normality" in these women.
38. In a second study by the same group (Bates *et al* 1982) 60 subjects living in two Gambian villages were given either 2 mg riboflavin or a placebo daily on a double-blind basis for 12 weeks. Their riboflavin intake from dietary sources was about 0.5 mg/day. In the supplemented group, the mean EGRAC fell from 1.62 to 1.19 within 3 weeks, and 90% had mean EGRAC < 1.3 throughout supplementation, whereas the placebo group maintained mean EGRAC between 1.6 and 1.9. Clinical signs associated with riboflavin deficiency improved more rapidly in the supplemented group; their breast milk riboflavin levels increased, and the mean EGRAC in their infants was reduced, compared with those of the placebo group. After withdrawal of the supplement, the maternal and infant EGRACs rose toward those of the placebo group. A total riboflavin intake of about 2.5 mg/day during lactation was thought to be sufficient to maintain normal biochemical status.
39. Riboflavin and vitamin B₆ status of mothers from a low-income group were assessed by erythrocyte glutathione reductase activation and erythrocyte aspartate aminotransferase activation tests respectively, at different stages of lactation (Bamji *et al* 1986). Levels of these vitamins in milk were also measured. The

majority of the women showed an increase in EGRAC, indicative of riboflavin deficiency. Levels of riboflavin in milk were in general satisfactory.

Unusual diets

40. Some unusual diets (e.g. reducing diets that drastically restrict food selection) may not supply minimum daily requirements of riboflavin. Supplementation is necessary in patients receiving total parenteral nutrition (TPN) or undergoing rapid weight loss or in those with malnutrition, because of inadequate dietary intake (United States Pharmacopeia 1994).

Interactions

Therapeutic drugs

41. Alterations in various aspects of flavin disposition have been observed following administration of certain drugs, namely, antimalarial, antimicrobial, anticancer, and some tricyclic antidepressant and antipsychotic agents. However, none of these interactions has been reported to be hazardous (Dollery 1999).
42. Prior administration of probenecid has been shown to decrease renal clearance of riboflavin and to decrease gastrointestinal absorption of riboflavin. Requirements for riboflavin may therefore be increased in patients receiving probenecid (United States Pharmacopeia 1994).
43. Riboflavin reduces the antibiotic activity of solutions of streptomycin, erythromycin, tyrothricin, carbomycin and tetracyclines. For tetracyclines, the reaction is a photochemical oxidation. No inactivation has been shown with chloramphenicol, penicillin or neomycin (Rivlin 1976).
44. Phenothiazine drugs may increase riboflavin excretion (Pelliccione *et al* 1983, Pinto and Rivlin 1987). Treatment with chlorpromazine accelerates the depletion of tissue stores of flavin adenine dinucleotide during dietary riboflavin deficiency. Earlier findings showed that low doses of chlorpromazine in rats fed abundant riboflavin increase urinary riboflavin excretion and reduce hepatic flavin stores. From 6 to 10 days after beginning to feed on a riboflavin-deficient diet, rats treated with chlorpromazine, 2 mg/kg body weight twice daily, had approximately twice the urinary riboflavin excretion of that of pair-fed saline-treated controls. When the riboflavin-deficient diets and chlorpromazine treatments were extended for 3 weeks and the animals killed, FAD levels in liver, kidney, and heart were markedly lower in drug-treated than in saline-treated animals. Brain levels of FAD by contrast were relatively resistant to both dietary riboflavin withdrawal and treatment with chlorpromazine. Urinary riboflavin excretion began to increase within 6 h of treatment with chlorpromazine.
45. The phenothiazine ring of chlorpromazine and the isoalloxazine ring of riboflavin have a number of structural features in common and have been shown to form a

molecular complex *in vitro*. In animals treated for a 3- and 7-week period with chlorpromazine, urinary levels of riboflavin are twice that of pair-fed, saline-treated animals. Recent studies have extended these findings to humans.

46. The effect of aluminium hydroxide, magnesium hydroxide, and a combination of aluminium-magnesium hydroxide suspensions on the oral absorption of riboflavin was examined in five subjects (Feldman and Hedrick 1983). Coadministration of aluminium hydroxide or magnesium hydroxide suspension with riboflavin (30 mg) resulted in an increase in time of peak urinary excretion rate of riboflavin when compared with control studies. There was no increase in the peak excretion rate or total urinary excretion of riboflavin when the antacid-treated subjects were compared to the control studies. *In vitro* experiments indicated that significant binding of riboflavin to the aluminium hydroxide and magnesium hydroxide suspensions occurred. The results of the present investigation are consistent with the reported effect of aluminium ion on GI motility and the known influence of gastric emptying on the absorption of riboflavin from the GI tract.
47. Thyroid hormones, corticotrophin and aldosterone enhance the formation of FMN and FAD (Rivlin 1979).
48. Boric acid increases the excretion of riboflavin (Rivlin 1979, Pinto and Rivlin 1987). Boric acid complexes with the polyhydroxyl ribitol side chain of riboflavin and greatly increases its water solubility. Individuals who have accidentally consumed boric acid or one of its derivatives excrete high levels of riboflavin within the first 24 to 48 hours following ingestion.
49. Riboflavin depletion has been reported in some studies in women on oral contraceptives (OC). A study by Roe *et al* (1982) compared the riboflavin requirements of healthy OC users and non-users on diets prepared in a metabolic unit. The basic diet providing riboflavin at a level of 0.6 mg/1000 kcal was used in the period of acclimation and period 1. In periods 2 and 3, the riboflavin content of the diet was increased to 0.8 and 1.0 mg/1000 kcal, respectively. The riboflavin status of subjects was monitored by erythrocyte glutathione reductase assay and urinary riboflavin excretion. Eight women on OC and 10 non-users participated. Erythrocyte glutathione reductase assay values and urinary riboflavin excretion showed intersubject and interperiod differences, but no significant group differences (OC versus non-OC) in erythrocyte glutathione reductase values or in urinary riboflavin per g creatinine. When dietary intake is controlled, OC do not significantly influence riboflavin status.

Alcohol

50. Alcohol has been reported to impair intestinal absorption of riboflavin (United States Pharmacopeia 1994), and chronic alcoholism is associated with a high prevalence of riboflavin deficiency. In the study of Pinto *et al.* (1987), rats received by gavage a liver homogenate to which either [¹⁴C]riboflavin or [¹⁴C]FAD was added with either ethanol or isocaloric sucrose solutions. Ethanol markedly diminished the bioavailability of [¹⁴C]FAD to a greater degree than that of [¹⁴C]riboflavin. Corroboration of ethanol-impaired intraluminal hydrolysis of

FAD was provided by using everted jejunal segments and measuring mucosal uptake of [¹⁴C]riboflavin together with non-radiolabelled FAD. In subsequent studies with mucosal cell extracts, ethanol markedly inhibited activities of FAD pyrophosphatase and flavin mononucleotide (FMN) phosphatase. These findings suggest that dietary sources of riboflavin (FMN and FAD) are not absorbed as well in the presence of ethanol as are vitamin preparations containing riboflavin, which is utilized more readily.

Calcium

51. Riboflavin nutrition status was evaluated in 24 healthy elderly female care home residents in the US (Alexander *et al* 1984). Riboflavin intake by history was greater than or equal to the recommended dietary allowances (RDA) for this nutrient in all but three subjects, and the average intake in the group as a whole was 50% greater than the RDA, suggesting that the nutritional value of the subjects' diet was adequate. However, calcium intake was greater than or equal to the RDA (US RDA for calcium 800 mg/day, National Research Council, 1989) in only four of the 24 subjects. The adequacy of calcium intake was found to be dependent upon a sufficiently high percentage of the total dietary intake of riboflavin being derived from milk and dairy products. It was observed that individual calcium intakes were less than 80% of the RDA unless 40% or more of the total intake of riboflavin was derived from milk and dairy products rather than from other food sources. In those subjects taking daily supplementation with a single multivitamin tablet containing low levels of riboflavin, the total intake of riboflavin and its urinary excretion were increased similarly, suggesting that even small amounts of riboflavin are not retained by elderly subjects consuming a diet adequate in riboflavin.

