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The role of vitamins in the prevention and control of anaemia

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Abstract

Objective: While iron deficiency is regarded as the major cause of nutritional anaemia, changes in vitamins A, B_{12}, C and E, folic acid and riboflavin status have also been linked to its development and control. This paper provides a systematic review of vitamin supplementation trials relating to the control of nutritional anaemia.
Methods: A MEDLINE search was used to find reports of vitamin supplementation trials that reported changes in anaemia or iron status.
Results: Vitamin A can improve haematological indicators and enhance the efficacy of iron supplementation. Both folate and vitamin B_{12} can cure and prevent megaloblastic anaemia. Riboflavin enhances the haematological response to iron, and its deficiency may account for a significant proportion of anaemia in many populations. Vitamin C enhances the absorption of dietary iron, although population-based data showing its efficacy in reducing anaemia or iron deficiency are lacking. Vitamin E supplementation given to preterm infants has not reduced the severity of the anaemia of prematurity. Vitamin B_6 effectively treats sideroblastic anaemia. Multivitamin supplementation may raise haemoglobin (Hb) concentration, but few studies have isolated the effect of multivitamins from iron on haematological status.
Conclusions: In general, the public health impact of vitamin supplementation in controlling anaemia is not clear. Neither are the complex interactions involving multiple vitamins in haematopoiesis sufficiently understood to explain the observed variability in haematological responses to vitamins by age, population, vitamin mixture and dosages. Further research is needed to understand the roles of individual and combined vitamin deficiencies on anaemia to design appropriate micronutrient interventions to prevent anaemia.

Keywords
Vitamin
Nutrient
Supplement
Anaemia
Haemoglobin

More than two billion people in the world, including an estimated two-thirds of children and women of reproductive age in developing countries, suffer from iron deficiency[1]. Half of those deficient in iron have or will develop anaemia, clinically defined as low blood Hb concentration or low haematocrit (Hct), the volume fraction of packed red cells, using various cut-offs suggested for different life-stage groups (Table 1)[2]. While low intake of bioavailable iron may be regarded as the underlying cause of anaemia in most instances, other widespread factors can produce or contribute to the disorder, including infections such as malaria and hookworm, dietary deficiencies of other nutrients, malabsorption, blood loss, acquired immune deficiency syndrome (AIDS), genetic defects such as sickle cell disease, metabolic disorders and repeated pregnancy[3–5]. Approximately 50% of women and children in Africa and South Asia, 25% in Latin America, and 10% in industrialized nations are anaemic[6]. Anaemia has been associated with numerous, poor health-related outcomes such as impaired cognition, reduced work capacity, increased maternal morbidity and mortality, low birth weight, and increased fetal and neonatal death[7–10].

Controlled trials provide evidence that adequate iron supplementation improves iron status and prevents anaemia, but there are various physiological, economic, social and logistical obstacles to achieving its effectiveness in practice[5]. The maintenance of normal haematopoietic function also requires adequate levels of many other nutrients acting in concert. While deficiencies of such 'accessory' nutrients may occur in isolation, they usually exist in combination. Unfortunately, the roles and mechanisms by which many nutrients influence the pathogenesis or prevention of anaemia remain obscure. Figure 1 illustrates some of the basic features of iron metabolism and erythropoiesis, emphasizing points in the process at which certain vitamins may influence iron deficiency and anaemia. Vitamins such as vitamin A, folic acid, vitamin B_{12}, riboflavin and vitamin B_6, are necessary for the normal production of red blood cells, while others such as vitamins C and E protect mature red blood cells from premature destruction by free radical oxidation (Table 2). Riboflavin, vitamin A and vitamin C may also prevent anaemia by improving intestinal absorption of iron, or by facilitating its mobilization from body stores. This paper explores the effects of these vitamins in the treatment and

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Table 1 Haemoglobin and haematocrit cut-offs used to define anaemia among different population groups. (From WHO/UNICEF/UNU[2])

Group	Haemoglobin below	Haematocrit below
Children 6 months to 5 years	110 g l^{-1}	0.33
Children 5–11 years	115 g l^{-1}	0.34
Children 12–13 years	120 g l^{-1}	0.36
Non-pregnant women	120 g l^{-1}	0.36
Pregnant women	110 g l^{-1}	0.33
Men	130 g l^{-1}	0.39

prevention of anaemia in human populations and identifies areas for future research.

Methods

Controlled vitamin supplementation and fortification trials that reported changes in anaemia (by Hb or Hct indicators) or iron status were considered for review. Studies were identified, first, by a MEDLINE search using combinations of the following keywords: vitamin, multivitamin, nutrient, anaemia, haemoglobin, iron deficiency and supplement. This was followed by a search of references cited by relevant studies, and a search of recent editions of non-MEDLINE nutrition journals. The search focused primarily on English-language human studies published since 1967,

Table 2 Mechanisms by which vitamin deficiencies can play roles in the development of anaemia

Vitamin deficiency	Possible role in anaemia through:
Vitamin A	Impaired mobilization of iron stores Impaired erythropoiesis Increased susceptibility to infection
Folic acid	Impaired DNA synthesis, leading to ineffective erythropoiesis
Vitamin B_{12}	Impaired metabolism of folate, leading to ineffective erythropoiesis
Riboflavin	Impaired iron mobilization Impaired globin production, leading to impaired erythropoiesis Reduced intestinal absorptive capacity
Vitamin C	Reduced absorption of iron Reduced mobilization of iron from stores Impaired folate metabolism Oxidant damage to erythrocytes, leading to haemolysis Capillary haemorrhaging, leading to blood loss
Vitamin E	Oxidant damage to erythrocytes, leading to haemolysis
Vitamin B_6	Impaired haem synthesis, leading to impaired erythropoiesis

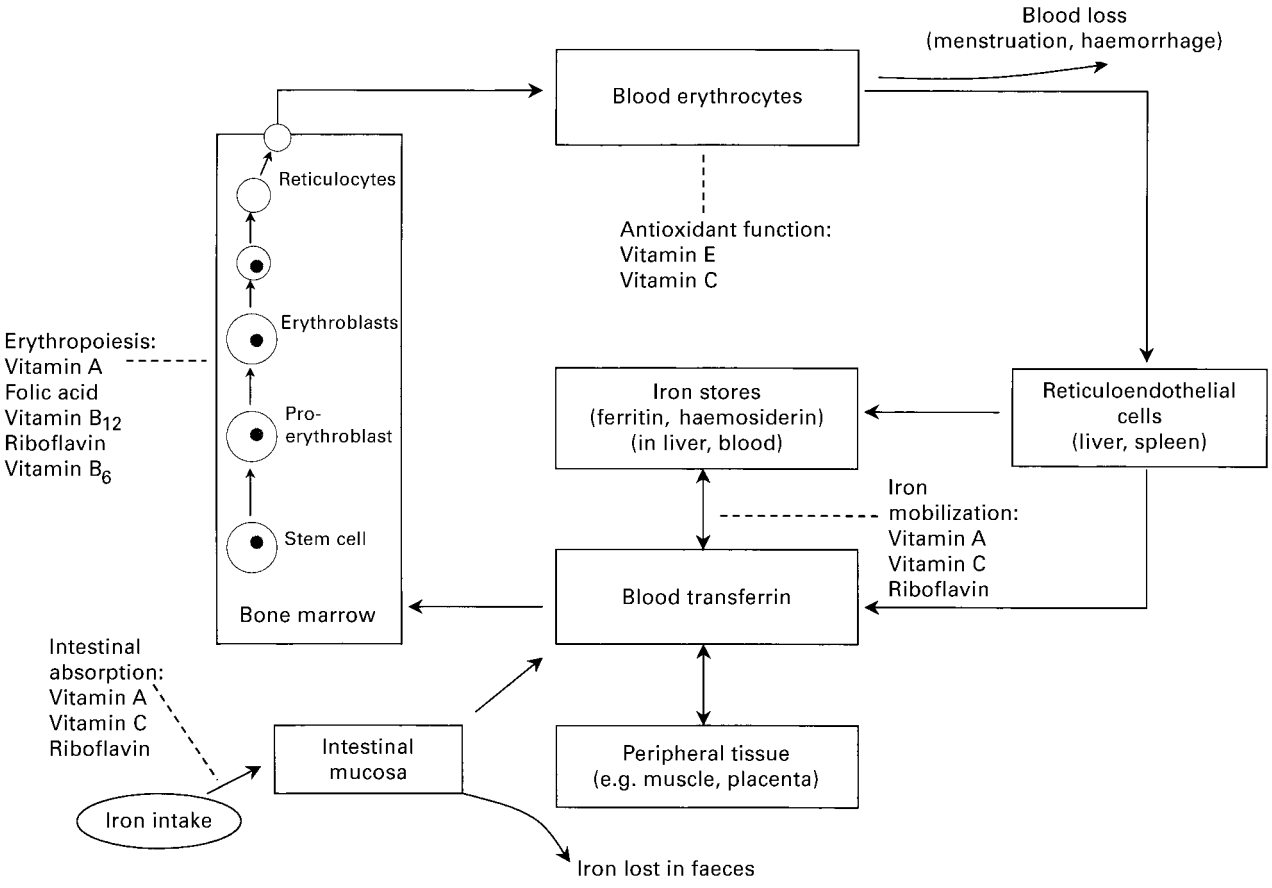

Fig. 1 Vitamin roles in iron metabolism and erythropoiesis. (Adapted from Hughes-Jones & Wickramasinghe[57])

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although a number of seminal early papers are cited to provide historical perspective.

