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Abbreviations:
EGRac, erythrocyte glutathione reductase activation coefficient; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; Hb, haemoglobin; NDNS, National Diet and Nutrition Survey; PLP, pyridoxal 5'-phosphate; RCTs, Randomised Controlled Trials

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Anaemia during pregnancy: could riboflavin deficiency be implicated?

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Abstract

Anaemia affects more than 36 % of all pregnancies globally and is associated with significant maternal and neonatal morbidity and mortality. Iron deficiency is widely recognised as the most common nutritional cause of anaemia but other nutrient deficiencies are also implicated, including the B vitamin riboflavin, albeit its role is largely under-investigated and thus typically overlooked. Riboflavin, in its co-factor forms flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), is required for numerous oxidation-reduction reactions, antioxidant function and in the metabolism of other B vitamins and iron. While clinical deficiency of riboflavin is largely confined to low-income countries, sub-clinical (functional) deficiency is much more widespread, including in high-income countries, and is particularly common among women of reproductive age and during pregnancy. Limited observational evidence from high-income populations suggests that suboptimal riboflavin status contributes to an increased risk of anaemia. Furthermore, randomised controlled trials in pregnant women from low- and middle-income countries have demonstrated beneficial effects of riboflavin on haematological status and anaemia. Various mechanisms have been proposed to explain the contribution of riboflavin deficiency to anaemia, with the strongest evidence pointing to an adverse effect on iron metabolism, given that riboflavin co-factors are required for the release of iron from storage ferritin in the production of red blood cells. Overall, this review investigates riboflavin intakes and status during pregnancy in different populations and evaluates the available evidence for the under-recognised role of riboflavin in the maintenance of haemoglobin concentrations together with its potential to protect against the development of anaemia during pregnancy.

Anaemia affects 36 % of pregnancies globally, presenting a major public health problem across low, middle and high-income populations[1]. Given the global burden of the condition, and the inadequacy of public health efforts to substantially reduce prevalence rates globally for more than three decades, a better understanding regarding the role of nutritional factors other than iron is required, with the potential to offer new and innovative strategies to manage anaemia in pregnancy. The B vitamin riboflavin is involved in iron metabolism, although its role in the prevention of anaemia is largely overlooked. This review aims to explore riboflavin intakes and status during pregnancy in different populations, to evaluate the evidence for the under-recognised role of riboflavin in the maintenance of haemoglobin (Hb) concentrations and its potential to protect against the development of anaemia during pregnancy.

Burden of anaemia in pregnancy

Anaemia in pregnancy is associated with maternal morbidity and impaired quality of life, an increased risk of post-partum haemorrhage, greater need for blood transfusion and a high rate of maternal mortality[2–4]. Anaemia is also associated with adverse offspring outcomes including preterm birth, low birthweight, small for gestational age, stillbirth and a higher risk of perinatal death[3,4]. In addition, there is evidence of impaired growth and neuro-developmental outcomes in children born to anaemic mothers[5]. Furthermore, iron deficiency, even within high-resource settings, is associated with poorer cognitive performance in children[6]. Apart from the adverse health impacts for the mother and baby, anaemia in pregnancy may also pose a significant economic burden, with evidence that it is associated with reduced work capacity and productivity, and increased economic loss[7,8]. Iron deficiency is responsible for the majority of anaemia cases globally, and is the most common micronutrient deficiency worldwide[9].

The double burden of malnutrition continues to be a major global health problem, whereby the presence of micronutrient deficiencies exist even among seemingly over-nourished populations. Iron deficiency is common among women of reproductive age in the UK. Some 49 % of British girls (11–18 years) and 25 % of women (19–64 years) report iron intakes below the lower reference nutrient intake, with anaemia and low iron stores presenting in 9 %

and 5 % of girls and women, respectively[10]. The average daily iron intake among women in the UK is 10 mg/d, 10–15 % of which is estimated to be absorbed[11]. Although iron absorption is enhanced during pregnancy, requirements also increase to 27 mg/d, almost double that of a non-pregnant woman[11]. The late foetal and early postnatal periods are critical stages of rapid brain development, increased neural plasticity and high nutritional requirements[12]. As pregnancies progress, the risk of developing anaemia increases, owing to higher iron requirements, increased maternal erythropoiesis and haemodilution[13]. Plasma volume and red blood cell mass are both known to expand during pregnancy, but as plasma volume expansion exceeds the rate of red blood cell mass production, haemodilution occurs[14]. This leads to the characteristic fall in Hb concentrations that commences during the first trimester, reaching the lowest levels at the end of the second trimester[14].

Increased iron requirements during pregnancy, coupled with poor dietary intake, makes pregnancy a vulnerable time for the development of anaemia. In particular, vegans, vegetarians, those with short interpregnancy intervals, multiparous women, teenagers and women from lower socio-economic backgrounds are all at a higher risk for developing anaemia[11]. In low- and middle-income populations, hereditary blood disorders and infections such as hookworm and malaria also contribute substantially to the overall prevalence[15]. Apart from iron deficiency, which is the most common and well-known nutritional cause of anaemia, other nutrients are also implicated; namely, folate, vitamin B_{12}, vitamin B_6 and riboflavin, with the latter two B vitamins typically receiving less research and public health attention.