Iron

52. Fairweather-Tait *et al* (1992) measured iron absorption in riboflavin-deficient Gambian men with haemoglobin (Hb) <11.5 g/dl before and after oral riboflavin therapy, and the results were compared with a group not receiving riboflavin. Riboflavin status (as determined by erythrocyte glutathione reductase activation coefficient) and Hb increased in the riboflavin-supplemented but not placebo group. Plasma ferritin levels were low and did not change in either group. There were very wide variations in percentage iron absorption between individuals and also within single individuals on two separate occasions but no measurable change with riboflavin supplementation. The efficiency of iron utilization was impaired in riboflavin deficiency, although iron absorption was unaffected.
53. Two earlier studies by the same group (Powers *et al* 1988 1991) measured iron absorption and daily loss of iron in riboflavin-deficient Norwegian hooded rats and controls. Powers *et al* (1988) measured iron absorption in the rat by monitoring whole-body retention of a dose of radio-Fe (⁵⁹Fe) using a small-animal gamma-counter. Female Norwegian Hooded rats were fed on a diet deficient in riboflavin from 5 weeks of age. Control animals, fed on a complete diet, were weight-matched to rats fed on the riboflavin-deficient diet. After 7 weeks all rats were fed on a test meal extrinsically labelled with ⁵⁹Fe and whole-body

radioactivity measured for 15 days. Riboflavin deficiency was associated with a reduction in the percentage of the dose of iron absorbed and an increase in the rate of loss of iron post-absorption. A smaller percentage of the absorbed dose was present in the livers of the riboflavin-deficient animals.

54. In the study of Powers *et al* (1991) animals were fed on a test meal extrinsically labelled with ^{59}Fe and whole-body radioactivity measured for 15 days. Riboflavin deficiency led to a reduction in the percentage of the ^{59}Fe dose absorbed and an increased rate of ^{59}Fe loss. All post-absorption ^{59}Fe loss could be accounted for by faecal ^{59}Fe , confirming that the loss was gastrointestinal. Fe concentrations and ^{59}Fe as a percentage of retained whole-body ^{59}Fe were higher in the small intestine of riboflavin-deficient animals than their controls, 14 days after the test meal. A separate experiment demonstrated that riboflavin deficiency was associated with a significant proliferative response of the duodenal crypts of the small intestine. These observations may explain the enhanced Fe loss in riboflavin deficiency.
55. In the study of Butler and Topham (1993) riboflavin deficiency in rats resulted in a reduction in the transfer of ^{59}Fe from an intragastric dose to plasma compared to age- or weight-matched controls. The uptake of iron by brush-border membrane vesicles made from intestinal mucosa of riboflavin-deficient rats was much less than by identically prepared vesicles from control animals. Although the mucosal content of ^{59}Fe was smaller in riboflavin-deficient rats 30 min after dosing, the relative distribution of ^{59}Fe between the mucosal iron-binding proteins, ferritin and transferrin, was not changed compared to the control groups. These studies suggest that the impairment in iron absorption in riboflavin deficiency is primarily the result of a reduced uptake of iron into the mucosal cell and not a redistribution of iron between iron-binding proteins inside the mucosal cell.
56. Adelekan and Thurnham (1986) measured iron absorption in weanling riboflavin-deficient rats or weight-matched controls fed on appropriate diets for 7 weeks. Concentrations of ^{59}Fe in plasma were monitored every 30 min for 4 h following intragastric administration. Total Fe absorption in riboflavin-deficient rats was significantly lower than that in controls. In a separate experiment, ferritin-Fe concentrations were measured in the livers of four groups of rats (ad-lib, pair-fed and weight matched controls and riboflavin deficient) at day 0, and subsequently at days 14, 21, 28, 35 and 49. Liver ferritin-Fe concentration was significantly lower ($P < 0.05$) in RD rats than in all other controls after 3 weeks on the respective diets and remained lower for the remainder of the experiment.

Zinc

57. Riboflavin can form complexes with some metals, and supplementation with riboflavin may result in increased absorption of zinc. Agte *et al* (1998) reported the results of experiments on pregnant and lactating mice. Two groups, each of 12 mice (9 females and 3 males), were observed on a low-riboflavin rice-based diet (adequate in all other nutrients), one with and one without supplementation of 10 mg riboflavin/kg diet. There was significant improvement in growth parameters such as percent conception, mean weight gain in pregnancy, mean weight of pups at the age of 21 days, and percentage haemoglobin due to riboflavin

supplementation ($P < 0.05$). The percentages of zinc absorbed, for the low-riboflavin diet, the supplemented diet, and the synthetic control diet were 16.4 ± 5.7 , 33.7 ± 8.9 , and 44.6 ± 4.0 , respectively, indicating the beneficial effect of riboflavin supplementation on zinc utilization.

Absorption, distribution and excretion

General pharmacokinetics

58. The pharmacokinetics and utilization (flavocoenzyme synthesis) of orally and intravenously administered riboflavin in healthy humans were assessed in a study by Zemleni *et al* (1996a). After the determination of circadian rhythms of riboflavin concentrations in plasma and urine of four males and five females (control period), each subject received three different oral riboflavin doses (20, 40, and 60 mg) and one intravenous bolus injection of riboflavin (11.6 mg). Pharmacokinetic variables (Table 3) were calculated using a two-compartment open model. The maximal amount of riboflavin absorbed from a single dose was 27 mg per adult. Half-life of absorption was 1.1 h. First-order rate constants describing distribution and elimination of riboflavin were significantly higher after intravenous than after oral administration ($P < 0.01$). Release of flavocoenzymes into plasma was low compared with the increase of riboflavin concentrations. 7- α -Hydroxyriboflavin was identified in plasma. Clearance data indicated that urinary excretion of riboflavin contributes to one-half of the overall removal of riboflavin from plasma. No sex differences were observed for any of the pharmacokinetic variables ($P > 0.05$).

Table 3

Riboflavin dose (mg)	C_{\max} (nmol/l)	t_{\max} (h)	AUC_{0-11h} (nmol.h/l)	AUC_{0-24h} (nmol.h/l)	AUC_{0-48h} (nmol.h/l)
<i>Oral</i>					
20	217.7 ^a (173.5-277.0)	1.5 (1.0-2.5)	572.8 (515.6-924.2)	674.2 (535.1-1266.2)	883.9 (535.1-1412.3)
40	2.06.7 ^a (170.0-262.8)	1.4 (1.0-1.5)	751.1 (593.1-863.4)	788.2 (677.4-917.1)	886.4 (757.3-1030.5)
60	308.2 ^a (199.6-338.2)	2.0 (1.0-2.2)	801.1 (739.6-1102.9)	911.9 ^b (751.1-1191.6)	981.6 ^b (754.3-1369.6)
<i>Intravenous</i>					
11.6	1209.4 (1007.1-1371.0)	-	532.1 (486.5-726.4)	5321. (499.2-745.2)	532.1 (499.2-775.3)

^a $P < 0.01$, ^b $P < 0.05$ compared with intravenous administration.

Absorption

59. Riboflavin is readily absorbed from the small intestine and distributed to all tissues; however, very little is stored (American Medical Association 1994). Riboflavin is absorbed largely by a specialized transport mechanism that involves

phosphorylation of the vitamin to flavin mononucleotide, with only a small amount absorbed by passive diffusion. Large doses may not be absorbed quantitatively, due to rapid movement past the absorption sites (American Pharmaceutical Association 1976, Gilman *et al* 1990).