Vitamin A

An estimated 190–255 million preschool-aged children throughout the world are vitamin A deficient, with some 3–5 million having xerophthalmia, and 500 000 becoming blind and dying each year[11–14]. Vitamin A deficiency may be responsible for 25–35% of all early childhood deaths in high risk regions of the developing world, attributed to increased severity of infection in a deficient state[15–17].

There appears to be a causal relationship between vitamin A deficiency and anaemia. Early studies of vitamin A-deficient rats reported haematological disturbances such as losses of haematopoietic tissue in bone marrow, hypochromia, depressed Hb concentration and splenic accumulation of haemosiderin. Interpretation of these effects was complicated by results from other studies showing that initial declines in Hb levels and erythrocyte counts were followed by increases in packed cell volumes and Hb levels as deficiency progressed, creating apparent polycythaemia rather than anaemia[18–21]. The increase in blood Hb level seen in some studies has been attributed to haemoconcentration resulting from dehydration and diarrhoea associated with severe vitamin A deficiency[20]. Restoration of vitamin A to the diet of deficient animals was followed by regeneration of the bone marrow, disappearance of haemosiderin from the spleen and liver, and enhanced erythroblastic activity[22].

In humans, cross-sectional studies show positive correlations between serum retinol concentration and Hb that are more apparent with poorer vitamin A status and possibly age, at least in children. Chronically mild to moderately vitamin A-deficient children are more likely to be anaemic than their non-deficient peers[13]. Six Central American nutrition surveys and biochemical studies in Ethiopia and Bangladesh observed modest, positive correlations between circulating retinol and Hb levels in children (r *c*.0.21), suggesting that serum retinol accounts for 4–10% of the variation in Hb concentration[23–25]. The correlation was slightly stronger among severely vitamin A-deficient school-aged children (r *c*.0.31)[23]. A weaker relationship was observed in Central American children aged 1–4 years (r *c*.0.13, $P > 0.05$)[23]. Although no association was observed among 1–8-year-old hyporetinolaemic Thai children[26], a strong correlation (r *c*.0.52) between Hb and plasma retinol concentration was observed among anaemic (Hb $< 110\,g\,l^{-1}$), malnourished school-aged Indian children[27]. An even stronger mean correlation (overall r= 0.78) between Hb and plasma retinol was reported from nutritional surveys of non-pregnant, non-lactating women of reproductive age in eight developing countries[28]. Intervention trials among women, however, suggest a more complex relationship (see below).

Positive haematological responses to vitamin A, most consistently reflected in increased Hb and serum iron concentrations, have been observed among children and pregnant women, whether the vitamin A was delivered as a regular supplement, a single dose or a fortified food item. Mejia and Arroyave found that 6 months after the start of a vitamin A sugar-fortification programme that provided approximately 330–360 μg retinol equivalents (RE) per child per day, serum iron levels of preschool children had increased (+0.81 $\mu mol\,l^{-1}$) and serum ferritin concentrations had declined (−3.0 $\mu g\,l^{-1}$), suggesting that existing body iron stores were mobilized to increase iron availability to tissues[29]. After 18 and 24 months, serum iron, transferrin saturation and serum ferritin were higher than baseline levels[29,30]. While strongly suggestive of a vitamin A response, there was no comparison group against which these changes could be judged, and Hb concentrations were not measured.

Vitamin A trials employing concurrent comparison groups to evaluate impact on anaemia are summarized in Table 3. Among Indonesian preschoolers, consuming *c*.240 μg RE day^{-1} from vitamin A-fortified monosodium glutamate (MSG) for 5 months significantly increased Hb concentration by *c*.10 $g\,l^{-1}$, while Hb concentrations in a concurrent control group remained about the same (−2 $g\,l^{-1}$)[31]. Hb did not increase further after six additional months of vitamin A-fortified MSG intake despite continued improvement in vitamin A status, suggesting that dietary iron, or possibly other anaemia prevention measures, may have been required to further improve Hb concentration[13,31].

Mejia and Chew studied the effect of supplementing anaemic Guatemalan children aged 1–8 years daily with vitamin A (1500–3000 μg RE) or iron (3 $mg\,kg^{-1}$) for 2 months[32]. Supplementation with vitamin A alone elevated the concentration of serum iron by 2 $\mu mol\,l^{-1}$, transferrin saturation by 3%, and Hb by 9 $g\,l^{-1}$ but had no effect on serum ferritin (i.e. apparent iron stores). Vitamin A plus iron produced positive gains in Hb (14 $g\,l^{-1}$) and ferritin (5 $\mu g\,l^{-1}$), but these increments were similar to the responses observed with iron alone. Vitamin A and iron combined, however, increased transferrin saturation (by another *c*.5%) and serum iron (by another 4 $\mu mol\,l^{-1}$) more than either supplement alone. The findings suggest that adequate vitamin A status can help maintain adequacy of plasma iron to supply body tissues, including bone marrow, which may in turn enhance haematopoiesis[32]. Supporting this inference are the significant increases in Hb (+6 $g\,l^{-1}$), Hct (+0.02) and plasma iron (+2.33 $\mu mol\,l^{-1}$) reported among xerophthalmic Indian children aged 4–12 years[27] who were given 8 mg of retinyl palmitate daily for 2–3 weeks and the improved Hb concentrations following weekly vitamin A supplementation (3030 μg RE $week^{-1}$) among refugee preschool-aged children in Belize (an increase of *c*.12 $g\,l^{-1}$ vs. 4 $g\,l^{-1}$ in the placebo group)[33].

The effect of vitamin A on risk of anaemia appears to be more variable in pregnancy than in childhood. Panth *et al.* observed a significant, but transient, rise in Hb

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Table 3 Vitamin A supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Muhilal *et al.* (1988)[31]	Indonesia, preschool children (445)	5 months	Unfortified MSG 240 μg RE VA/day fortified MSG	–2.0 10.0**	Not reported	Randomization of villages not specified
Mejia & Chew (1988)[32]	Guatemala, children 1–8 years (115)	2 months	Placebo 1500–3000 μg RE VA/day 3 mg/kg/day Fe 3 mg/kg/day Fe + 1500–3000 μg RE VA/day	3.2 9.3 13.8** 14.2**	Not reported	Anaemic population Blinding status unknown
Smith *et al.* (1999)[33]	Belize, preschool children (51)	6 months	Placebo 70 mg Zn/week 3030 μg RE VA/week 70 mg Zn + 3030 μg RE VA/week	4.0 8.0* 12.0** 11.0**	Not reported	Children selected for low/marginal initial serum Zn and VA concentrations
Panth *et al.* (1990)[34]	India, pregnant women (450)	6–24 weeks	60 mg Fe/day 1800 μg RE VA/day + 60 mg Fe/day	Not reported	Not reported	Analysis of Hb changes was cross-sectional
Suharno *et al.* (1993)[35]	Indonesia, pregnant women (305)	8 weeks	Placebo 2400 μg RE VA/day 60 mg Fe/day 2400 μg RE VA/day + 60 mg Fe/day	2.0 6.0** 10.0** 15.0**	0.01 0.02** 0.03** 0.05**	Anaemic population
Shatrugna *et al.* (1997)[36]	India, pregnant women (145)	12–16 weeks	500 μg folic acid + 120 mg Fe/day 500 μg folic acid + 60 mg Fe/day 500 μg folic acid + 60 mg Fe + 1800 μg RE VA/day	9.2 8.5 8.9	Not reported	Randomization and blinding not clear
Fawzi *et al.* (1998)[37]	Tanzania, HIV+ pregnant women (1075)	13–28 weeks	Placebo 6500 μg RE VA/day 6500 μg RE VA/day + multivitamins Multivitamins w/o VA	Group means not reported	Not reported	No significant Hb difference between groups given VA and groups not given VA

Bloem *et al.* (1990)[39]	Thailand, children 3–9 years (134)	Single dose 2 week follow-up	No supplement 110 mg RE VA	–0.8 2.2*	–0.01 –0.001*	VA deficient Not double blinded
Semba *et al.* (1992)[40]	Indonesia, children 3–6 years (236)	Single dose 5 week follow-up	*Xerophthalmic children:* Placebo 60 000 μg RE VA *Clinically normal children:* Placebo 60 000 μg RE VA *All children with Hb $<11.0\,g\,l^{-1}$:* Placebo 60 000 μg RE VA	 5.0 5.0 5.0 5.0 14.0 21.0*	Not reported	VA deficient Majority non-anaemic
Bloem *et al.* (1989)[26]	Thailand, children 1–6 years (166)	Single dose 4 month follow-up	No supplement 110 mg RE VA + 40 mg VE	2.4 2.0	0.01 0.01	Anaemic population Not double blinded VA dietary intake increased over course of study among both groups
Chawla & Puri (1995)[41]	India, pregnant women (81)	15 weeks	No supplement 60 mg Fe + 500 μg folic acid/day 60 mg Fe + 500 μg folic acid/day + 60 000 μg RE VA (1×)	–6.0 3.0** 5.0**	–0.018 0.007** 0.015**	Not randomized Not double blinded
Kolsteren *et al.* (1999)[42]	Bangladesh, non-pregnant women (216)	2 months	60 mg Fe/day 60 mg Fe/day + 60 000 μg RE VA (1×) 60 mg Fe/day + 15 mg Zn/day + 60 000 μg RE VA (1×)	13.4 15.9 17.9*	Not reported	Anaemic population Not VA deficient Double blinded?