Riboflavin intake and status

Riboflavin, also known as vitamin B_2, is an essential nutrient which exists in two co-factor forms, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). As reviewed extensively elsewhere[16], these co-factors play crucial roles in oxidation-reduction reactions involved in energy metabolism, intermediary metabolism, the maintenance of antioxidant status, as well as the metabolism of iron. Riboflavin co-factors are also involved in the metabolism of other B vitamins including folate, vitamin B_{12}, vitamin B_6 and niacin, which are important for the normal functioning of one-carbon metabolism[16]. Riboflavin status is determined using the functional biomarker assay, erythrocyte glutathione reductase activation coefficient (EGRac), which is considered the gold standard measurement of status. However, due to the rather laborious and time-consuming nature of this assay, including the requirement for thrice-washing of cells at the blood processing stage, this assay is rarely performed in human studies or as part of national nutrition surveys. An EGRac value $\geq$1·40 is the most commonly accepted cut-off to indicate a riboflavin deficiency[16].

Riboflavin status during pregnancy is very rarely reported in human studies. In fact, even among non-pregnant populations, very little is known about the global prevalence of riboflavin deficiency, with most studies (including population-based surveys) relying on reported dietary intake data only, without corresponding biomarker assessment. Clinical riboflavin deficiency has been described in low- and middle-income countries[16]. Using convenience sampling, riboflavin deficiency has been reported among an estimated 90 % of adults in China[17], 76 % of adults in Guatemala[18], 71 % of reproductive-aged women in Malaysia[19], and up to 92 % of reproductive-aged women in Cambodia[20]. In high-income populations, whilst such severe deficiency is rare, a growing body of evidence suggests that sub-clinical (functional) deficiency is more widespread than generally recognised, particularly among reproductive-aged women[16,21]. In the most recent UK National Diet and Nutrition Survey, 67 % of girls and 58 % of women were found to have a suboptimal or deficient riboflavin status[22].

Table 1. Food sources of riboflavin in Irish adults*

Food	Riboflavin (mg/100 g)	Riboflavin (mg/serving)
Cow's milk	0·23	0·46/200 ml glass
Fortified breakfast cereal	1·30	0·39/30 g bowl
Fortified cereal bars	0·78	0·35/1 bar
Yoghurt	0·27	0·34/125 g pot
Eggs	0·47	0·24/1 egg
Chicken (breast, grilled)	0·19	0·23/120 g
Yoghurt drinks	0·22	0·22/100 ml
Cheese	0·43	0·13/30 g

*Source: Data from the National Adult Nutrition Survey (NANS); 2008–2010.

Clinical signs and symptoms of riboflavin deficiency (ariboflavinosis) may occur following prolonged periods of having dietary intakes below 0·5 mg/d, and typically present in tissues with rapid cellular turnover such as the epithelia. A sore throat, hyperaemia and oedema of the mouth and tongue, cheilosis, angular stomatitis, glossitis and normochromic, normocytic anaemia are among the reported signs of deficiency[23]. Riboflavin deficiency rarely occurs in isolation and is often accompanied by other nutritional deficiencies. While there are no clinically obvious signs and symptoms associated with a biochemical riboflavin deficiency, these are common in many high-income populations and have been associated with an increased risk of hypertension, disturbed metabolism of other B vitamins within the one-carbon cycle, and impaired iron metabolism[16].

In typical Western diets, milk and dairy products make the largest contributions to riboflavin intakes, accounting for 22 % and 27 % of total riboflavin intakes in Irish and UK diets respectively[24,25]. Other rich dietary sources of riboflavin have been summarised in Table 1 and include fortified cereal products. Riboflavin is highly bioavailable, with an estimated absorption of up to 95 %, and a maximal uptake of up to 27 mg per meal, with little or no absorption at doses higher than this[26]. Furthermore, although certain bacteria within the gut microbiome have been shown to synthesise riboflavin, it remains unclear whether this can make a meaningful contribution to help meet dietary needs in the host[27]. In the US and Canada, mandatory riboflavin enrichment policies have been in place over many years, at levels of 4 mg/kg for flour, with the aim of replacing the riboflavin lost from grain during milling[28]. Population-based data from the US suggests dietary riboflavin intakes among reproductive-aged women are sufficient to meet requirements, and are higher than estimates from similar populations in the UK and Ireland (Table 2). In the absence of nationally representative biomarker data, however, the

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Table 2. Official dietary riboflavin recommendations (mg/d) in pregnant and non-pregnant women globally

	UK (1991)	US (1998)	Netherlands (2000)	France (2001)	WHO (2004)	Nordic* (2014)	DACH† (2015)	EFSA‡ (2017)
Pregnant women (mg/d)	1·4	1·4	1·4	1·6	1·4	1·6	1·3 (2nd trimester) 1·4 (3rd trimester)	1·9
Non-pregnant women (mg/d)	1·1	1·1	1·1	1·5	1·1	1·3	1·0	1·6

*Nordic: Denmark, Finland, Iceland, Norway, Sweden.
†D-A-CH: Germany, Austria, Switzerland.
‡EFSA: European Food Safety Authority.

adequacy of riboflavin status in the US and Canada remains unclear, though analysis of a convenience sample of reproductive-aged women from Canada recently revealed functional riboflavin deficiency among 40 %[29].