Animal studies

60. Feder *et al.* (1991) investigated absorption kinetics of riboflavin under *in vivo* conditions. The small intestine of anaesthetized rats was perfused with [¹⁴C]riboflavin in a concentration range between 0.31 and 10.00 µmol/l. Apart from the uptake of riboflavin from the perfusate, passage of the vitamin into the portal (vena portae) and peripheral (vena femoralis) blood was determined. Absorption proved to be a dual process: at low substrate concentrations (<2 µmol/l) a saturable component predominated; at higher concentrations simple diffusion was found to be the prevailing uptake mechanism. The apparent transport constant of the saturable component was calculated to be 0.38 µmol/l. [¹⁴C]Flavin concentrations in the portal and peripheral blood were estimated as a function of the riboflavin concentration of the perfusion medium. The dual character of the absorption was reflected by the portal blood flavin levels. Elimination constants were independent of the concentration of riboflavin in the perfusion media.
61. Middleton (1990) studied the uptake of riboflavin in everted sacs of rat intestine using [¹⁴C]riboflavin and [³H]polyethylene glycol to define the mechanism of mucosal membrane transport. Initial studies indicated the presence of saturable uptake in duodenum, jejunum and ileum. Studies in jejunum at low riboflavin concentrations demonstrated saturable uptake [$K_m = 0.154-0.177 \mu\text{mol/l}$, $V_{\text{max}} = 19.6-25.8 \text{ pmol}/(100 \text{ mg dry tissue}\cdot\text{min})$]. In contrast, uptake was linear with respect to higher concentrations of vitamin (10-50 µmol/l). Uptake at low (0.1 µmol/l) but not high (20 µmol/l) riboflavin concentrations was inhibited by 50 µmol/l lumiflavin, anoxia, 5 mmol/l indoacetamide, Na⁺-free buffer and low temperature. It was concluded that saturable uptake of riboflavin occurs throughout the rat small intestine, that jejunal uptake is consistent with a transport carrier located in the brush border membrane, that saturable uptake is energy-dependent and may be directly or indirectly driven by a Na⁺ gradient, and that riboflavin is also taken up by rat intestinal mucosa by a non-saturable, energy-independent mechanism consistent with simple, passive diffusion.
62. Vaziri *et al* (1985) examined the intestinal absorption of riboflavin in rats made uraemic by subtotal nephrectomy and sham-operated (control) rats *in vivo* using the recycling perfusion technique and *in vitro* using the everted-sac technique. The results showed a significant impairment of intestinal absorption of riboflavin *in vivo* in uraemic rats compared to the control group.
63. Riboflavin deficiency has been reported in older individuals. The cause of this deficiency is not known but could include a decrease in the intestinal absorptive capacity for riboflavin. The intestinal absorption of riboflavin in young (3 month) and old (26 month) rats was examined by Said and Hollander (1985). The kinetic parameters of riboflavin absorption measured were an apparent K_m of 0.37 and

0.43 μM and V_{max} of 37 and 38 pmol/g initial tissue wet wt/20 min in young and old rats, respectively. These data do not demonstrate an age-associated change in the intestinal transport capacity for riboflavin in the rat.

Human studies

64. Roe *et al.* (1988) studied the effect of dietary fibre from wheat bran and psyllium gum on the apparent absorption of riboflavin in 12 healthy women. The test fibre was consumed in crackers that contained approximately 7.5 g fibre from psyllium gum, wheat bran, or a combination of the two sources. Each subject was given a riboflavin load test after consuming one of the three different fibre supplements or a control supplement that contained 1.3 g fibre. Fractional urine collections were made for 24 h, and riboflavin was measured by fluorometric techniques. The psyllium gum and combination supplements reduced the 24-h apparent absorption of riboflavin from 31.8% to 25.4% and 26.1%, respectively ($P < 0.01$). No effect of the wheat bran supplement on riboflavin was detected.
65. The transport of riboflavin across the brush border membrane of human intestine was studied by Said and Arianas (1991). When an inwardly directed Na^+ gradient was imposed, transport of riboflavin was linear with time for approximately 20 seconds of incubation and was significantly higher than in the presence of an identical K^+ gradient. Initial rate of transport of riboflavin as a function of concentration was found to include a saturable component in the presence of an inwardly directed Na^+ gradient but was linear in the presence of an identical K^+ gradient. The apparent K_m and V_{max} of the Na^+ stimulated transport process were found to be 7.26 $\mu\text{mol/l}$ and 0.97 pmol/mg protein per 10 seconds, respectively. The addition of high concentrations of unlabelled riboflavin and its structural analogue lumiflavin to the incubation medium caused significant inhibition in the transport of ^3H -riboflavin in the brush border membrane vesicle incubated in the presence of an inwardly directed Na^+ gradient but not in vesicles incubated in the presence of an identical K^+ gradient. Inducing a more positive intravesicular space with the use of valinomycin and an inwardly directed K^+ gradient caused significant inhibition in the transport of riboflavin. On the other hand, inducing a more negative intravesicular space with the use of anions of different lipid permeabilities caused significant stimulation in the transport of riboflavin. These results demonstrate that riboflavin transport in human intestinal brush border membrane vesicle is through a carrier-mediated system. This system functions in the presence of a Na^+ -gradient and seems to transport the substrate by an electrogenic process.

66. Riboflavin can also be absorbed through the skin (Hayes and Laws, 1991).

Distribution

67. Riboflavin has been shown to be present in red blood cells (Osol and Pratt 1973).
68. The half-life of riboflavin is about 66-84 min following oral or intramuscular administration of a single large dose in healthy individuals (McEvoy 1994).

69. Riboflavin binding by plasma proteins from healthy human subjects has been examined by equilibrium dialysis using a physiological concentration of [2-¹⁴C]riboflavin (0.04 μM) (Innis *et al.*, 1985). Binding ranged from 0.080 to 0.917 pmole of riboflavin/mg protein (mean ± SD of 0.274 ± 0.206), which corresponded to 4.14 to 49.4 (15.5 ± 11.0) pmol/ml of plasma (*n* = 34). Males and females showed similar results. Albumin bound riboflavin only very weakly (K_d = 3.8-10.4 mM), although FMN and photochemical degradation products (e.g., lumiflavin and lumichrome) were more tightly bound. Binding in the gamma-globulin fraction was attributed to IgG and IgA because the binding protein(s) and immunoglobulins co-purified using various methods were removed by treatment of plasma with conjugated protein A-agarose, and were coincident upon immunoelectrophoresis followed by autoradiography to detect [2-¹⁴C]riboflavin. Differences among the plasma samples correlated with the binding recovered with the immunoglobulins. Binding was not directly related to the total IgG or IgA levels of subjects, and it appeared that the binding is due to a subfraction of these proteins.