MSG, monosodium glutamate; RE, retinol equivalents; VA, vitamin A; w/o, without.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.

concentration at 26–28 weeks of gestation among Indian women supplemented with 1800 μg RE plus 60 mg of iron day^{-1} compared to iron alone[34]. In Indonesia, mid-gestational anaemic women received 2400 μg RE of vitamin A, oral iron, vitamin A plus iron, or placebo daily for 8 weeks[35]. Mean Hb concentrations increased by 6, 10 and 15 g l^{-1} as the prevalence of anaemia declined by 23%, 62% and 98% in the three treatment groups, respectively, suggesting that about a quarter of the prevalence of anaemia in this population could be prevented with vitamin A alone. Combining vitamin A with iron increased serum iron and transferrin saturation values more than either nutrient alone. In contrast, the addition of vitamin A (1800 μg RE) to daily iron (60 mg) had no additional effect on Hb concentration in pregnant Indian women[36]. And, among pregnant women infected with human immunodeficiency virus (HIV)-1, in Tanzania, daily supplementation with *c.*6500 μg RE (as β-carotene and preformed vitamin A) lacked a measurable effect on Hb concentration[37]. Reasons for a variable haematological response to vitamin A in pregnant women are not well understood, but may relate to inadequate dosage in the presence of poor absorption and increased requirements in malnourished and diseased states, such as HIV or AIDS. Plasma volume expansion and haemodilution during the first two trimesters of pregnancy may also obscure haematological responses to supplementation[38].

Large, single dose supplements of vitamin A have produced positive haematological effects. For example, randomized trials among preschool and early school-aged children in Thailand[39] and Indonesia[40] have shown 60–110 mg RE doses to increase serum or plasma ferritin, and transferrin saturation, without affecting Hb or Hct, except among children with low initial Hb concentrations (<110 g l^{-1})[26,39,40]. In contrast, other studies have shown high-potency vitamin A to elevate Hb and serum iron but not serum ferritin[39]. Among anaemic and mildly vitamin A-deficient pregnant women in India, a single 60 000 μg RE dose of vitamin A added to daily supplementation of iron and folic acid resulted in a mean increase in Hb concentration (of 2 g l^{-1}) and Hct, and, compared to treatment with iron and folic acid alone, lessened the severity of the decline in serum iron[41]. A similar (but in this case, not statistically significant) rise in Hb concentration of 3 g l^{-1} was obtained in non-pregnant, anaemic Bangladeshi women in response to a large, single oral dose of vitamin A (200 000 IU) when given with daily iron relative to iron alone[42]. A combination of vitamin A with daily iron and zinc raised Hb concentration by 5 g l^{-1} ($P < 0.05$) above that associated with iron alone. The greater response observed in the presence of zinc could reflect increased vitamin A mobilization, as zinc supplementation has been associated with increases in plasma vitamin A and retinol-binding protein[43].

Parasitic infections may modify the impact of vitamin A on anaemia. Among predominantly anaemic pregnant women in Nepal receiving 7000 μg RE week^{-1}, anaemia was reduced by *c.*9% during pregnancy and postpartum relative to a placebo group[44]. Vitamin A, however, was unable to compensate for the effect of blood loss associated with hookworm infection: there was no measurable effect of vitamin A among heavily hookworm-infected women (>1000 eggs g^{-1}). Among women having light or no worm load, the prevalence of iron deficiency anaemia (Hb < 110 g l^{-1} with erythrocyte protoporphyrin > 90 μmol mol^{-1} or serum ferritin < 12 μg l^{-1}) was 46% lower in the vitamin A group relative to the placebo group.

Vitamin A deficiency may induce anaemia by impairing the differentiation and proliferation of pluripotent haematopoietic cells[13,45,46]; disturbing renal and hepatic erythropoietin synthesis[47]; reducing mobilization of body iron stores and disturbing iron and haem metabolism[13,48]; through sequestration of iron during the acute phase response to infection[49,50]; or via other mechanisms such as iron absorption (Fig. 1). In Venezuela, for example, provitamin A carotenoid enrichment increased iron absorption from cereals such as corn, rice and wheat, and appeared to counteract inhibitory effects of tea and coffee served with meals[51,52].

In summary, vitamin A deficiency is consistent in its association with anaemia. Vitamin A supplementation can generally be expected to:

1. Increase Hb and serum ferritin concentrations of anaemic children and pregnant women.
2. Improve the iron supply to haematopoietic tissue, possibly by enhancing the mobilization of iron delivery, and increasing plasma iron and transferrin saturation.

Folate

Alongside iron and vitamin B_{12}, folate is a central component of human erythropoiesis, and although widely distributed in foods, especially green leaves ('foliage'), dietary folate deficiency is the leading cause of megaloblastic anaemia in the world[53]. When deficient in folate, the synthesis phase of cell division is prolonged, and germ cell maturation is retarded, leading, in the case of bone marrow, to abnormal red cell precursors (megaloblasts) that have larger than normal cell and nuclear diameters[54–57]. Megaloblasts undergo grossly disturbed cell proliferation, and those that mature are often ingested and degraded by bone marrow macrophages. As a result, erythropoiesis is ineffective, the rate of delivery of new erythrocytes into circulation is depressed, and a macrocytic anaemia gradually develops (Fig. 1). Haematologically, this may be reflected in a high mean (corpuscular) cell volume (MCV) and low Hb concentration[57].

Pregnant women are at high risk for folate deficiency and megaloblastic anaemia during pregnancy[58–60]. Preterm infants have lower folate body stores at birth and

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higher growth demands as almost two-thirds of preterm infants experience low serum folate levels between 1 and 3 months of age[53,61–63]. Populations in malaria-endemic regions are at a high risk of folate deficiency, as well. The extensive haemolysis brought on by malaria stimulates erythroid hyperplasia and drastically increases the requirement for folate, making malaria during pregnancy the most common cause of megaloblastic erythropoiesis in West Africa[64].

Table 4 summarizes trials that have investigated the effects of folic acid supplementation on Hb concentration and Hct, while a few others have reported effects on neutrophil hypersegmentation, a functional measure of abnormal folate metabolism. Folic acid supplementation can prevent megaloblastic erythropoiesis among severely folate-deficient individuals, but the extent to which this translates into increases in Hb concentrations of public health importance among generally malnourished and subclinically deficient populations is not known. Folate trials have focused predominantly on effects during pregnancy. Although a few studies have noted improvement in Hb concentrations, most studies have been unable to demonstrate this effect in the absence of severe, overt folic acid deficiency or megaloblastic erythropoiesis. Modest and statistically non-significant increases in Hb concentrations of 1–6 $g\,l^{-1}$ have been consistently reported among studies of anaemic and non-anaemic, pregnant women in Burma[65], Thailand[66,67], India[68–71], Nigeria[72], Liberia[73] and Australia[74], employing supplemental doses of folic acid ranging from 0.5 to 5 mg day^{-1}, compared to placebo, iron alone or iron in combination with vitamin B_{12}. One study, in South Africa, has reported a significant improvement in Hb[75]. Women receiving 300–1000 μg day^{-1} of folate, as fortified maize, during the last month of pregnancy exhibited Hb gains of 5.0–8.5 $g\,l^{-1}$ compared to a Hb decline of –6.9 $g\,l^{-1}$ among women receiving unfortified maize. These results would be unexpected, given that women were not anaemic at baseline, and the study lasted for only a few weeks.

Although these trials indicate that folate supplementation fails to raise Hb concentration or lower the risk of anaemia, it can prevent development of megaloblastosis. For example, in a randomized, placebo-controlled trial among non-anaemic pregnant women in Australia, folic acid supplementation significantly reduced the percentage of hypersegmented neutrophils by the time of delivery[74]. In a second trial, among 200 primigravids in Nigeria, 8% of women receiving daily folic acid with antimalarial prophylaxis exhibited megaloblastic erythropoiesis (based on blood examination of red cell morphology) at follow-up compared to 25% receiving antimalarial prophylaxis without folic acid and 56% in the placebo group[76].

Folic acid has also had little effect on Hb concentration among non-pregnant women. Three months of daily supplementation with 1 mg folic acid and multivitamin during the postpartum period produced slight, though significant, increases in mean Hb and Hct levels (2 $g\,l^{-1}$ and and 0.008, respectively) compared to multivitamin use alone among lactating American women[77]. However, no benefit of folic acid supplementation on Hb response was observed in trials among either non-pregnant Thai women[67] or Malaysian adolescent girls[78]. In the latter study, however, plasma ferritin increased significantly following supplementation with iron and folate, but decreased in the folate-alone group, suggesting that folate may have stimulated synthesis of Hb from existing iron stores. In Thai school-aged children, hospitalized for malaria, 5 weeks of folic acid supplementation (15 mg day^{-1}), failed to increase Hb and Hct levels beyond those achieved by placebo[79].

Premature and low birth weight infants are highly susceptible to folate deficiency in the first year of life, and megaloblastic anaemia is common among them by 6–8 weeks of age[80]. However, in this age group as well, Hb appears to respond poorly to folate supplementation. In Britain, parenteral folic acid[61] and oral folic acid[81] given to low birth weight infants failed to improve Hb concentrations, while in a third trial[82], oral folic acid (100 μg) appeared to temper the decline in Hb at 8 weeks and significantly increase Hb by 23 $g\,l^{-1}$ at 6 months. However, the folate group had significantly higher Hb levels at baseline, the infants were not randomized, and the groups were fed differently[82].