Metabolic roles of riboflavin in pregnancy

Pregnancy is associated with a progressive fall in riboflavin status as maternal riboflavin is accreted for the placenta and developing foetus[23]. In fact, the status of most B vitamins has been shown to fall over the course of pregnancy due to increased requirements for the developing foetus, accretion of vitamins by the placenta, hormonal changes and haemodilution, as reviewed elsewhere[30] (Fig. 1). Infants born to deficient mothers in The Gambia were found to be riboflavin deficient at birth, and to remain so throughout the first two years of their life in the absence of any intervention[31]. Given the higher requirements for riboflavin in pregnancy and poor status among women of reproductive age generally, riboflavin deficiency during pregnancy is likely to be a significant but largely unrecognised problem, with consequences for both maternal and offspring health; the extent of which have not been fully investigated.

Very few studies have considered the impact of maternal riboflavin deficiency in pregnancy on maternal or offspring outcomes. However, in our recent observational analysis of the OptiPREG study, involving more than 2000 pregnancies, we provide new evidence that riboflavin may be important for the maintenance of blood pressure during pregnancy[32]. A small study of 372 pregnant women from the Netherlands reported that dietary riboflavin intake was positively associated with foetal growth, though in the absence of biomarker measurements, the nature of this association remains unconfirmed[33]. Maternal riboflavin intake around the time of conception was previously associated with alternations in the DNA methylation of offspring[34], although further research would be required to elucidate the phenotypic consequences of such alterations. Historical studies from pregnant animals showed skeletal and soft tissue abnormalities among offspring born to riboflavin-deficient rats and mice[35]. Other animal studies documented impaired gastrointestinal development in the presence of a riboflavin-deficient diet during weaning[36], suggesting that riboflavin in early life, possibly including the in-utero period, may play a role in gastrointestinal maturation[37]. Impairments in the gastrointestinal tract may lead to altered nutrient absorption and thus could explain the possible relationship between riboflavin deficiency and foetal growth.

Dietary riboflavin recommendations during pregnancy

The recommendations for riboflavin intake are increased during pregnancy compared to non-pregnant women, albeit with significant variations in estimated requirements from different countries/regions (ranging from 1·3 to 1·9 mg/d, as summarised in Table 3). Recommendations for riboflavin have often been based on limited, outdated evidence, using extrapolations from adult data rather than evidence from pregnancy cohorts, making this an area clearly deserving more research[23]. In a sample of 156 Gambian pregnant women with low riboflavin intakes (0·5 mg/d), riboflavin deficiency was reported (mean EGRac of 1·78), with a further marked deterioration of status near parturition, suggesting that late pregnancy may be associated with especially high riboflavin requirements[38]. Interestingly, when a fortified dietary

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Table 3. Dietary intakes and status of riboflavin among reproductive-aged women from population-based cohorts

Population	Years	Sample size	Method of dietary assessment	Riboflavin intakes (mg/d)	Riboflavin status (% deficient)
USA (*National Health and Nutrition Examination Survey; NHANES*)*	2017–2020	*n* 3962	2 × 24-h dietary recall	Mean (SE): 1·76 (0·04)	Not identified. No riboflavin biomarkers assessed
UK (*National Diet and Nutrition Survey; NDNS*)†	2014–2016	*n* 632	Four-day estimated food diary	Mean (SD): 1·42 (0·58) Median intake: 1·39	58 % with riboflavin deficiency (EGRac >1·30)
ROI (*The National Adult Nutrition Survey; NANS*)‡	2008–2010	*n* 640	Four-day weighed food diary	Mean (SD): 1·60 (0·60) Median intake: 1·50	64 % with riboflavin deficiency (EGRac >1·30)

*Women aged 20 years or above.
†Women aged 19–64 years.
‡Women aged 18–64 years.
Abbreviations: EGRac, erythrocyte glutathione reductase activation coefficient; ROI, Republic of Ireland; UK, United Kingdom; USA, United States of America.

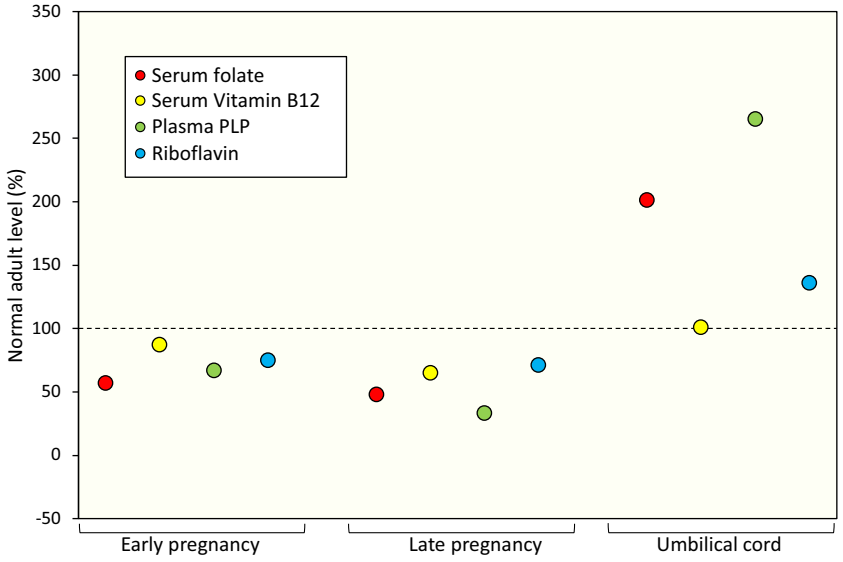


Figure 1. B vitamin biomarkers during pregnancy in unsupplemented mothers and in umbilical cord blood. B-vitamin biomarker values are represented as a percentage of the biomarker values in non-pregnant, non-lactating unsupplemented women (punctured line). Early pregnancy and Late pregnancy are defined as <14 and >36 gestational weeks, respectively. Adapted from Pentieva *et al.* (2024)[30].