Excretion

70. When riboflavin is ingested in amounts approximately equivalent to the minimal daily requirement, only about 10-20% appears in the urine (Osol and Pratt 1973). As the intake is increased above minimal requirements, larger proportions are excreted unchanged (Gilman *et al* 1990). Riboflavin has been shown to undergo active secretion into and saturable reabsorption from the kidney tubules in rat, dog and human (Chemical Society 1972). Probenecid appears to inhibit both tubular secretion and reabsorption of riboflavin, while decreasing its apparent volume of distribution by about one-half. However, probenecid had no effect on riboflavin elimination during haemodialysis of two functionally anephric patients, suggesting that the change in the volume of distribution in normal subjects is only an indirect result of its effect on the renal excretion of the vitamin (Chemical Society 1972).
71. Riboflavin is the primary flavin excreted in human urine but significant amounts of 7 alpha-hydroxyriboflavin and lesser amounts of 8 alpha-hydroxyriboflavin are present and reflect tissue endoplasmic reticular oxidations (Chastain and McCormick 1987). A newly found flavin catabolite of an 8 alpha-sulphonyl type may reflect intake and/or turnover of such thioether-linked flavin as occurs in monoamine oxidase. In addition, smaller amounts of 10-hydroxyethylflavin (indicative of intestinal microbial action on the vitamin) and traces of lumiflavin (arising from photodecomposition) constitute part of the remaining flavin, which reflects level of intake.
72. Biliary excretion of riboflavin in rats has been shown to be biphasic at high concentrations and monophasic at low concentrations. In biphasic excretion the initial phase lasted approximately 1 h after intravenous doses and the first order elimination rate constant was approximately 1-3 times that of the second phase (Chemical Society 1972). However, biliary excretion of riboflavin in humans has been reported as negligible (Chemical Society 1975).

73. Riboflavin is present in faeces, but this probably represents vitamin synthesized by intestinal microorganisms since, when intake is low, the amount excreted in the faeces exceeds that ingested (Gilman *et al* 1990). There is no evidence that riboflavin synthesized by the bacteria in colon can be absorbed.

Metabolism

74. Biochemically, free riboflavin is transformed in the liver to form the flavin coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). The functional moiety in both coenzymes is riboflavin's isoalloxazine ring system, which serves as a two-electron acceptor in enzymatic reductions. Enzymes that use a flavin cofactor are termed "flavoproteins".
75. The metabolism of riboflavin was studied in five female patients with liver cirrhosis (Zempleni *et al* 1996b). Following the oral administration of 40 mg riboflavin, plasma concentrations of riboflavin and flavo-coenzymes as well as urinary riboflavin excretion were analyzed over a period of 48 h. Results were compared to data obtained for healthy controls (subjects in Zempleni *et al* 1996a). About 18% of the administered vitamin was recovered from patients' urine, indicating an absorption similar to healthy subjects ($P > 0.05$). The area under the riboflavin plasma concentration versus time curve was 1.2-fold larger among patients than controls, but the difference was not significant ($P > 0.05$). Riboflavin peak concentrations in plasma (315.6 nmol/l) and times when those concentrations were achieved (3.0 h) were similar to those found for healthy subjects ($P > 0.05$). Distribution and elimination kinetics of riboflavin were also not different from controls ($P > 0.05$). No differences between the groups were found regarding renal excretion (renal clearance, first-order rate constants for renal excretion; $P > 0.05$). It was concluded that patients with liver cirrhosis did not show alterations of riboflavin turnover.

Toxicity

Human toxicity

76. No toxic or adverse reactions to riboflavin in humans have been identified, with the exception of the harmless yellow discolouration of urine at high doses (Dollery 1999), and no toxic symptoms have been reported. Because riboflavin is a water-soluble vitamin, excess amounts are excreted (Osol and Pratt 1973, American Pharmaceutical Association 1976).

Carcinogenicity studies

77. Rivlin (1986) has reviewed the effects of riboflavin nutritional status on tumour growth. Little is known regarding the possible role of riboflavin in the aetiology of human cancer. Because the mixed function oxidase system requires flavin coenzymes, it might be expected that riboflavin deficiency would retard the inactivation of carcinogens, thereby enhancing their delivery to susceptible

tissues. However, Roe (1962) studied the effects of riboflavin 0.6% in the diet in rats treated with high doses of PABA for 22 weeks and 2-BP for 24 weeks and found no increase in tumour incidence in the animals fed with riboflavin.

78. Ziegler *et al* (1986) evaluated the role of nutritional factors in the aetiology of oesophageal cancer among black men in Washington D.C. Low intake of riboflavin, carotene and vitamin C slightly increased the relative risk of oesophageal cancer (Table 4).

Table 4

Micronutrients	Relative risk by consumption level		
	High	Moderate	Low
Vitamin A	1.0	1.4	1.6
Carotene	1.0	1.4	1.6 ^a
Vitamin C	1.0	1.3	2.1 ^b
Thiamin	1.0	1.2	1.1
Riboflavin	1.0	1.1	1.6 ^b

^a $P < 0.10$, ^b $P < 0.01$.

79. In the study of Thurnham *et al* (1985), blood samples were collected for nutritional studies in two counties in China where the risks of oesophageal cancer are very different. The blood samples were used to measure the nutritional status of riboflavin (erythrocyte glutathione reductase activation coefficient), vitamin A (retinol and carotene concentrations), and zinc (plasma and hair zinc concentrations). Only riboflavin status was significantly different in the two communities. The distribution of erythrocyte glutathione reductase activation coefficient values suggested that riboflavin status was much better in the low-risk community. Both communities consumed very little in the way of animal products or fruit, but intake of these items was higher in the community with a lower risk of oesophageal cancer and higher riboflavin levels.

80. The study of Marshall *et al* (1992) compared the smoking, alcohol consumption, dental hygiene and diet of 290 cases of oral cancer with those of 290 sex-, age-, and neighbourhood-matched controls. The results suggested that among micronutrients, calcium, sodium, riboflavin and retinol were associated with increased risk, while thiamin, niacin, and dietary fibre were associated with decreased risk. Although patterns of dietary effects were discernable, the authors stressed that these effects were in general much weaker than are those of smoking and alcohol consumption.

81. The study of Olsen *et al* (1991) assessed the role of dietary factors in the aetiology of pancreatic cancer. Cases were white males aged 40 to 84 whose death certificate listed pancreatic cancer (exocrine only). White male controls were

ascertained through random-digit dialing. Family members were interviewed about the subject's dietary usage in the 2 years prior to death (cases, $n = 212$) or prior to interview (controls, $n = 220$). Energy-adjusted, nutrient-intake, risk estimates were calculated. Among all respondents, negative trends were observed for polyunsaturated fat, linoleic acid, vitamin C, and beta-carotene, but positive trends were observed for riboflavin and retinol. The nutrients associated with a decreased risk for pancreatic cancer occur primarily in vegetables and fruits, of which the consumption of cruciferous and beta-carotene-rich vegetables and citrus fruits provided the greatest reduction in risk.

82. The study of Kaul *et al* (1987) involved 55 histologically confirmed black prostate cancer patients and 55 controls who were seen at three major hospitals in Washington, DC from 1982 to 1984. Personal interviews were conducted to obtain the number of times food items of specified serving size were consumed per week by cases and controls; the subjects were grouped according to the age periods 30-49 and 50 years and older. The average daily consumption of each of 18 nutrients per 1000 calories was calculated. There was a significant negative association between linoleic acid ($P < 0.04$) for the 50 years and older group, thiamin ($P < 0.05$) for those 30-49 years old, riboflavin ($P < 0.03$) for the 50 and older group, and iron ($P < 0.05$) for those 30-49 years old. The results of this study suggest that the intake of thiamin and iron (in subjects 30-49 years old), linoleic acid and riboflavin (in subjects 50 years and over) could be protective because control subjects consumed more of these nutrients than did patients with prostate cancer.
83. Liu *et al* (1993) analysed 257 cases of cervical dysplasia, confirmed both by cytological examination and colposcopic findings and 133 controls. The study controlled for factors such as alcohol intake and smoking. Riboflavin deficiency showed increased risk at the two lower quartiles of intake with a trend test P value of 0.04, and therefore low intake of riboflavin appeared to be associated with an increased risk of cervical dysplasia.

Vulnerable groups

84. No precautions are required in groups normally regarded as high risk, i.e. neonates, children, pregnant women and the elderly.