Stronger evidence of Hb improvement has been observed. In southwest England, infants weighing < 2.5 kg received either 100 $\mu g\,day^{-1}$ oral folic acid with 10 mg day^{-1} iron or iron alone for 12 months[83]. At 6 and 9 months, mean Hb was significantly higher in the iron plus folate group compared with those receiving iron alone (by *c.* 4–5 $g\,l^{-1}$) and still slightly, but not significantly, higher at 12 months. In a trial of 0.1 mg oral folic acid with or without 100 μg parenteral vitamin B_{12} among premature infants weighing <1800 g in the USA, Hb declined among all infants, reaching a nadir at age 10–12 weeks. Relative to a mean Hb drop of 70 $g\,l^{-1}$ in the control group, however, folic acid supplementation significantly reduced the severity of the decline (–51 $g\,l^{-1}$), though by 6 months of age Hb concentrations were comparable in both folate-supplemented and control infants[84].

To conclude, folic acid deficiency contributes to anaemia primarily by disrupting cell division which compromises erythropoiesis. Supplementation with folic acid is effective in treating and preventing severe folate deficiency and overt megaloblastic anaemia. However, trials to date indicate that folic acid supplementation:

1. Has little effect on Hb concentration or Hct status among pregnant women.
2. May lessen the severity of anaemia of prematurity among young infants, although no large trials have

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Table 4 Folic acid supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Batu *et al.* (1976)[65]	Burma, pregnant women (133)	16 weeks	Placebo 120 mg Fe/day 10 mg folic acid/day 120 mg Fe + 10 mg folic acid/day	−7.0 4.0 −7.0 7.0	Not reported	Predominantly anaemic population
Srisupandit *et al.* (1983)[66]	Thailand, pregnant women (567)	12 weeks	60 mg Fe/day 180 mg Fe/day 180 mg Fe + 5 mg folic acid/day	7.6 9.0 8.3	Not reported	Not placebo controlled Blinded?
Valyasevi *et al.* (1988)[67]†	Thailand, pregnant women (325)	15 weeks	Placebo 5 mg folic acid + 120 mg Fe/day 5 mg folic acid + 240 mg Fe/day 240 mg Fe/day 5 mg folic acid + 120 mg Fe/day (unsupervised) 5 mg folic acid + 240 mg Fe/day (unsupervised)	−0.7 14.4** 13.0** 12.1** 12.7** 12.9**	Not reported	Predominantly anaemic population Not double blinded
Thanangkul *et al.* (1988)[67]†	Thailand, non-pregnant women (377)	3 months	*Village A:* Placebo 120 mg Fe/day 240 mg Fe/day 240 mg Fe + 5 mg folic acid/day *Village B:* Placebo 120 mg Fe/day 240 mg Fe/day 240 mg Fe + 5 mg folic acid/day	 0.2 11.8** 6.9* 11.4** −2.5 3.3 5.0 0.5	Not reported	Village A had 27% prevalence of anaemia, population was largely vegetarian and area was malaria endemic Village B had 7% prevalence of anaemia, population was largely non-vegetarian and area was not malaria endemic
Thane Toe *et al.* (1988)[67]†	Burma, pregnant women (306)	12 weeks	5 mg folic acid + 60 mg Fe/day 5 mg folic acid + 120 mg Fe/day (divided dose) 5 mg folic acid + 120 mg Fe/day 5 mg folic acid + 240 mg Fe/day (divided dose) 5 mg folic acid + 240 mg Fe/day 240 mg Fe/day (divided dose) 5 mg folic acid +120 mg Fe/day (unsupervised) 5 mg folic acid + 240 mg Fe/day (divided dose, unsupervised)	5.4 6.6 5.5 7.7 4.7 5.8 7.4 2.0	Not reported	No significant folic acid effect Blinded?
Basu *et al.* (1973)[68]	India, pregnant women (112)	4 weeks	Placebo 75 mg Fe/day 10 μg B_{12}/day 500 μg folic acid/day 75 mg Fe + 10 μg B_{12}/day 75 mg Fe + 500 μg folic acid/day 10 μg B_{12} + 500 μg folic acid/day 75 mg Fe +10 μg B_{12} + 500 μg folic acid/day	Not reported per group	Not reported	Women receiving Fe had mean Hb rise of 1.46 $g\,l^{-1}$ Folid acid enhanced this response by 4.2 $g\,l^{-1}$ Anaemic population not blinded Short duration of supplementation

Sood *et al.* (1975)[69]	India, pregnant women (647)	10–12 weeks	Placebo 100 μg B_{12}/qow + 5 mg folic acid/day 100 μg B_{12}/qow + 5 mg folic acid +30 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 60 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 120 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 240 mg Fe/day 120 mg Fe/day	–3.7 –2.2 8.3 9.8 12.6 13.9 7.2	–0.004 0.0 0.025 0.027 0.033 0.038 0.025	Predominantly anaemic population
Iyengar & Rajalakshmi (1975)[70]	India, pregnant women (500)	12–16 weeks	60 mg Fe/day 60 mg Fe + 500 μg folic acid/day	Change not reported	Not reported	Predominantly non-anaemic population Double blinded? High drop-out Hb higher among folate group at 38 weeks
Iyengar & Apte (1970)[71]	India, pregnant women (768)	12–16 weeks	Placebo 30 mg Fe/day 30 mg Fe + 500 μg folic acid/day 30 mg Fe + 500 μg folic acid + 2 μg B_{12}/day	Not reported	Not reported	No apparent added haematological benefit from folic acid
Osifo (1970)[72]	Nigeria, pregnant women (52)	From enrolment to delivery	120 mg Fe/day 120 mg Fe + 5 mg folic acid/day 120 mg Fe + 5 mg folic acid + antimalarial	10.0 12.0 15.0	0.021 0.044 0.046	Non-anaemic population not randomized, blinded or placebo controlled
Jackson & Latham (1982)[73]	Liberia, pregnant women (621)	12 weeks	40 mg Fe/day 120 mg Fe/day 120 mg Fe + 5 mg folic acid/day Antimalarial + 120 mg Fe + 5 mg folic acid/day	6.0 13.0 13.0 16.0	Not reported	Not placebo controlled High drop-out
Fleming *et al.* (1974)[74]	Australia, pregnant women (146)	From mid-pregnancy to 6–8 weeks postpartum	Placebo 60 mg Fe/day 0.5 mg folic acid/day 60 mg Fe + 0.5 mg folic acid/day	10.2 15.3** 12.5 17.9**	0.040 0.048* 0.046 0.060*	Non-anaemic population
Colman *et al.* (1975)[75]	South Africa, pregnant women (122)	4 weeks	Unfortified maize 1000 μg folic acid-fortified maize/day 500 μg folic acid-fortified maize/day 300 μg folic acid-fortified maize/day 300 μg folic acid tablet/day	–6.9 5.0** 8.5** 5.2** 16.1**	Not reported	Non-anaemic population
Fleming *et al.* (1986)[76]	Nigeria, pregnant women (200)	16 weeks + 6 week follow-up	Placebo Antimalarial Antimalarial + 60 mg Fe/day Antimalarial + 1 mg folic acid/day Antimalarial + 60 mg Fe + 1 mg folic acid/day	11.0 16.5 21.5 9.0 16.5	Not reported	Small sample size High drop-out
Mackey & Picciano (1999)[77]	USA, lactating women (42)	12 weeks	Multivitamin + placebo Multivitamin + 1 mg folic acid	0.0 2.0*	–0.003 0.008*	Non-anaemic population Not folate deficient
Tee *et al.* (1999)[78]	Malaysia, adolescent girls (624)	22 weeks	*Initial Hb 80–119.9 g l^{-1}:* 60 mg Fe + 3.5 mg folic acid/week 120 mg Fe + 3.5 mg folic acid/week *Initial Hb 120–130 g l^{-1}:* 60 mg Fe + 3.5 mg folic acid/week 120 mg Fe + 3.5 mg folic acid/week 5 mg folic acid/week	 21.4 23.1 11.4 13.0 9.3	Not reported	Plasma ferritin increased in Fe-supplemented groups and decreased in folate-only group

Table 4 *Continued*

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Areekul *et al.* (1980)[79]	Thailand, children 8–12 years (10)	5 weeks	Placebo 15 mg folic acid/day	20.0 1.0	0.04 0.004	Randomized? Small sample size
Burland *et al.* (1971)[61]	England, premature infants (30)	4 weeks +8 month follow-up	Untreated 100 μg folic acid/qod	−47.0 −62.0	Not reported	Not randomized, not blinded and small sample size Folate levels at baseline not comparable
Kendall *et al.* (1974)[81]	Wales, Low birth weight infants (<2500 g) at 2 weeks of age (130)	6 months	Placebo 50 μg folic acid/day	−42.0 −52.0	Not reported	High drop-out
Roberts *et al.* (1972)[82]	England, premature infants at 1 month of age (110)	5 months	Untreated 100 μg folic acid/day	−34.0 0.0**	Not reported	Not randomized Groups fed differently Folate group had higher baseline Hb
Stevens *et al.* (1979)[83]	England, Low birth weight infants (<2500 g) at 3 weeks of age (246)	12 months	10 mg Fe/day 10 mg Fe/day + 100 μg folic acid	−28.5 −18.4	−0.087 −0.064	Not randomized, not blinded Hb was significantly higher in folate group at 6 and 9 months
Worthington-White *et al.* (1994)[84]	USA, premature infants (184)	4 months +2 month follow-up	No supplement 0.1 mg folic acid/day 0.1 mg folic acid/day + 100 μg B_{12} IM/month 100 μg B_{12} IM/month	−45.0 −40.0 −30.0** −27.0**	Not reported	Blinded?