supplement (which increased mean riboflavin intakes to 1·5 mg/d) was provided to these women for 8 months during lactation, riboflavin status improved (mean EGRac of 1·42), albeit biomarker values remained within the deficient range. It could be speculated, therefore, that intakes greater than the current UK recommendations (1·4 mg/d) would be needed to achieve biochemical and functional normality in these women[38]. Moreover, a small, non-randomised intervention from the Philippines reported higher riboflavin requirements among pregnant compared to non-pregnant women[39]. In support of these earlier findings, we recently reported biochemical riboflavin deficiency among 31 % of Irish pregnant women, despite 64 % reporting to take a riboflavin supplement (mean dose of 2 mg/d, predominantly in the form of a prenatal multivitamin)[32]. A noteworthy randomised trial of 1729 women from the UK, Singapore and New Zealand showed modest reductions in plasma concentrations of riboflavin from preconception to early pregnancy, and again from early to late pregnancy. In this study, intervention with 1·8 mg/d of riboflavin from preconception through to delivery led to substantial improvements in status compared to the control group, though some 67 % and 82 % of participants were still deemed to have insufficient status in early and late pregnancy, respectively[40]. Overall, these findings call into question the adequacy of current riboflavin intake recommendations in meeting the higher requirements during pregnancy, particularly so for countries such as the UK and USA where requirements are set at only 1·4 mg/d, suggesting that official dietary riboflavin recommendations may need to be reconsidered.

Riboflavin and anaemia

Biochemically confirmed riboflavin deficiency has been implicated in the development of anaemia, and the observational data linking riboflavin with anaemia are summarised in Table 4. Preliminary findings from our OptiPREG study showed for the first time within a high-income population of over 2000 pregnancies, that riboflavin status was a predictor of Hb concentrations and risk of anaemia[42]. Notably, riboflavin status was reported to be a significant predictor of anaemia risk (Hb < 120 g/l) in a sample of over 400 non-pregnant women from The Lao People's Democratic Republic, where poor riboflavin status (EGRac > 1·30) was found among 97 % of women sampled[41]. Likewise, in a cohort of reproductive-aged women in Canada and Malaysia, a deficient status of

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Table 4. Observational studies investigating riboflavin in relation to haematological status or anaemia in women

Author (y)	Population	Measures	Findings
Hess *et al.*, 2023[41]	Women (*n* 436) and their children (*n* 427) from Lao People's Democratic Republic	Riboflavin status (EGRac) Haematological indicators Ferritin Soluble transferrin receptor Thiamine diphosphate Retinol-binding protein	• Poor riboflavin status (EGRac >1·30) was present among 97·1 % of women and 71·1 % of children. • EGRac was associated with anaemia in women but not children.
Duffy *et al.*, 2021[42]	Pregnant women from Northern Ireland and the Republic of Ireland (*n* 2153)	Riboflavin status (EGRac) Haematological indicators Ferritin Vitamin B_6 Folate Vitamin B_{12}	• Riboflavin status (EGRac) was a significant predictor of Hb and risk of anaemia during pregnancy.
Aljaadi *et al.*, 2019[19]	Women aged 19–45y from Canada (*n* 206) and Malaysia (*n* 210)	Riboflavin status (EGRac) Haematological indicators Ferritin Soluble transferrin receptor Vitamin A Folate Vitamin B_{12}	• Riboflavin deficiency (EGRac ≥ 1·40) was present in 39·8 % and 71·4 % of Canadian and Malaysian women. • EGRac was correlated with Hb. • Riboflavin deficiency was associated with a 2-fold greater risk of developing anaemia.
Shi *et al.*, 2014[43]	Women (*n* 733) and Men (*n* 520) from China	3-day food diary Validated FFQ Ferritin Hb	• Riboflavin intakes below the Chinese EAR were found in 98 % of women. • Inadequate riboflavin intakes were associated with an increased risk of anaemia after 5 years of follow up.

Abbreviations: EAR, estimated average requirement; EGRac, erythrocyte glutathione reductase activation coefficient; FFQ, food frequency questionnaire; Hb, haemoglobin.

riboflavin was found to be a significant predictor of Hb concentrations and associated with a 2-fold greater risk of anaemia[44]. Moreover, evidence from over 700 Chinese women showed than inadequate dietary riboflavin intakes were associated with an increased risk of anaemia after 5 years of follow-up[43].

Randomised controlled trials (RCTs) from pregnant populations in low- and middle-income countries have demonstrated favourable effects on haematological status when riboflavin was administered alone or in combination with other nutrients (such as iron, folate or vitamin A; summarised in Table 5). An RCT of Indonesian pregnant women found that the greatest improvements in Hb concentrations were observed among those supplemented with riboflavin (5 mg) in combination with iron, compared with those supplemented with iron alone, iron and folate, or iron and vitamin A[48]. Similarly, Ma *et al.* (2008), found additional improvements in Hb concentrations and a reduced prevalence of anaemia among pregnant women from rural China when riboflavin (1 mg) and vitamin A were administered in addition to iron and folate supplementation, compared to iron and folate alone[47]. Decker *et al.* (1977) documented a higher erythrocyte count among pregnant women after supplementation with riboflavin (9 mg) and iron, compared with iron alone. Furthermore, the fall in Hb concentrations and haematocrit over the course of pregnancy was smaller (though insignificant) when riboflavin was administered in combination with iron[50]. A study of over 800 Cambodian women found that daily iron supplementation for 12 weeks increased Hb concentrations; however, the addition of multiple micronutrient supplementation containing riboflavin (1·4 mg) did not confer additional significant benefit[45]. Inconsistencies in results may be attributed to differences in baseline riboflavin status which were not measured in all studies, differences in the additional micronutrients included in trials, the riboflavin dose and the duration of interventions.