Adverse drug reactions

85. Suspected adverse drug reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting". A number of reactions have been reported for products containing riboflavin, but the presence of other nutrients makes interpretation difficult.

Toxicity in laboratory animals

86. Riboflavin appears to be virtually non-toxic when administered orally or by injection in animals. Furia (1972) reported that 10 g/kg administered orally to rats resulted in no systemic toxic effects.

Genotoxicity

87. Riboflavin was not mutagenic in the Ames Salmonella test (see Table 5 below). Kale *et al.* (1992a) have also reported negative results with the umu test and SOS chromotest, but no further reports of results with test systems other than Ames Salmonella could be located.

Table 5. Results of mutagenicity tests with riboflavin

<i>Test</i>	<i>Strain</i>	<i>Metabolic activation</i>	<i>Method</i>	<i>Dose</i>	<i>Results</i>	<i>Reference</i>
<i>Rat</i>						
Ames <i>S. typhimurium</i>	TA97A	None	Preincubation	0.05-5 mg/plate (test material solvent: distilled water)	-ve	Fujita and Sasaki (1986)
Ames <i>S. typhimurium</i>	TA102	None	Preincubation	0.05-5 mg/plate (test material solvent: distilled water)	-ve	Fujita and Sasaki (1986)
Ames <i>S. typhimurium</i>	TA97A	Rat, liver, S-9, Arochlor 1254	Preincubation	0.05-5 mg/plate (test material solvent: distilled water)	-ve	Fujita and Sasaki (1986)
Ames <i>S. typhimurium</i>	TA102	Rat, liver, S-9, Arochlor 1254	Preincubation	0.05-5 mg/plate (test material solvent: distilled water)	-ve	Fujita and Sasaki (1986)
Ames <i>S. typhimurium</i>	TA102	None	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992b)
Ames <i>S. typhimurium</i>	TA102	None	Preincubation	25-100 µg/ml (illuminated for 5 min at 20 J/m ²)	+ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA100	None	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA100	Rat, caecal cell-free	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)

		extract				
Ames <i>S. typhimurium</i>	TA98	None	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA98	Rat, liver, S-9, Aroclor 1254	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA98	Rat, caecal cell-free extract	Preincubation	25-100 µg/ml	-ve	Kale <i>et al.</i> (1992a)
Ames <i>S. typhimurium</i>	TA97A	None	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA97A	Rat, liver, S-9, Aroclor 1254	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)
Ames <i>S. typhimurium</i>	TA97A	Rat, caecal cell-free extract	Preincubation	25-100 µg/ml	-ve	Kale <i>et al</i> (1992a)

88. Kawaguchi *et al* (1997) found induction of polyploidy by needle crystals of riboflavin in cultured Chinese hamster lung cells. However, there was no induction of polyploidy when riboflavin was used in solution. The needle crystals were shown by electron microscopy to adhere to the cell surface and to be enclosed in viscous cellular materials, and the authors therefore concluded that the induction of polyploidy was a "mechanical" effect of the crystal structure, which physically fixed the shape of the cells and prevented normal mitosis. The observation that riboflavin in solution had no such effect confirms that riboflavin itself does not appear to cause polyploidy.
89. Jazzar and Naseem (1996) examined the genotoxic effects of photoilluminated riboflavin in the presence of Cu(II). Using the phage inactivation assay, they observed a significant decline in plaque-forming unit (PFU). Results of Ames testing suggested that a frameshift mutation was caused by a riboflavin-Cu(II)-mediated reaction. Using neocuproine as a Cu(I) sequestering reagent, Cu(I) has been shown to be an essential intermediate generated in the reaction between Cu(II), photoilluminated riboflavin, and DNA. Results obtained with various scavengers of active oxygen species suggested that the species predominantly responsible for DNA damage is oxygen (O_2) in the singlet or triplet state, together with H_2O_2 , hydroxyl radical, and hydroxyl ion, to a lesser extent. In the case of riboflavin, a ternary complex of DNA-drug-Cu(II) is likely to be formed. A redox reaction, involving riboflavin and Cu(II) in the complex, may then occur with the formation of a DNA-oxidized riboflavin-Cu(I) complex. This probably acts as a catalyst for the oxidation of Cu(I) to Cu(II), during which molecular oxygen is reduced to generate a variety of active oxygen species.
90. Riboflavin was found by Joshi (1985) to generate singlet oxygen (1O_2) and superoxide anion radicals $O_2^{\cdot -}$ on exposure to UV-A (320-400 nm) and UV-B (290-320 nm) light. Azide ions (N_3^-) and 1,4 diazabicyclo-[2.2.2]-octane (DABCO) produced over 90% inhibition of deoxyguanosine (dGuo) photo-oxidation, whereas superoxide dismutase did not show any noticeable quenching effect under similar conditions. These studies indicated that dGuo is particularly sensitive to damage by reactive oxygen species and that 1O_2 was the species responsible for riboflavin-sensitized photodegradation of the guanine base of DNA and RNA.
91. Riboflavin was found by Florsheim (1994) to increase the response of mice to ionizing radiation. A dose of 10.5 Gy of gamma rays from a ^{60}Co source resulted in a dose-dependent shortening of survival times after pretreatment with riboflavin, administered at doses which were well tolerated alone. Riboflavin (120 mg/kg) reduced the survival rate to approximately 65% in the dose range from 3 to 6 Gy. In mice treated with riboflavin and irradiated with non-lethal exposures (from radiation alone) mortality occurred within the first few days after irradiation, suggesting a different type of injury than is usually associated with radiation death. Doses of riboflavin used clinically are lower than those providing enhanced radiation response in these experiments, although subtle injury caused by combined exposure to riboflavin and radiation could not be completely excluded.

92. Seekamp and Hultquist (1999) found that riboflavin had a protective effect against oxidant-mediated acute lung injury in rats. Pulmonary injury was induced by intravenous injection of cobra venom factor (CVF), by the intrapulmonary deposition of IgG immune complexes, or by hind limb ischaemia-reperfusion. In each of the three models, injury was characterized by increases in vascular permeability (leakage of ^{125}I -labeled bovine serum albumin), alveolar haemorrhage (extravasation of ^{51}Cr -labeled rat erythrocytes), and neutrophil accumulation (myeloperoxidase activity). Intraperitoneal administration of riboflavin at a dose of 6 $\mu\text{mol/kg}$ body weight reduced vascular leakage by 56% in the CVF model, by 31% in the immune complex model, and by 53% in the lung injury model following ischaemia-reperfusion of the hind limbs. Similar treatment reduced haemorrhage by 76%, 51%, and 70% in the three models of lung injury. In the CVF model, riboflavin was also shown to decrease products of lipid peroxidation (conjugated dienes) in lungs (by 45%) and in plasma (by 74%). The authors concluded that riboflavin could provide significant protection against oxidant-mediated inflammatory organ injury.
93. Activated oxygen species have been implicated in skin photosensitization and tumour promotion; nevertheless, there is no evidence that riboflavin causes such effects *in vivo*. Since excess riboflavin is rapidly eliminated from the body, the concentrations required to produce these effects in the living organism are unlikely to be achieved.