IM, intramuscular; qod, every other day; qow, every other week.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.
† Published in Charoenlarp *et al.* (1988)[67].

assessed the haematological effect of folate delivery to children.

Vitamin B_{12}

A second nutritional cause of megaloblastic anaemia is vitamin B_{12} (cobalamin) deficiency, which can produce macrocytic anaemia, as seen in folate deficiency, as well as extensive neurological impairment. Vitamin B_{12} is an essential cofactor in at least two key transmethylation reactions, one of which closely interrelates with folate in DNA synthesis and haematopoiesis. The conversion of homocysteine to the amino acid methionine requires a B_{12}-dependent enzyme as well as a methyl group donated by the folate compound 5-methyltetrahydrofolate (5-methylTHFA). With deficiency of vitamin B_{12}, the enzyme function is disrupted, methionine formation is impaired, and both 5-methylTHFA and homocysteine accumulate. Through either the trapping of folate in the form of 5-methylTHFA or the failure of methionine synthesis, the levels of the folate compound 5,10-methyleneTHFA are reduced, ultimately leading to impaired synthesis of thymidine. An inadequate supply of thymidine, in turn, impairs DNA synthesis, potentially leading to megaloblastosis and anaemia (Fig. 1)[57].

Dietary B_{12} deficiency occurs less frequently than folate deficiency, usually resulting from defective absorption rather than insufficient intake[85]. In particular, it is commonly the result of a pathological failure or reduction in the secretion of intrinsic factor, the glycoprotein that binds to and facilitates the transport of vitamin B_{12} into the epithelial cells of the small intestine, a condition referred to as pernicious anaemia[57]. The only natural source of vitamin B_{12} is its synthesis by certain algae, fungi and bacteria. The best dietary sources are meat products in which B_{12} has accumulated, via either the animal's ingestion of B_{12}-containing microorganisms or the synthesis of B_{12} by the animal's gut flora; higher plants contain virtually no vitamin B_{12} unless contaminated by microorganisms[86].

Body stores of B_{12} among normal, healthy adults are large and would take an estimated 3–4 years of zero intake (and perhaps 20 years of low intake) to deplete, due to an efficient enterohepatic circulation that recycles B_{12} from bile and other intestinal secretions[87]. However, several studies have observed that pregnant women who are strict vegetarians or who consume only minimal amounts of meat products are at high risk for becoming B_{12} deficient during pregnancy and lactation[88,89].

Few studies have assessed the haematological benefit of prophylactic vitamin B_{12} supplementation (Table 5), and those studies that have addressed anaemia have either not been designed to isolate the effects of B_{12} from those of iron or folate, or have shown no additional haematological improvement associated with B_{12}. In Israel, 90% of anaemic pregnant women supplemented with 100 mg iron, 5 mg folic acid and 100 μg B_{12} had an increase in Hb concentration of at least 5 g l^{-1}, compared with only 22% in the placebo group, although the specific effect of B_{12} remained unknown[90]. Among pregnant women in Hyderabad, India, 2 μg oral B_{12} added to 30 mg iron and 500 μg folic acid did not produce a response in Hb concentration significantly different from that of iron and folic acid alone[71]. Among anaemic pregnant women in New Delhi, 10 μg B_{12}, either alone or in combination with iron and folate, appeared to have no effect on Hb concentration, although the sample size was small and supplementation lasted only 4 weeks[68]. A study in New Delhi and Vellore demonstrated a slight, yet statistically significant, additional increase in Hb concentration (*c.* 5 g l^{-1}) from a combination of parenteral B_{12} and folate when given with iron, but the study was not designed to distinguish between the effects of B_{12} and folate[69].

The strongest evidence of haematological benefit appears to be among premature infants. In Florida, premature, low birth weight infants were randomized to receive, in addition to their standard treatment of iron and vitamin E, 0.1 mg day^{-1} oral folate, 100 μg $month^{-1}$ parenteral B_{12}, folate with B_{12} or no additional supplementation, in order to assess differences in the severity of decline in Hb concentration that typically occurs in such infants[84]. Groups receiving B_{12} experienced the least decline, with Hb concentrations falling 10–18 g l^{-1} less than unsupplemented or folate-alone groups. By 6 months of age, the infants who had received B_{12}, either with or without folate, had a significantly higher mean Hb level than both the unsupplemented and folate-alone groups.

To summarize, deficiency of vitamin B_{12} is less common than that of folate, but treatment of megaloblastic anaemia with folate alone can mask concomitant vitamin B_{12} deficiency, which can lead to severe neurological sequelae. Thus, megaloblastic anaemia should be treated with both folate and vitamin B_{12}. Few studies have reported the haematological effects of vitamin B_{12} beyond preventing megaloblastosis. Those conducted suggest that B_{12} supplementation:

1. Has no effect on the Hb level of pregnant women.
2. May improve Hb status and reduce the severity of the anaemia of prematurity among premature and low birth weight infants.

Riboflavin

Riboflavin (vitamin B_2) deficiency has been associated with the development of normochromic, normocytic anaemia that responds favourably to riboflavin supplementation[91,92]. Although riboflavin is ubiquitous in foodstuffs, riboflavin deficiency may be one of the most common vitamin deficiencies among the people of developing nations, particularly in those regions where diets are predominantly rice-based and contain insufficient milk, meat, fish, fresh fruit or vegetables[93].

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Table 5 Vitamin B_{12} supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Iyengar & Apte (1970)[71]	India, pregnant women (768)	12–16 weeks	Placebo 30 mg Fe/day 30 mg Fe + 500 μg folic acid/day 30 mg Fe + 500 μg folic acid + 2 μg B_{12}/day	Not reported	Not reported	No apparent added haematological benefit from folic acid or B_{12}
Basu *et al.* (1973)[68]	India, pregnant women (112)	4 weeks	Placebo 75 mg Fe/day 10 μg B_{12}/day 500 μg folic acid/day 75 mg Fe + 10 μg B_{12}/day 75 mg Fe + 500 μg folic acid/day 10 μg B_{12} + 500 μg folic acid/day 75 mg Fe +10 μg B_{12} + 500 μg folic acid/day	Not reported per group	Not reported	Anaemic population Not blinded Short duration of supplementation
Sood *et al.* (1975)[69]	India, pregnant women (647)	10–12 weeks	Placebo 100 μg B_{12}/qow + 5 mg folic acid/day 100 μg B_{12}/qow + 5 mg folic acid +30 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 60 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 120 mg Fe/day 100 μg B_{12}/qow + 5 mg folic acid + 240 mg Fe/day 120 mg Fe/day	–3.7 –2.2 8.3 9.8 12.6 13.9 7.2	–0.004 0.0 0.025 0.027 0.033 0.038 0.025	Predominantly anaemic population
Worthington-White *et al.* (1994)[84]	USA, premature infants (184)	4 months +2 month follow-up	No supplement 0.1 mg folic acid/day 100 μg B_{12} IM/month 0.1 mg folic acid/day + 100 μg B_{12} IM/month	–45.0 –40.0 –27.0** –30.0**	Not reported	Blinded?

IM, intramuscular; qow, every other week.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.

In vitro and *in vivo* studies have described a riboflavin-dependent mechanism for iron mobilization in which a flavin mononucleotide (FMN)-dependent oxidoreductase catalyses the removal of iron from storage ferritin and makes it available for utilization in haem synthesis (Fig. 1)[94,95]. There is also an FMN-dependent oxidase instrumental in the conversion of vitamin B_6 to its active form, which ultimately stimulates globin production. In one clinical study, riboflavin supplementation produced a three-fold increase in erythrocyte B_6 conversion, followed by a rise in α- and β-globin chain synthesis[96]. Another possibility suggested by animal studies is that riboflavin affects iron absorption by maintaining the absorptive capacity of gastrointestinal villi, but studies among humans have not yet observed measurable change in iron absorption following riboflavin supplementation[97,98].

Table 6 summarizes riboflavin supplementation trials that have assessed effects on anaemia. Results have been mixed, but several have shown that riboflavin can significantly improve haematological status and augment the response to iron supplementation. A European study of mildly anaemic pregnant women showed that those who had received daily riboflavin (9 mg) along with iron (60 mg) maintained their erythrocyte counts and Hb and Hct levels while an iron-alone group showed significant reductions in all three indices[99]. In the Gambia, marginally anaemic pregnant or lactating women were randomly allocated to receive daily iron (30 mg), riboflavin (5 mg), or both, for 6 weeks[100]. There were no significant responses in Hb or Hct to any iron or riboflavin regimen. Unexpectedly, riboflavin appeared to lower Hb by *c.* 10 g l^{-1} among pregnant women, although a small sample size limited the difference from reaching statistical significance. Lactating women receiving both iron and riboflavin had significant increases in plasma iron and ferritin, whereas those receiving iron or riboflavin alone did not. The 6-week duration of supplementation may have been too brief to elicit more substantial responses.