The majority of studies to date investigating the relationship between riboflavin and anaemia risk have been limited to low- and middle-income populations where clinical riboflavin deficiency is endemic owing to low dietary intakes of dairy products. Less is known about the role of riboflavin in cases where deficiency is less severe; however, one RCT has been conducted within a high-income population, albeit in young women, rather than pregnant women. Women aged 19–25 years who were riboflavin deficient, non-supplement users were randomised to either placebo or riboflavin (at doses of either 2 mg/d or 4 mg/d). There were no significant effects of treatment on haematological status when data for all participants were analysed together. However, when changes in Hb concentrations were examined relative to EGRac values at baseline, a clear pattern emerged, such that women with the worst riboflavin status at baseline (EGRac > 1·65) showed the greatest increase in Hb in response to riboflavin intervention[46]. This indicates that riboflavin supplementation may be effective in improving haematological status only under the condition of riboflavin deficiency.

Mechanisms linking riboflavin with iron metabolism and anaemia

Various mechanisms have been proposed to explain the relationship between riboflavin status and Hb. Evidence from animal studies shows that riboflavin deficiency can reduce iron absorption. In riboflavin-deficient rats, the total radiolabelled iron absorbed from a test meal was significantly lower than that which was absorbed from control rats[51,52]. When similar experiments were conducted in humans, however, these findings could not be confirmed, possibly due to the large variability in iron absorption among participants, thus requiring further investigation[46,53].

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Table 5. Randomised controlled trials investigating riboflavin intervention (alone or combined supplementation) in relation to haematological parameters and anaemia among pregnant, lactating and reproductive-aged women

Author (y)	Population	Intervention	Duration	Outcomes
Karakochuk *et al.*, 2017[45]	Women aged 18–45y from Cambodia (*n* 808)	Iron MMN containing Riboflavin (1·4 mg) Iron+MMN Placebo	12 wk	• Iron supplement (+/–MMN) increased Hb. • MMN did not appear to confer any additional benefit.
Powers *et al.*, 2011[46]	Women aged 19–25y with poor riboflavin status (EGRac >1·40) from The United Kingdom (*n* 117)	Riboflavin (2 mg) Riboflavin (4 mg) Placebo	8 wk	• Women with worst riboflavin status (EGRac >1·65) had greatest increase in Hb and RBCs in response to riboflavin.
Ma *et al.*, 2008[47]	Anaemic pregnant women from Rural China (*n* 366)	Iron-FA Iron-FA + Vit A Iron-FA + Riboflavin (1 mg) Iron-FA + Riboflavin (1 mg)+Vit A	2 mo	• Reduced anaemia prevalence and greatest Hb increase in iron-FA+ riboflavin + vit A group. • Increased reported wellbeing in the riboflavin group.
Suprato *et al.*, 2002[48]	Anaemic pregnant women from Indonesia (*n* 103)	Iron-FA Iron-FA + Vit A Iron-FA + Riboflavin (5 mg) Iron-FA + Riboflavin (5 mg)+Vit A	60 d	• Greatest Hb improvement in iron-FA+riboflavin group (Hb increased 0·8 g/dl)
Powers *et al.*, 1985[49]	Pregnant (*n* 18) and lactating (*n* 63) women from The Gambia (*n* 81)	Iron Iron+Riboflavin (5 mg) Riboflavin (5 mg/d) Placebo	6 wks	• Increased plasma iron and plasma ferritin among lactating women in riboflavin+iron group compared to placebo.
Decker *et al.*, 1977[50]	Pregnant women with poor riboflavin status (*n* 200)	Iron Iron+Riboflavin (9 mg)	2 mo	• Increase in erythrocyte count in riboflavin+iron group compared to iron alone.

Abbreviations: EGRac, erythrocyte glutathione reductase activation coefficient; FA, folic acid; Hb, haemoglobin; MMN, multiple micronutrient; RBC, red blood cell; vit, vitamin.

Animal studies have suggested that riboflavin deficiency may increase gastrointestinal iron losses via increased shedding of mucosal cells from the gastrointestinal tract[54,55]. Moreover, young rats fed a riboflavin-deficient diet upon weaning exhibited morphological changes within the intestinal villi compared with controls[36]. It is thought that the gastrointestinal development which occurs during critical periods in utero and during weaning may be irreversible and that riboflavin deficiency during these periods can precede lifelong changes such as a reduction in the number of villi, leading to a reduction in absorptive surface area[56]. Thus, impaired gastrointestinal development is another route by which riboflavin deficiency may contribute to reduced iron absorption, but this requires further research.

Of all the mechanisms which have been proposed, the evidence linking riboflavin to iron utilisation or mobilisation is the most convincing. This involvement is almost certainly due to a flavin-dependent release of iron from ferritin[57,58]. Iron is retained in the protein shell of ferritin and its release is dependent upon the reduction of ferritin from Fe^{+3} to Fe^{+2} by a reducing agent[59]. The reduction and release of ferritin-bound iron is crucial for the production of red blood cells. Numerous reducing agents have been found to be capable of mobilising iron from ferritin, however, only reduced riboflavin and riboflavin co-factors; FMN and FAD, have been shown to be effective in doing so at a physiologically relevant rate in vitro[58,60]. For this reason, riboflavin deficiency may impair iron handling, resulting in lower Hb concentrations.