Carcinogenicity

94. Riboflavin deficiency may decrease the development of spontaneous tumours in experimental animals but may increase carcinogenesis due to certain agents (Rivlin *et al.*, 1983).
95. According to the comprehensive review of Rivlin (1986), riboflavin deficiency diminishes the rate of growth of spontaneous tumours in experimental animals but enhances the carcinogenicity of specific drugs such as the azo dyes, which are degraded by a microsomal hydroxylase system requiring riboflavin.
96. van Rensburg *et al.* (1986), found that oesophageal cancer induced by *N*-nitrosomethylbenzylamine (MBN) in rats was inhibited by riboflavin. Marked reductions in the number of tumours and tumour-bearing rats were recorded in groups of rats given supplements of riboflavin, nicotinic acid, zinc, magnesium, selenium, and molybdenum. Various combinations of nutrients did not distinctly reduce the tumour yield further; however, tissue analyses suggest that individual supplements could enhance the status of other marginally deficient nutrients. When the experiment was repeated, but was varied by commencing the supplements only well after the cessation of MBN exposure, inhibitory effects on tumorigenesis were still exerted by most nutrients. However, the authors cautioned that supplementation only appeared to be effective in the early stages of the disease, and treatment of premalignant oesophageal changes by high doses of these nutrients was not recommended.

97. A similar result was reported by Foy and Kondi (1984). Eleven male baboons fed a synthetic diet completely lacking in riboflavin developed, after 15-36 weeks, profound macroscopic and microscopic architectural disorganization of the skin, mouth, and oesophagus. The cutaneous lesions showed hyperkeratosis, gross derangement of keratinization with acanthosis, and pseudocarcinomatous hyperplasia. In five baboons that died or were killed, there were large penetrating lesions having raised epithelial edges at the lower third of the oesophagus or at the cardio-oesophageal junction, with gross epithelial hyperplasia and grossly deranged and thickened keratinization and numerous mitotic figures. None of the eight control animals given riboflavin showed these abnormalities.
98. Formation of single strand breaks in nuclear DNA induced by hepatocarcinogens aflatoxin B1 and N-nitrosodimethylamine was observed by Webster *et al.* (1996) to be more pronounced in rats maintained on a riboflavin-deficient diet compared to that on a normal diet. This increased damage was reversed on riboflavin supplementation. The induction of repair enzymes poly(ADP-ribose) polymerase, DNA polymerase beta and DNA ligase was significantly greater in riboflavin-deficient rats following DNA damage caused by the administration of carcinogens. Riboflavin supplementation brought down the induction to the levels found in rats maintained on a normal diet. Since damage to DNA and its altered repair may relate to carcinogenesis, modulation of these parameters by riboflavin suggests a potential chemopreventive role.
99. Pacernick *et al.* (1975) found that riboflavin had no effect against UV-induced skin tumours in hairless mice (*hr/hr*). Three groups of HR-hairless mice were studied. Group I served as control. Group II was painted daily with a 15 mg per ml solution of riboflavin. In Group III, drinking water was replaced with a 15 mg per ml solution of riboflavin in water. All three groups were simultaneously irradiated in a light box with two Westinghouse FS20 sunlamps from a distance of 30 cm for 5 min daily 6 days a week throughout the experiment. By the 11th month all surviving mice developed several histologically-proven squamous cell carcinomas. The total numbers and times of onset of tumours did not vary in the three groups. Thus, riboflavin in high doses had no effect, either protective or exacerbating, on ultraviolet-induced carcinogenesis in the hairless mouse when such high dose rates of UV were used.

Regulatory considerations

100. The Infant Formula and Follow-on Formula (1995) recommend an upper limit of 60µg riboflavin/100kcal for infant formula. The Foods Intended for use in Energy Restricted Diets for Weight Reduction Regulations (1997) recommend that whole diet products should provide 1.6 mg riboflavin and meal replacement products 0.48 mg. The Recommended Daily Allowance, used for food labelling purposes for riboflavin is 1.6mg.

Existing recommendations on maximum intake levels

101. COMA (1991) concluded that the low solubility of riboflavin prevents its absorption from the gastrointestinal tract in amounts sufficient to produce toxic

effects. When 120mg/day were used for 10 months to treat congenital methaemoglobinaemia, no adverse effects were reported (COMA 1991)

Existing recommendations on maximum supplementation levels

102. The European Federation of Health Product Manufacturers suggest an upper safe level of 200 mg (EHPM 1997). No upper limit for short term consumption was established

Summary

101. Riboflavin is a water-soluble vitamin (vitamin B₂) of the B group. It is widely distributed in nature, in plants and animals, as an essential constituent of all living cells, and is therefore widely distributed in small amounts in foods.
102. The syndrome associated with riboflavin deficiency is known as ariboflavinosis. Individuals who have inadequate food intake are at risk, particularly children in developing countries. Other groups prone to riboflavin deficiency include elderly persons with poor diet, chronic dieters, patients taking tranquillizers, persons who use fibre-based laxatives regularly, hypothyroidism patients and women who exercise excessively. Riboflavin deficiency may occur as a result of inadequate nutrition or intestinal malabsorption but is not common in the developed world because this vitamin is plentiful in the food supply.
103. The minimal requirement for riboflavin to prevent clinical signs of deficiency appears to be less than 0.35 mg/1000 kcal. Horwitt *et al* (1949 & 1950) found that subjects fed 0.55 mg/day of riboflavin for 4 months developed clinical symptoms of deficiency. From the evidence assessed by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) it was concluded that group intakes of 0.5-0.8 mg/day are necessary to meet the requirements of men and women (COMA, 1991). The Reference Nutrient Intake set by COMA for adult men and women (19-50 years) is 1.3 mg/day and 1.1 mg/day respectively (COMA 1991).
104. Riboflavin is readily absorbed from the small intestine and distributed to all tissues; however, very little is stored. Riboflavin is absorbed largely by a specialised transport mechanism that involves phosphorylation of the vitamin to flavin mononucleotide, rather than by passive diffusion. When riboflavin is ingested in amounts approximately equivalent to the minimal daily requirement, only about 10-20% appears in the urine (Osol and Pratt 1973). As the intake is increased above minimal requirements, larger proportions are excreted unchanged.
105. Riboflavin is virtually non-toxic when administered orally or by injection in animals. It has been reported that 10 g/kg administered orally to rats resulted in no toxic effects.
106. Photodegradation of riboflavin may result in the formation of active oxygen species, which can cause damage to DNA. However, there is no evidence that such damage occurs *in vivo*, either in humans or in animals. Since excess riboflavin is rapidly eliminated from the body, the concentrations required to produce these effects in the living organism are unlikely to be achieved.
107. No toxic or adverse reactions to riboflavin in humans have been identified, with the exception of the harmless yellow discolouration of urine at high doses, and no toxic symptoms have been reported. Because riboflavin is a water-soluble vitamin, excess amounts are excreted.

108. Riboflavin has not been shown to be carcinogenic; rather, deficiency of riboflavin may predispose to the development of some tumours, and in certain circumstances, the early correction of a riboflavin deficiency state may be beneficial in preventing tumour formation. A study (Roe 1962) on the effects of riboflavin administered by a number of routes, including orally, on the carcinogenicity of high doses of known chemical carcinogens in mice over several months showed that riboflavin did not increase the incidence of tumour formation in these animals. With regard to photodegradation of riboflavin, a single report (Florsheim, 1994) has found riboflavin to increase the response of mice to ionizing radiation. However, Pacernick *et al* (1975) found that riboflavin had no effect against UV-induced skin tumours in hairless mice.
109. There are no reports of any adverse reproductive effects of riboflavin in humans. Riboflavin has also been reported to be of low developmental toxicity in experimental animals. The current literature suggests that no precautions are required regarding the administration of riboflavin in groups normally regarded as high risk, i.e. neonates, children, pregnant women and the elderly. There is a theoretical possibility that neonates undergoing phototherapy for hyperbilirubinaemia may be at risk at this time from photoactivation of riboflavin.
110. Riboflavin has a long history of use with no reports of adverse effects in humans, although there have been no specific studies designed to investigate its tolerability. A prophylactic study of migraine in 55 patients with placebo control showed that high doses (400 mg per day) of riboflavin for at least 3 months were very well tolerated, with only two minor, non-specific adverse events reported (Schoenen *et al* 1998). Data on administration of high doses of riboflavin to animals are also very sparse. The only peer-reviewed report found in the literature is that of Furia (1972), which states that administration of 10 g/kg riboflavin orally to rats produced no adverse effects. Extrapolated to humans, this represents approximately 500,000 times the recommended daily dose of 1.5 mg on average.