Riboflavin-deficient European children aged 9–12 years receiving 3 mg riboflavin exhibited a non-significant increase of 3 g l^{-1} in Hb concentration after 3 months, compared with a 4 g l^{-1} decrease in the control group[101]. The Hb increase (7 g l^{-1}) was statistically significant among children with an initial Hb below 135 g l^{-1}. In a placebo-controlled trial among mostly anaemic 6–12-year-old Thai children, 6 mg of daily riboflavin with 40 mg iron increased mean Hb by 4 g l^{-1} above that achieved with iron alone ($P < 0.005$)[102]. Other studies among children have not observed significant haematological effects. For example, in the Gambia, iron and riboflavin given at two different doses to riboflavin-deficient 4–12-year-old children had no impact on iron status beyond that of iron supplementation alone[103]. Likewise, adolescents in Yugoslavia showed no response in mean Hb or Hct to 2 months of 2 mg day^{-1} riboflavin supplementation[104].

Unlike the negative findings in Gambian children, however, riboflavin-deficient Gambian men who received 5 mg of riboflavin with 40 mg day^{-1} of iron showed comparable changes in Hb concentration, but higher Hct, erythrocyte counts and serum ferritin after 6 weeks than men supplemented with iron alone[103]. In a subgroup of anaemic men, the benefit of riboflavin appeared to be greater, producing a 23 g l^{-1} increase in mean Hb relative to 19 g l^{-1} in the iron-alone group. Improvement was also seen in Nigeria among 27 men and women who received placebo or 5 mg of riboflavin with or without 50 mg of ascorbic acid for 8 weeks in the absence of iron supplementation[105]. Erythrocyte counts, Hct and Hb levels all increased significantly in the riboflavin-treated groups, with the greatest Hb increase (18 g l^{-1}) produced by the combination of riboflavin and vitamin C.

Thus, riboflavin deficiency may impair iron mobilization, globin synthesis and, possibly, iron absorption. Supplementation with riboflavin may:

1. Enhance the Hb, Hct and erythrocyte count response to iron supplementation during pregnancy.
2. Improve the haematological status of anaemic children and adults.

Vitamin C

Vitamin C deficiency has been associated with various forms of anaemia, but it is still unclear whether vitamin C (ascorbate) is directly involved in haematopoiesis or if anaemia arises indirectly through the interactions of vitamin C with folate and iron metabolism[106]. In its role as a reducing agent, vitamin C can facilitate iron absorption from the gastrointestinal tract and enable its mobilization from storage (Fig. 1). Iron and ascorbate form an iron chelate complex that is more soluble in the alkaline environment of the small intestine and, as a result, more easily taken up[107–110]. Supplementation with vitamin C may augment the absorption of dietary iron. The simultaneous consumption of 25–75 mg of vitamin C has been shown to enhance four-fold or more the absorption of the less bioavailable, but more common, non-haem iron[109]. However, ascorbic acid must be consumed at about the same time as iron to be effective[111]. In addition, vitamin C may counteract the inhibition of iron absorption produced by dietary phytates and tannins[109]. Ascorbic acid also activates the enzyme folic acid reductase, to form tetrahydrofolic acid, the active form of folic acid, which prevents megaloblastic anaemia[106,112]. Vitamin C may also prevent iron loss due to haemorrhaging associated with vitamin C deficiency, or, possibly, prevent haemolysis resulting from compromised cellular antioxidant defence mechanisms[106,113]. Vitamin C deficiency is evident when serum ascorbate falls below 11.4 μmol l^{-1}. Inadequate status[114–116] is reflected by a serum ascorbate concentration of 11.5–17 μmol l^{-1}. Groups that have been identified as being at risk of vitamin C deficiency include pregnant

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Table 6 Riboflavin (B_2) supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Decker *et al.* (1977)[99]	Austria, pregnant women (200)	2 months	60 mg Fe/day	−3.0	−0.007	
			60 mg Fe + 9 mg B_2/day	0.0	−0.002	
Powers *et al.* (1985)[100]	The Gambia, pregnant or lactating women (81)	6 weeks	*Pregnant women (n = 18):*			Small sample size
			Placebo	7.3	0.038	Folate deficiency common
			5 mg B_2/day	−9.7	−0.002	Short duration of supplementation
			30 mg Fe/day	7.2	0.051	
			30 mg Fe + 5 mg B_2/day	−10.2	−0.023	
			Lactating women (n = 63):			
			Placebo	2.2	0.032	
			5 mg B_2/day	3.4	0.029	
			30 mg Fe/day	6.8	0.030	
			30 mg Fe + 5 mg B_2/day	6.6	0.042	
Buzina *et al.* (1979)[101]	Yugoslavia, children 9–12 years (58)	3 months	No supplement	−4.0	−0.009	Small sample size
			3 mg B_2/day	3.0	0.006	Allocation not random or placebo controlled
Charoenlarp *et al.* (1980)[102]	Thailand, children 6–12 years (101)	5 months	Placebo	−1.2	−0.011	Predominantly anaemic population
			40 mg Fe/day	4.8**	0.004**	
			40 mg Fe + 6 mg B_2/day	8.6**	0.014**	
Powers *et al.* (1983)[103]	The Gambia, children 4–12 years (80) and adult men (80)	6 weeks	*Children:*			Predominantly anaemic population
			Placebo	0.6	0.026	
			20 or 40 mg Fe/day	14.5**	0.051*	
			20 or 40 mg Fe + 2.5 or 5 mg B_2/day	8.5**	0.048**	
			Adult men:			
			Placebo	−2.0	−0.020	
			40 mg Fe/day	12.9**	0.015**	
			40 mg Fe + 5 mg B_2/day	12.2**	0.022**	
Suboticanec *et al.* (1990)[104]	Croatia, school children 12–14 years (115)	2 months	Placebo	2.0	−0.007	Non-anaemic population
			2 mg B_6/day	1.0	−0.012	
			2 mg B_2/day	−1.0	0.0	
Ajayi *et al.* (1990)[105]	Nigeria, adult men and women (27)	8 weeks	Placebo	−4.0	−0.02	Controlled experimental study
			5 mg B_2/day	14.8**	0.04**	
			5 mg B_2 + 50 mg VC/day	17.8**	0.05**	

VC, vitamin C.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.

and lactating women, infants fed exclusively cow's milk, elderly men and smokers[116–118].

A number of trials have assessed the effects of vitamin C supplementation on iron status and anaemia in children and adult pregnant and non-pregnant women (Table 7). Anaemic preschool Indian children receiving 200 mg day^{-1} of ascorbic acid for 2 months showed improved red blood cell morphology and a significant increase in mean Hb of 19 g l^{-1}, whereas Hb changed little among placebo controls[119]. In northeastern China, Hb increased significantly by 3–6 g l^{-1} in a dose-responsive manner and serum ferritin rose by 14–28 μg l^{-1}, compared to placebo, among mildly anaemic preschool children receiving 50 mg or more of vitamin C daily for 2 months[120]. These studies support an adjunct role for vitamin C in modulating the risk of anaemia in malnourished child populations. Findings of no impact arise from a study of anaemic preschool Indonesian children whose Hb concentration failed to rise following 2 months of receiving 20 mg of vitamin C; however, lack of an adequate control group weakens the inference to be drawn from this study[121]. In Yugoslavia, 70 mg day^{-1} of ascorbic acid, given also for 2 months, failed to increase Hb or Hct among adolescent males, but the study population was not anaemic[122].

Vitamin C may exert a measurable haematological effect in non-pregnant women. Among 32 non-anaemic, Nigerian women, receipt of 50 mg or 100 mg day^{-1} of ascorbic acid significantly raised Hb concentration by 18 and 20 g l^{-1}, respectively, compared to a 4 g l^{-1} decline in unsupplemented controls[123]. In a controlled dietary experiment, 11 American women aged 22–36 years underwent iron stores depletion through a low-iron diet and phlebotomy and then were placed on an iron-replete diet, supplemented with either placebo or 1500 mg of ascorbic acid day^{-1} for 5.5 weeks[124]. Vitamin C recipients showed a slight, yet significant, rise in Hb concentration (+0.5 g l^{-1}) compared with the placebo group, whose mean Hb concentration declined by *c.* 3 g l^{-1}. Apparent iron absorption also significantly rose among vitamin C recipients (to 38% vs. 27% for placebos), who retained an additional 2.3 mg day^{-1} of iron from their diets. However, serum ferritin concentration remained unaffected by ascorbic acid.

Non-anaemic, iron-deficient women in Mexico were randomly assigned either lime juice containing 25 mg ascorbic acid or a lime-flavoured placebo beverage to be consumed twice per day within 1 hour of meals[125]. After 6 months, gain in serum ferritin was consistently higher among supplemented women, representing an increase in iron absorption of up to 0.5 mg day^{-1}. Hb concentration was unaffected by supplementation, possibly due to inadequacy of the daily 50 mg vitamin C dose or to the initial absence of anaemia in subjects. However, negative findings emerged from a trial among anaemic and non-anaemic pregnant Filipina women, where the addition of 100–300 mg of ascorbic acid to iron supplements daily for 16–18 weeks conferred no effect beyond that of iron alone in improving Hb concentration, Hct, serum iron or transferrin saturation by the time of delivery[126].