Finally, riboflavin may be implicated indirectly in Hb synthesis through its metabolic relationship with vitamin B_6 and the requirement for riboflavin to generate the active form of B6 in tissues. Specifically, pyridoxine 5′-phosphate oxidase requires riboflavin in its co-factor form FMN for the conversion of pyridoxine 5′-phosphate and pyridoxamine 5′-phosphate to pyridoxal 5′-phosphate (PLP), the active vitamin B_6 form[61]. PLP is a co-factor for δ-aminolevulinate synthase in the erythrocytes, which is a crucial enzyme that catalyses the first step of haem biosynthesis[62]. A prospective study of Japanese pregnant women who were both anaemic and B6 deficient demonstrated improvements in Hb concentrations following intervention with vitamin B_6[63]. Moreover, within a recent, non-randomised trial of anaemic, Egyptian pregnant women, vitamin B_6 supplementation in combination with iron resulted in greater increases in Hb concentrations, compared to iron therapy alone over a short 3-week period of intervention[64]. This study, however, did not include measurements of vitamin B_6 status. The metabolic dependency of PLP on riboflavin was demonstrated in recent studies from our centre, including a large-scale investigation of over 5600 Irish adults which showed that PLP concentrations decreased in a stepwise manner according to riboflavin status from

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optimal, to suboptimal to deficient, independently of B6 intake[65,66]. It could be speculated, therefore, that riboflavin may be a rate-limiting nutrient for the maintenance of normal plasma PLP concentrations, with knock-on effects for haem biosynthesis. Further research is required to determine the metabolic interaction between riboflavin and vitamin B_6 in pregnancy and to understand the extent of their roles both independently and together in the context of anaemia during pregnancy.

Conclusions

Riboflavin deficiency during pregnancy is a significant, but unrecognised, problem globally, with associated adverse maternal and foetal health outcomes, the full extent of which is not known primarily because riboflavin biomarkers are rarely measured in human studies. Although overall dietary riboflavin intakes are often reported to be sufficient in most populations, the lowest intakes are typically found in young women. Correspondingly, high rates of functional (subclinical) riboflavin deficiency have been reported in reproductive-aged women in convenience samples and in the very few nationally representative samples from high-income populations where riboflavin status has been assessed. Poor riboflavin status among women entering pregnancy, coupled with increased requirements during pregnancy, makes this an especially vulnerable time for the development and progression of riboflavin deficiency. The limited available data suggests that current dietary riboflavin recommendations during pregnancy may be insufficient to meet the increased demands. In low- and middle-income countries, where status is poorest, RCTs in pregnant women have demonstrated beneficial effects of riboflavin on haematological status. In high-income populations, limited observational evidence suggests that suboptimal riboflavin status contributes to an increased risk of anaemia, but further investigation, in the form of randomised trials in women before and during pregnancy, is warranted. If shown to be effective, interventions to improve riboflavin status - through improved diet, food fortification or supplementation – could offer a novel and effective means of reducing the global burden of anaemia in pregnancy.