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ANNEX 1 TO EVM/00/15.REVISED AUG2001

INTAKES OF RIBOFLAVIN FROM FOOD AND SUPPLEMENTS

The data presented on riboflavin intakes are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years^{1,2,3,4,5}. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

Total intakes of riboflavin

Table 1 provides information on the absolute intakes of riboflavin by the British population, classified by age and sex. Mean and median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively) are given. In addition, intakes of riboflavin from food and supplements for older people are presented both including and excluding prescribed riboflavin supplements, for comparison. Although these prescribed preparations were only taken by a small minority of participants within this group, intakes from them were found to have a disproportionate impact on mean daily intakes of some nutrients.

Average intake of riboflavin from food was lowest for pre-school children and highest in males aged 16 to 64 years. Intakes of riboflavin from food increased significantly with age for males aged 4 to 18 years, females aged 16 to 64 years and decreased significantly with age for females aged 65 years and over free-living in the community. In addition, intakes of riboflavin from all sources (food and supplements) increased significantly with age for females aged 16 to 64 years.

Intakes from food only, and from food and supplements at the 97.5%ile were about 1½-4½ times the median in all groups (except infants where data regarding intakes of riboflavin supplements is unavailable). Median riboflavin intakes (from food sources, and from all sources including prescribed supplements) were above the Reference Nutrient Intakes for each group.

Table 2 provides information on riboflavin intakes adjusted for body weight classified by age and sex. Riboflavin intakes adjusted for body weight showed a trend to decrease with age up to the 15 to 18 year age group.

¹ Food and nutrient intakes of British infants. 1986

² National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3

³ National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8

⁴ Dietary and nutritional survey of British adults. 1986/7

⁵ National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

Sources of riboflavin in the diet

Table 3 indicates the contribution made by different types of food to average intakes of riboflavin by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices.

The main source of riboflavin in this age group is milk and milk products (contributing 32% of intake, about half of which is derived from semi-skimmed milk). This is closely followed by cereal and cereal products (contributing 31%, about two thirds of which is derived from breakfast cereals), then meat and meat products (14%).

The main sources of riboflavin are similar for all other groups. Pre-school children obtain half, infants and older people about a third, and adults about a quarter of their riboflavin intake from milk and milk products. For infants, after milk and milk products, the main source of riboflavin is infant formula.

Riboflavin is often added voluntarily by manufacturers to cereal products such as cereal bars, breakfast cereals, and to some soft drinks.

Riboflavin intakes from supplements

For pre-school children dietary supplements containing riboflavin provided a negligible contribution to population average intakes of riboflavin. Dietary supplements containing riboflavin provided 2% of population average intakes for both young people and older people living in institutions. For adults and older people free-living in the community, dietary supplements containing riboflavin provided about 12% of population average intake, and 38% in females aged 65 to 74 years, falling to 26% after trimming⁶. Contributions to intakes from prescribed riboflavin supplements were small. Equivalent data for infants are not available. Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately.

Table 4 shows the number of consumers of dietary supplements containing riboflavin in each age group, together with the mean, median and range of intakes of riboflavin from supplements for those who consumed them. The highest prevalence of riboflavin supplement use was in females aged 16 to 64 years. 8% of this group took vitamin supplements containing riboflavin.

The range of intakes from supplements was wide. Maximum intakes from this source were 50mg and 71mg per day in adult females and adult males respectively, and 100mg in older people free-living in the community. These high intakes of riboflavin from supplements taken by older people were due in part to the use of high strength B complex multivitamin tablets, multivitamin and mineral supplements and yeast based supplements.

⁶ The riboflavin intake from food and supplements for one woman in the 65-74 year age group was very high and was trimmed. See table 1 for further details.

It should be borne in mind that the data for adults aged 16-64 years was collected in 1986/87 and use of supplements may have changed since then.

Diet and Nutrition Surveys Branch
Nutrition Division
Food Standards Agency
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Table 1: Total intakes of riboflavin

*data not available

Age/sex	Absolute riboflavin intake (mg/day) ⁷							
	Food Only				Food and Supplements			
	2.5% ile	Mean	Median	97.5% ile	2.5% ile	Mean	Median	97.5% ile
Infants (1986) 6-12mths M&F	0.6	1.5	1.5	2.8	*	*	*	*
Pre-school children (1992/3)								
1½-2½ yrs/M&F	0.5	1.2	1.2	2.2	0.5	1.2	1.2	2.2
2½-3½ yrs/M&F	0.4	1.2	1.1	2.3	0.4	1.2	1.1	2.5
3½-4½ yrs/M	0.5	1.2	1.2	2.0	0.5	1.2	1.2	2.1
3½-4½ yrs/F	0.5	1.1	1.1	2.0	0.5	1.2	1.1	2.2
Young people(1997/8)								
4-6 yrs/M	0.58	1.56	1.49	2.96	0.58	1.57	1.50	3.05
4-6 yrs/F	0.62	1.40	1.35	2.44	0.62	1.43	1.39	2.55
7-10 yrs/M	0.80	1.62	1.52	2.83	0.80	1.64	1.52	2.92
7-10 yrs/F	0.57	1.37	1.34	2.58	0.57	1.38	1.35	2.62
11-14 yrs/M	0.63	1.73	1.62	3.49	0.63	1.74	1.65	3.49
11-14 yrs/F	0.45	1.32	1.30	2.69	0.45	1.35	1.31	2.79
15-18 yrs/M	0.68	1.92	1.82	3.88	0.68	1.95	0.86	3.88
15-18 yrs/F	0.39	1.30	1.22	3.00	0.39	1.34	1.25	3.00
Adults (1986/7)								
16-24 yrs/M	0.83	1.96	1.91	3.66	0.85	2.18	1.93	4.56
16-24 yrs/F	0.57	1.45	1.33	2.79	0.58	1.53	1.37	3.07
25-34 yrs/M	0.92	2.08	1.95	3.54	0.92	2.43	2.00	4.02
25-34 yrs/F	0.52	1.50	1.41	2.96	0.52	1.67	1.47	3.46
35-49 yrs/M	1.00	2.14	2.03	3.69	1.00	2.24	2.05	4.28
35-49 yrs/F	0.66	1.64	1.54	3.05	0.66	1.98	1.61	4.16
50-64 yrs/M	0.92	2.11	2.08	3.59	0.92	2.33	2.08	4.32
50-64 yrs/F	0.68	1.63	1.59	2.77	0.70	2.00	1.63	5.19
Older people free-living in the community (1994/5)								
65-74yrs/M	0.70	1.78	1.70	3.28	0.71 (0.70)	1.86 (1.85)	1.73 (1.72)	4.01 (3.81)
65-74yrs/F ⁸	0.66	1.46	1.38	2.94	0.65 (0.66)	2.36 (1.96)	1.44 (1.44)	3.30 (3.31)
75-84 yrs/M	0.68	1.69	1.60	3.04	0.70 (0.69)	1.78 (1.76)	1.61 (1.61)	4.03 (3.93)
75-84 yrs/F	0.59	1.41	1.27	2.84	0.59 (0.59)	1.57 (1.56)	1.30 (1.30)	3.76 (3.76)
85 & over/M	0.71	1.64	1.55	3.34	0.71 (0.71)	1.74 (1.72)	1.57 (1.55)	4.39 (4.06)
85 & over/F	0.47	1.29	1.21	2.55	0.47 (0.47)	1.37 (1.34)	1.23 (1.22)	3.15 (2.91)
Older people living in institutions (1994/5)								
65-84 yrs/M	0.72	1.73	1.68	2.96	0.72 (0.72)	1.73 (1.73)	1.66 (1.68)	2.83 (3.00)
65-84 yrs/F	0.91	1.70	1.63	3.24	0.91 (0.91)	1.71 (1.71)	1.64 (1.66)	3.23 (3.24)
85 & over/M	0.76	1.90	1.81	3.77	0.77 (0.76)	1.93 (1.90)	1.81 (1.81)	3.76 (3.77)
85 & over/F	0.67	1.55	1.55	2.57	0.68 (0.67)	1.61 (1.61)	1.55 (1.55)	2.67 (2.58)

⁷ Data in brackets = intakes from food and supplements, excluding prescribed riboflavin supplements⁸ The riboflavin intake including supplements for 1 woman in the 65-74 year age group was very high and was trimmed. The value trimmed was 101.49mg/day. Intake for the group after trimming is given in brackets (there was no contribution from prescribed supplements in this group).