Non-experimental studies support a modest effect of vitamin C. For example, a study among Indian vegetarians observed significant increases in Hb (by 8%), serum iron (by 17%) and serum ferritin (by 12%) from baseline following receipt of 500 mg of vitamin C after lunch and dinner for 2 months[127]. Providing well-nourished Turkish subjects with vitamin C (2 g) daily was associated with rises in Hb concentration (+11 g l^{-1}) and serum iron (+6 μmol l^{-1}) after 1 month; however, levels were comparable to baseline after a second month of supplementation[128]. Lack of concurrent comparison groups weaken the results of both of these studies.

In summary, evidence is lacking to support a clear role for vitamin C in improving the haematological status of pregnant women. Small studies to date do suggest that vitamin C may:

1. Improve absorption of non-haem iron, protect against oxidative damage and counteract the effects of iron absorption inhibitors.
2. Increase serum iron, ferritin and Hb concentrations among children and non-pregnant subjects.

Vitamin E

Vitamin E (α-tocopherol) is a lipid-soluble compound that functions in humans primarily as an antioxidant, scavenging highly reactive free radicals and protecting the polyunsaturated fatty acids (PUFAs) of cellular membranes from oxidative destruction. Nutritional deficiency of vitamin E is thought to be uncommon as it is widely distributed in foods, particularly vegetable and seed oils such as almond, sunflower, corn, soybean and wheat germ[106]. Susceptibility to deficiency is largely limited to premature and low birth weight newborns and to various pathological malabsorption syndromes such as cystic fibrosis, biliary atresia and abetalipoproteinaemia[129].

Animal studies have observed the development of severe anaemia and morphological abnormalities of the bone marrow among primates on long-term vitamin E-deficient diets[130,131]. Treatment with vitamin E stimulated reticulocytosis and improved blood parameters among these animals[132]. Abnormal erythropoiesis, impaired iron metabolism and decreased erythrocyte survival times have also been observed in vitamin E-deficient animals[130,133,134]. In humans, vitamin E supplementation has been shown to increase the reticulocyte count[135].

Preterm and low birth weight infants are born with low serum and tissue concentrations of vitamin E, due in part to limited placental transport of tocopherols and to scarcity of storage adipose tissue[129,136]. Vitamin E deficiency-induced anaemia in infants 6–12 weeks of age has been characterized by red blood cell haemolysis, reticulocytosis,

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Table 7 Vitamin C supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Seshadri *et al.* (1985)[119]	India, preschool children (54)	60 days	Placebo 200 mg VC/day	1.0 19.2**	Not reported	Anaemic population Small sample size
Mao & Yao (1992)[120]	China, preschool children 3–5 years (65)	8 weeks	Placebo 25 mg VC/day 50 mg VC/day 100 mg VC/day 150 mg VC/day	11.4 14.8 14.5* 15.2* 16.9*	0.014 0.012 0.014 0.015 0.026	Anaemic population Blinded?
Angeles *et al.* (1993)[121]	Indonesia, preschool children 2–5 years (80)	8 weeks	20 mg VC/day 30 mg Fe + 20 mg VC/day	1.0 10.0**	Not reported	Anaemic population Not designed to assess the effect of VC Control group experienced greater morbidity
Suboticanec-Buzina *et al.* (1984)[122]	Yugoslavia, adolescent males 11–13 years (91)	2 months	2 mg riboflavin + 2 mg B_6/day 2 mg riboflavin + 2 mg B_6 + 70 mg VC/day	1.0 –1.0	–0.007 –0.002	Non-anaemic population Blinded? Randomized?
Ajayi & Nnaji (1990)[123]	Nigeria, young adult women (32)	8 weeks	No supplement 50 mg VC/day 100 mg VC/day	–4.3 17.9** 19.6**	–0.018 0.057** 0.047**	Non-anaemic population Blinded? Small sample size
Hunt *et al.* (1990)[124]	USA, young adult women (11)	5.5 weeks	Placebo 1500 mg VC/day	–3.0 0.5*	Not reported	Controlled experimental study
Garcia *et al.* (1998)[125]	Mexico, adult women (36)	8 months	Placebo Lime juice with 25 mg VC 2×/day	Not reported	Not reported	Serum ferritin was consistently higher among VC-supplemented group VC increased Fe absorption 0.5 mg day^{-1}
Kuizon *et al.* (1979)[126]	Philippines, pregnant women (335)	16–18 weeks with 7–8 month follow-up	*Non-anaemic women:* Placebo 65 mg Fe/day 100 mg VC/day 65 mg Fe + 100 mg VC/day *Anaemic women:* Placebo 195 mg Fe/day 300 mg VC/day 195 mg Fe + 300 mg VC/day	 –7.8 3.9 –2.9 4.6 –4.0 14.4 –1.2 11.1	 –0.016 0.017 –0.008 0.017 0.002 0.031 0.003 0.020	

VC, vitamin C.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.

thrombocytosis and oedema that resolves promptly following vitamin E treatment[137–141]. However, in these landmark studies, improvement in Hb status following vitamin E supplementation occurred only among infants consuming a low tocopherol to PUFA ratio in their diet and receiving concurrent iron supplementation[139,142–144]. It was soon recognized that infant formula diets rich in PUFAs and low in α-tocopherol, especially in the presence of oxidant compounds such as iron, potentiated the severity of deficiency and haemolytic anaemia. Promotion of early breast-feeding, modifications in modern infant formulas to lower PUFA and iron levels, and routine vitamin E supplementation have virtually eliminated severe vitamin E deficiency in premature infants[106,145].

Randomized, placebo-controlled trials have examined the effect of vitamin E supplementation in preventing anaemia of prematurity among infants fed modern diets relatively low in PUFAs and iron (Table 8). Two, in Canada, among low birth weight infants, failed to improve Hb concentration, reticulocyte count or erythrocyte morphology after 6 weeks of supplementation with 16 mg day^{-1} of vitamin E[146–148]. In England, preterm, low birth weight infants receiving either 5 or 15 mg day^{-1} of vitamin E had higher, albeit not significantly, Hb values than the control group at 10 weeks of age[149]. Similarly, a small Brazilian trial failed to find significant differences in Hb concentration, Hct or indicators of reticulocytosis among premature low birth weight infants treated for 6 weeks with iron, vitamin E or both[150]. The potential benefits of vitamin E supplementation may have been masked in these trials because the diets of premature, low birth weight infants commonly contain vitamin E.

Non-experimental studies among anaemic, malnourished infants and children in Jordan[151] and Thailand[152] observed reticulocytosis and increases in Hb concentration and Hct following supplementation with oral vitamin E, but subsequent studies in India and Lebanon could not corroborate the response to vitamin E supplementation[153,154]. Lack of randomization and concurrent control groups leads to caution in interpreting the findings of these studies. However, a randomized, controlled trial among anaemic 1–3-year-old, protein-energy malnourished children in Thailand reported no additional improvements in Hb and reticulocyte counts from vitamin E given with iron relative to iron alone[155].

To summarize, vitamin E is routinely given to preterm infants in developed countries to protect against the potential oxidative damage caused by iron supplementation. Under existing regimens to provide vitamin E to premature infants, additional supplementation with vitamin E has not further reduced the severity of anaemia of prematurity.

Thiamine, niacin, pantothenic acid and vitamin B_6

Each of these four vitamins has been related to the development or treatment of anaemia during deficiency and supplementation, respectively, and warrants mention, although their public health significance with respect to anaemia is largely unknown. Thiamine-responsive megaloblastic anaemia, for example, is the product of a hereditary disorder of metabolism, part of a syndrome that is also characterized by diabetes mellitus and sensorineural deafness[156]. Niacin deficiency has produced macrocytic anaemia in some animal models, and normocytic anaemia has been reported among human patients with pellagra, but the anaemia cannot be specifically attributed to deficiency of niacin[157,158]. Animal studies have also observed anaemia following induced deficiency of pantothenic acid, but there has been only anecdotal evidence for the occurrence of pantothenic acid-responsive anaemia in humans[159–161]. No studies have been conducted to determine if these vitamins enhance erythropoiesis among malnourished populations.

Vitamin B_6 (pyridoxine) deficiency can disturb haem synthesis and lead to normocytic, microcytic or sideroblastic anaemia (Fig. 1). Treatment of sideroblastic anaemia with vitamin B_6 has resulted in the restored activity of erythroblastic δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in haem synthesis, followed by correction of the haematological abnormalities[162,163]. In Germany, after treating children hospitalized with iron deficiency anaemia for 8 days with iron plus vitamin B_6, there was an apparent acceleration of haem synthesis, reflected in Hb concentrations that were higher than observed in children who received only iron (Table 9)[164]. Perhaps not surprisingly, supplementation of non-anaemic adolescents in Yugoslavia with 2 mg of vitamin B_6 daily for 2 months had no significant effect on Hb or Hct status relative to placebo[104]. Vitamin B_6 may also inhibit sickling of erythrocytes in sickle-cell anaemia (SCA), possibly increasing erythrocyte counts, Hb concentrations and Hct among SCA patients[165].

To recapitulate:

1. Thiamine, niacin and pantothenic acid have been related to human anaemia, but their public health significance with respect to anaemia is questionable.
2. Vitamin B_6 deficiency is rare, but treatment with B_6 may be effective in correcting the haematological abnormalities of sideroblastic anaemia.