References

1. Stevens GA, Paciorek CJ, Flores-Urrutia MC, *et al.* (2022) National, regional, and global estimates of anaemia by severity in women and children for 2000–19: a pooled analysis of population-representative data. *Lancet Glob Health* **10**, 627–639.
2. Harrison RK, Lauhon SR, Colvin ZA, *et al.* (2021) Maternal anemia and severe maternal morbidity in a US cohort. *Am J Obstet Gynecol MFM* **3**, 1–23.
3. Nair M, Churchill D, Robinson S, *et al.* (2017) Association between maternal haemoglobin and stillbirth: a cohort study among a multi-ethnic population in England. *Br J Haematol* **179**, 829–837.
4. Randall DA, Patterson JA, Gallimore F, *et al.* (2019) The association between haemoglobin levels in the first 20 weeks of pregnancy and pregnancy outcomes. *PLoS One* **14**, 1–12.
5. Quezada-Pinedo HG, Cassel F, Duijts L, *et al.* (2017) Maternal iron status in pregnancy and child health outcomes after birth: a systematic review and meta-analysis. *Nutrients* **13**, 1–17.
6. McCarthy EK, Kiely ME, Hannon G, *et al.* (2017) Microcytosis is associated with low cognitive outcomes in healthy 2-year-olds in a high-resource setting. *Br J Nutr* **118**, 360–367.
7. Blakstad MM, Nevins JEH, Venkatramanan S, *et al.* (2020) Iron status is associated with worker productivity, independent of physical effort in indian tea estate workers. *Appl Physiol Nutr Metab* **45**, 1360–1367.
8. Haas JD & Brownlie T (2001) Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *J Nutr* **131**, 676–690.
9. De Benoist B, McLean E, Cogswell M, *et al.* (2008) Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. *Public Health Nutr* **12**, 444–454.
10. NDNS (2020) *National Diet and Nutrition Survey Rolling Programme Results from Years 9 to 11 (Combined)*. London: Public Health England.
11. Pavord S, Daru J, Prasannan N, *et al.* (2020) UK guidelines on the management of iron deficiency in pregnancy. *Br J Haematol* **188**, 819–830.
12. Georgieff MK, Brunette KE & Tran PV (2015) Early life nutrition and neural plasticity. *Dev Psychopathol* **27**, 411–423.
13. Scholl TO (2005) Iron status during pregnancy: setting the stage for mother and infant. *Am J Clin Nutr* **81**, 1218–1222.
14. Perry D & Lowndes K (2010) Blood disorders specific to pregnancy. In *Oxford Textbook of Medicine*. Oxford: Oxford University Press.
15. Petry N, Olofin I, Hurrell RF, *et al.* (2016) The proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: a systematic analysis of national surveys. *Nutrients* **8**, 1–17.
16. McNulty H, Pentieva K & Ward M (2023) Causes and clinical sequelae of riboflavin deficiency. *Annu Rev Nutr* **43**, 101–122.
17. Campbell T, Brun T & Junshi C (1990) Questioning riboflavin recommendations on the basis of a survey in China. *Am J Clin Nutr* **51**, 436–445.
18. Boisvert WA, Castaneda C, Mendoza I, *et al.* (1993) Prevalence of riboflavin deficiency among Guatemalan elderly people and its relationship to milk intake. *Am J Clin Nutr* **58**, 85–90.
19. Aljaadi AM, How RE, Loh SP, *et al.* (2019) Suboptimal biochemical riboflavin status is associated with lower hemoglobin and higher rates of anemia in a sample of Canadian and Malaysian women of reproductive age. *J Nutr* **149**, 1952–1959.
20. Whitfield KC, Karakochuk CD, Liu Y, *et al.* (2015) Poor thiamin and riboflavin status is common among women of childbearing age in rural and urban Cambodia. *J Nutr* **145**, 628–633.
21. McNulty H, Ward M, Hoey L, *et al.* (2019) Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges. *Proc Nutr Soc* **78**, 449–462.
22. Bates B, Cox L, Nicholson S, *et al.* (2016) National diet and nutrition survey: results from years 1–4 (combined) of the rolling programme. Executive summary. *Public Health Engl* **4**, 1–24.
23. EFSA (2017) Dietary reference values for riboflavin. *EFSA J* **15**, 1–65.
24. NDNS (2014) *National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009–2011/2012)*. London: Public Health England.
25. Kehoe L, Walton J, Hopkins SM, *et al.* (2018) Intake, status and dietary sources of riboflavin in a representative sample of Irish adults aged 18–90 years. *Proc Nutr Soc* **77**, 4919.
26. Zempleni J, Galloway JR & McCormick DB (1996) Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* **63**, 54–66.
27. Bedani R, Cucick ACC, Albuquerque M, *et al.* (2024) B-group vitamins as potential prebiotic candidates: their effects on the human gut microbiome. *J Nutr* **154**, 341–353.
28. FDA (2019) *Nutritional Quality Guidelines for Foods: Subpart B—Fortification Policy*. Silver Spring, MD: Food and Drug Administration.
29. Aljaadi AM, Wiedeman AM, Barr SI, *et al.* (2021) Dietary riboflavin intake and riboflavin status in young adult women living in metro Vancouver, Canada. *Curr Dev Nutr* **5**, 1–8.
30. Pentieva K, Caffrey A, Duffy B, *et al.* (2024) B-vitamins during pregnancy: health impacts and challenges. *Proc Nutr Soc* **0**, 1–15.
31. Bates CJ, Prentice AM, Paul AA, *et al.* (1982) Riboflavin status in infants born in rural Gambia, and the effect of a weaning food supplement. *Trans R Soc Trop Med Hyg* **76**, 253–258.
32. Duffy B, McNulty H, Pentieva K, *et al.* (2023) Riboflavin status in pregnancy and its relationship with blood pressure, heart rate and risk of hypertension: findings from the OptiPREG observational study. *Proceedings 14th Eur Nutr Conf FENS* **91**, 1–2

https://doi.org/10.1017/S0029665124007468 Published online by Cambridge University Press