Table 2: Bodyweight adjusted riboflavin intake

Age/sex	Bodyweight adjusted riboflavin intake (mg/kg bwt /day) ⁹		
	<i>intakes from food and supplements¹⁰</i>		
	Mean	Median	97.5% ile
Infants (1986)¹¹ 6-12mths/M&F	0.16	0.15	0.33
Pre-school children (1992/3) 1½-2½ yrs/M&F	0.10	0.10	0.19
2½-3½ yrs/M&F	0.08	0.08	0.15
3½-4½ yrs/M	0.07	0.07	0.12
3½-4½ yrs/F	0.07	0.07	0.14
Young people (1997/8) 4-6 yrs/M	0.07	0.07	0.13
4-6 yrs/F	0.07	0.07	0.13
7-10 yrs/M	0.05	0.05	0.11
7-10 yrs/F	0.05	0.04	0.09
11-14 yrs/M	0.04	0.04	0.08
11-14 yrs/F	0.03	0.03	0.06
15-18 yrs/M	0.03	0.03	0.06
15-18 yrs/F	0.02	0.02	0.05
Adults (1986/7) 16-24 yrs/M	0.03	0.03	0.07
16-24 yrs/F	0.03	0.02	0.06
25-34 yrs/M	0.03	0.03	0.05
25-34 yrs/F	0.03	0.02	0.06
35-49 yrs/M	0.03	0.03	0.05
35-49 yrs/F	0.03	0.03	0.07
50-64 yrs/M	0.03	0.03	0.05
50-64 yrs/F	0.03	0.03	0.08
Older people free-living in the community (1994/5) 65-74 yrs/M	0.02	0.02	0.06
65-74 yrs/F	0.03	0.02	0.07
75-84 yrs/M	0.02	0.02	0.05
75-84 yrs/F	0.03	0.02	0.07
85 and over/M	0.03	0.02	0.07
85 and over/F	0.02	0.02	0.06
Older people living in institutions (1994/5) 65-84 yrs/M	0.03	0.02	0.04
65-84 yrs/F	0.03	0.03	0.05
85 and over/M	0.03	0.03	0.05
85 and over/F	0.03	0.03	0.06

⁹ Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

¹⁰ Data includes intakes from prescribed riboflavin supplements.

¹¹ Intakes for infants aged 6-12 months are from food only.

Table 3: Sources of riboflavin in the diet¹²

Food Type	Contribution of food types to average daily intake of riboflavin		
	mg/day	% of total	
Cereal and cereal products	0.506	31	
<i>- of which breakfast cereals</i>	<i>0.331</i>		<i>20</i>
Milk and milk products	0.516	32	
<i>- of which semi-skimmed milk</i>	<i>0.247</i>		<i>15</i>
Egg and egg dishes	0.040	2	
Fat spreads	0.000	0	
Meat and meat products	0.224	14	
Fish and fish dishes	0.020	1	
Vegetables, potatoes and savoury snacks	0.104	6	
Fruits and nuts	0.016	1	
Sugar, confectionery and preserves	0.066	4	
Beverages	0.078	5	
Miscellaneous	0.050	3	
Total intake from food	1.62	100*	
<i>Intake from dietary supplements</i>	<i>0.03</i>	<i>2</i>	
Total intake from food and supplements	1.65	100	

*Total allows for rounding

¹² NDNS: young people aged 4-18 years. 1997/8. 15-18 year group.

Table 4: Riboflavin intake from supplements¹³

<i>Age/sex</i>	Consumers of riboflavin supplements		Riboflavin intake from supplements (consumers only) (mg/day)		
	<i>Number</i>	<i>%</i>	<i>Mean</i>	<i>Median</i>	<i>Range</i>
<i>Infants (1986)</i> 6-12 mths/M&F	*	*	*	*	*
<i>Pre-school children (1992/3)</i> 1½-4½ yrs/M&F	77	5	0.6	0.5	0.1 – 1.6
<i>Young people (1997/8)</i> 4-6 yrs/M&F	17	5	0.6	0.4	0.0 – 1.2
7-10 yrs/M&F	13	3	0.7	0.6	0.0 – 1.6
11-14 yrs/M	5	2	0.5	0.2	0.1 – 0.8
11-14 yrs/F	5	2	1.3	1.5	0.2 – 2.0
15-18 yrs/M	5	3	1.2	1.0	0.7 – 2.0
15-18 yrs/F	8	4	1.0	0.9	0.1 – 1.6
<i>Adults (1986/7)</i> 16-64 yrs/M	44	4	5.2	1.8	0.2 – 71.4
16-64 yrs/F	92	8	3.1	1.7	0.0 – 50.0
<i>Older people free-living in the community (1994/5)</i> 65 and over/M	35	5	1.7	1.6	0.1 – 6.6
65 and over/F	41	6	8.6	1.6	0.0 – 100.0
<i>Older people living in institutions (1994/5)</i> 65 and over/M	3	1	0.9	0.2	0.1 – 2.0
65 and over/F	6	3	1.0	0.1	0.1 – 3.3

* No data available

¹³ Data includes intakes from prescribed riboflavin supplements.

ANNEX 2 TO EVM/00/15.REVISED AUG2001

Riboflavin (B₂) : Summary table of selected nutrition related information and existing guidance on regulations

Unit of usage	mg/day		mg/100 kcal
	Male	Female	
<i>UK DRV¹⁴ for adults (19-50+)</i>			
LRNI	0.8	0.8	
EAR	1.0	0.9	
RNI	1.3	1.1	
<i>Mean adult UK dietary intake from food (all sources)</i>			
Adults 16-64 years ¹⁵	2.08 (2.29)	1.57 (1.84)	
Adults 65 years and over ¹⁶	1.74 (1.82)	1.43 (1.76)	
free living	1.80 (1.80)	1.62 (1.65)	
institutionalised			
EU labelling RDA ¹⁷	1.6		
Supplemental doses	0.2 – 100 mg/unit		
Regulations			60 µg/100 kcal
Infant formula ¹⁸			
Weight reduction ¹⁹	1.6		
whole daily diet replacement	0.48 mg/meal		
meal replacement			
<i>Maximum total safe daily intake</i>			
COMA 1991 ¹	120		
EHPM 1997 ²⁰	Upper safe level 1,500		
	Upper limit 3,000		

¹⁴ Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

¹⁵ Dietary and nutritional survey of British adults. 1986/7

¹⁶ National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

¹⁷ The Food Labelling Regulations 1996

¹⁸ The Infant Formula and Follow-on Formula Regulations 1995

¹⁹ The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.

²⁰ Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.