33. Badart-Smook A, Houwelingen AC, Monique D, *et al.* (1997) Fetal growth is associated positvely with maternal intake of riboflavin and negatively with maternal intake of linoleic acid. *J Am Diet Assoc* **97**, 886–870.
34. Dominguez-Salas P, Moore SE, Baker MS, *et al.* (2014) Maternal nutrition at conception modulates DNA methylation of human metastable epialleles. *Nat Commun* **5**, 1–7.
35. Warkany J & Nelson RC (1942) Congenital malformations induced in rats by maternal nutritional deficiency. *J Nutr* **23**, 321–333.
36. Williams EA & Powers HJ (1995) Morphological changes in the rat small intestine in response to riboflavin depletion. *Br J Nutr* **34**, 141–146.
37. Powers HJ (2003) Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* **77**, 1352–1360.
38. Bates CJ, Prentice AM, Paul AA, *et al.* (1981) Riboflavin status in Gambian pregnancy and lactating women and its implications for recommended dietary allowances. *Am J Clin Nutr* **5**, 928–935.
39. Kuizon MD (1998) Riboflavin requirements of Filipino children and non-pregnant, pregnant and lactating women: studied by the erthrocyte glutathione reductase activation test. *Asia Pac J Clin Nutr* **7**, 41–48.
40. Godfrey KM, Titcombe P, El-Heis S, *et al.* (2023) Maternal B-vitamin and vitamin D status before, during and after pregnancy, and the influence of supplementation preconception and during pregnancy: NiPPeR double-blind randomized controlled trial. *PLoS Med* **20**, 1–27.
41. Hess SY, Smith TJ, Sitthideth D, *et al.* (2023) Risk factors for anaemia among women and their young children hospitalised with suspected thiamine deficiency in northern Lao PDR. *Matern Child Nutr* **20**, 1–11.
42. Duffy B, Pentieva K, Ward M, *et al.* (2021) Impact of riboflavin status on haemoglobin and risk of anaemia in pregnancy. *Proc Nutr Soc* **80**, OCE1.
43. Shi Z, Zhen S, Wittert GA, *et al.* (2014) Inadequate riboflavin intake and anemia risk in a Chinese population: five-year follow up of the Jiangsu nutrition study. *PLoS One* **9**, 1–8.
44. Aljaadi AM, How RE, Loh SP, *et al.* (2019) Suboptimal biochemical riboflavin status is associated with lower hemoglobin and higher rates of anemia in a sample of Canadian and Malaysian women of reproductive age. *J Nutr* **149**, 1952–1959.
45. Karakochuk CD, Barker MK, Whitfield KC, *et al.* (2017) The effect of oral iron with or without multiple micronutrients on hemoglobin concentration and hemoglobin response among nonpregnant Cambodian women of reproductive age: a 2 × 2 factorial, double-blind, randomized controlled supplementation trial. *Am J Clin Nutr* **106**, 233–244.
46. Powers HJ, Hill MH, Mushtaq S, *et al.* (2011) Correcting a marginal riboflavin deficiency improves hematologic status in young women in the United Kingdom (RIBOFEM). *Am J Clin Nutr* **93**, 1274–1284.
47. Ma AG, Schouten EG, Zhang FZ, *et al.* (2008) Retinol and riboflavin supplementation decreases the prevalence of anemia in Chinese pregnant women taking iron and folic acid supplements. *J Nutr* **138**, 1946–1950.
48. Suprapto B, Widardo & Suhanantyo (2002) Effect of low-dosage vitamin A and riboflavin on iron-folate supplementation in anaemic pregnant women. *Asia Pac J Clin Nutr* **11**, 263–267.
49. Powers HJ, Bates CJ & Lamb WH (1985) Haematological response to supplements of iron and riboflavin to pregnant and lactating women in rural Gambia. *Hum Nutr Clin Nutr* **39**, 117–129.
50. Decker K, Doris B, Glatzle D, *et al.* (1977) Riboflavin status and anaemia in pregnant women. *Ann Nutr Metab* **21**, 17–19.
51. Adelekan DA & Thurnham DI (1986) The influence of riboflavin deficiency on absorption and liver storage of iron in the growing rat. *Br J Nutr* **56**, 171–179.
52. Powers HJ (1988) The effect of riboflavin deficiency in rats on the absorption and distribution of iron. *Br J Nutr* **58**, 381–387.
53. Fairweather-Tait SJ, Powers HJ, Minski MJ, *et al.* (1992) Riboflavin deficiency and iron absorption in adult gambian men. *Ann Nutr Metab* **36**, 34–40.
54. Miyaji K & Hara Y (1966) Effect of riboflavin or iron deficiency on the cell renewal rate of intestinal mucosa. *J Vitaminol* **12**, 89–97.
55. Powers HJ, Weaver LT, Austin S, *et al.* (1991) Riboflavin deficiency in the rat: effects on iron utilization and loss. *Br J Nutr* **65**, 487–496.
56. Yates CA, Evans GS & Powers HJ (2001) Riboflavin deficiency: early effects on post-weaning development of the duodenum in rats. *Br J Nutr* **86**, 593–599.
57. Zaman Z & Verwilghen RL (1977) Effect of riboflavin deficiency on activity of NADH-FMN oxidoreductase (ferriductase) and iron content of rat liver. *Biochem Soc Trans* **5**, 306–308.
58. Sirivech S, Frieden E & Osaki S (1974) The release of iron from horse spleen ferritin by reduced flavins. *Biochem J* **143**, 311–315.
59. Anderson GJ & Frazer DM (2017) Current understanding of iron homeostasis. *Am J Clin Nutr* **106**, 1559S–1566S.
60. Ulvik RJ (1983) Reduction of exogenous flavins and mobilization of iron from ferritin by isolated mitochondria. *J Bioenerg Biomembr* **15**, 151–160.
61. McCormick DB (1989) Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* **69**, 1170–1198.
62. EFSA (2016) Dietary reference values for vitamin B_6. *EFSA J* **14**, 1–79.
63. Hisano M, Suzuki R, Sago H, *et al.* (2010) Vitamin B_6 deficiency and anemia in pregnancy. *Eur J Clin Nutr* **64**, 221–223.
64. Ghannam MAMEEl, Hussain SH, Osman AMA, *et al.* (2023) Iron versus iron and vitamin B_6 supplementation in treatment of iron deficiency anemia during second trimester of pregnancy: quasi experimental trial. *Open J Obstet Gynecol* **13**, 814–824.
65. Jungert A, McNulty H, Hoey L, *et al.* (2020) Riboflavin is an important determinant of vitamin B-6 status in healthy adults. *J Nutr* **150**, 2699–2706.
66. Jarrett H, McNulty H, Hughes CF, *et al.* (2022) Vitamin B-6 and riboflavin, their metabolic interaction, and relationship with MTHFR genotype in adults aged 18–102 years. *Am J Clin Nutr* **116**, 1767–1778.

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