Multivitamin supplementation

Studies previously cited have assessed the haematological effects of a single vitamin or small number of vitamins combined, with or without iron, but few trials have examined the haematopoietic impact of multivitamin supplementation. Most trials that have assessed the impact of multivitamin supplementation have used multivitamins with iron, and have not differentiated the effects of the vitamins from those obtained from iron alone. Table 10 summarizes a complex series of

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Table 8 Vitamin E supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Blanchette *et al.* (1980)[146]	Canada, low birth weight infants (59)	6 weeks	Placebo 16 mg VE/day	Change not reported	Not reported	No difference in mean Hb between groups at 6 weeks
Zipursky *et al.* (1987)[148]	Canada, preterm infants <1500 g (178)	6 weeks	Placebo 16 mg VE/day	–59.0 –69.0	Not reported	Not VE deficient
Conway *et al.* (1986)[149]	England, preterm infants <1760 g (52)	10 weeks	Placebo 5 mg VE/day 15 mg VE/day	–92.0 –76.0 –66.0 (median)	Not reported	
Ferlin *et al.* (1998)[150]	Brazil, preterm infants <1600 g (40)	6 weeks	Placebo 4 mg/kg/day Fe 4 mg/kg/day Fe + 16 mg VE/day 16 mg VE/day	–105.0 –84.0 –94.0 –81.0 (median)	–0.292 –0.225 –0.222 –0.235	Blinded?
Kulapongs (1975)[155]	Thailand, children 1–3 years with PEM (70)	12 weeks	Placebo 10 mg/kg/day VE + 3 mg/kg/day Fe for 12 weeks VE alone for 6 weeks followed by VE + Fe for 6 weeks Fe alone for 6 weeks followed by VE + Fe for 6 weeks	12.0 38.0 23.0 23.5	Not reported	Hb and reticulocyte responses occurred only after Fe was given

PEM, protein-energy malnutrition; VE, vitamin E.

Table 9 Vitamin B_6 supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Reinken & Kurz (1975)[164]	Germany, children (32)	8 days + 4 day follow-up	100 mg Fe/day 100 mg Fe + 12.5 mg B_6/day	7.0 17.0	0.04 0.05	Anaemic population Small sample size Short duration
Suboticanec *et al.* (1990)[104]	Croatia, school children 12–14 years (115)	2 months	Placebo 2 mg B_6/day 2 mg B_2/day	2.0 1.0 –1.0	–0.007 –0.012 0.0	Non-anaemic population

multivitamin supplementation trials that have assessed outcomes relating to anaemia.

In Peru, 10 weeks of daily multivitamin supplement use (containing thiamin, riboflavin, B_{12}, folate and niacin) added to iron had no effect over iron alone on Hb levels of children aged 7–13 years[166]. Among preschool anaemic children in Germany, however, a combination of iron, folate, vitamin C, riboflavin, B_6 and B_{12} for just 9 days raised mean Hb concentration $5\,g\,l^{-1}$ above that of children receiving iron alone[167]. Russian school children were also reported to have shown significant increases in Hb concentration and lower morbidity rates during 5–7 months of multivitamin supplementation (composition not described) compared to unsupplemented controls[168].

Significant increases in serum iron and aerobic capacity were observed, compared with controls, in non-anaemic Yugoslav adolescents given daily ascorbic acid, riboflavin and B_6 for 3 months[169]. However, supplementation induced no significant changes in Hb or Hct. In India, among children aged 6 months to 6 years, 100% of the children receiving only folate and B_{12} for 12 weeks experienced some rise in Hb concentration, compared to 87% receiving only vitamins A and D, 92% receiving 40 mg of iron alone twice weekly, and 37% receiving placebo[170].

Among HIV-1-infected pregnant women in Tanzania, those taking multivitamins (either with or without vitamin A) had significantly higher increases in mean Hb concentration at 6 weeks postpartum than did women not taking multivitamins (13 vs. $6\,g\,l^{-1}$)[37]. All of the women received $120\,mg\,day^{-1}$ of iron, $5\,mg\,day^{-1}$ of folic acid and a weekly antimalarial, thus explaining the Hb increase in those not receiving the multivitamins.

Other multivitamin studies have demonstrated haematological improvements, but have not been designed to isolate the haematological effects of vitamins from iron. In the Gambia, a seasonal decline in Hb due to malaria and hookworm among vitamin-deficient prepubescent children appeared to be staunched somewhat by a multivitamin of thiamin, riboflavin, ascorbic acid and iron[171]. In China, a daily micronutrient-fortified weaning biscuit maintained the mean Hb concentration of infants aged 6–13 months, while Hb declined significantly ($-8\,g\,l^{-1}$) among infants receiving unfortified biscuits[172]. Among predominantly non-anaemic South African school children, biscuits fortified with iron, iodine and vitamin A along with a vitamin C-enriched drink significantly improved Hb concentration $3\,g\,l^{-1}$ more than supplementation with unfortified biscuits and placebo drink after 12 months[173].

Among 6–24-month-old Vietnamese children, a daily multivitamin (iron, vitamin A, vitamin C and zinc) or a higher-dose weekly multivitamin, produced highly significant increases in mean Hb of 16 and $13\,g\,l^{-1}$, respectively, and a reduction in the prevalence of anaemia from 50% to <10%, compared to no change in Hb in the placebo group[174]. In Indonesia, among non-pregnant adolescent girls, 12 weeks of multivitamins containing either lower

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Table 10 Multivitamin supplementation trials that examined effects on haematological indicators

Reference	Subject population (total sample size)	Duration of supplementation	Composition of multivitamin	Treatment groups and regimen	Change in mean haemoglobin ($g\,l^{-1}$)	Change in mean haematocrit	Comments
Bradfield *et al.* (1968)[166]	Peru, school children 7–13 years (156)	10 weeks	0.5 mg thiamine 1 mg riboflavin 3 μg B_{12} 0.2 mg folic acid 5 mg niacin	Placebo 5 mg Fe/day Multivitamin + 5 mg Fe/day Antihelminth + placebo Antihelminth + 5 mg Fe/day Antihelminth + multivitamin + 5 mg Fe/day	−12.0 3.0 3.0 13.0* 5.0* 11.0*	−0.01 0.01 0.02 0.02 0.03* 0.05*	34% of population anaemic at baseline
Reinken & Kurz (1978)[167]	Germany, preschool children (28)	9 days	104.4 mg Fe 0.9 mg folic acid 15 μg B_{12} 225 mg VC 4.5 mg riboflavin 12 mg B_6	104.4 mg Fe/day Multivitamin/day	9.0 14.0	0.026 0.042	
Buzina *et al.* (1982)[169]	Yugoslavia, male school children 12–15 years (201)	3 months		No supplement 70 mg VC + 2 mg riboflavin + 2 mg B_6/day	−2.0 1.0	−0.004 0.0	Non-anaemic population Not blinded Randomized?
Das *et al.* (1984)[170]	India, preschool children 0.5–6 years (175)	12 weeks		Placebo 360 μg RE VA + 200 IU VD 5×/week 1.4 μg B_{12} + 140 μg folic acid 5×/week 5 mg Fe 5×/week 10 mg Fe 5×/week 20 mg Fe 2×/week 40 mg Fe 2×/week 20 mg Fe 1×/week 40 mg Fe 1×/week	2.9 9.9* 20.8** 2.5 3.2 8.4* 14.8** 8.2* 8.4*	Not reported	
Fawzi *et al.* (1998)[37]	Tanzania, HIV+ pregnant women (1075)	2nd trimester enrolment to delivery (13–28 weeks)	20 mg B_1 20 mg riboflavin 25 mg B_6 100 mg niacin 50 μg B_{12} 500 mg VC 30 mg VE 0.8 mg folic acid	Placebo 6500 μg RE VA/day 6500 μg RE VA + multivitamin/day Multivitamin/day	Not reported per group	Not reported	Change in mean Hb at 6 weeks postpartum: multivitamins: 1.3*; no multivitamins: 0.6

Powers *et al.* (1985)[171]	The Gambia, school children 11–14.5 years (40)	10 weeks	60 mg Fe 23 mg thiamin 23 mg riboflavin 750 mg VC	Placebo Multivitamin/week	–6.4 –1.8	–0.077 –0.041	27.5% of population anaemic at baseline
Liu *et al.* (1993)[172]	China, infants 6–13 months (226)	3 months	5 mg Fe 25 μg folic acid 0.3 μg B_{12} 0.2 mg Riboflavin 224 μg VA 0.15 μg thiamin 2.5 mg niacin 3.0 mg zinc 300 mg calcium	Unfortified weaning biscuit/day Vitamin-fortified weaning biscuit/day	–7.9 –0.8**	Not reported	Double blinded?
Van Stuijvenberg *et al.* (1999)[173]	South Africa, school children 6–11 years (252)	43 weeks	*Fortified biscuit:* 5 mg Fe 2.1 mg VA 60 μg iodine	Unfortified biscuit + placebo drink 5 ×/week Fortified biscuit + 90 mg VC drink 5 ×/week	1.0 4.0** (median)	0.0 0.008* (median)	25–30% prevalence of anaemia at baseline 40% prevalence of low VA status
Thu *et al.* (1999)[174]	Vietnam, preschool children 6–24 months (163)	3 months	*Daily vitamin:* 333 μg VA 8 mg Fe 5 mg Zn 20 mg VC *Weekly vitamin:* 1700 μg VA 20 mg Fe 17 mg Zn 20 mg VC	Placebo Daily multivitamin 5 ×/week Weekly multivitamin 1 ×/week	–0.5 15.5** 13.2**	Not reported	41–48% of population anaemic at baseline
Angeles-Agdeppa *et al.* (1997)[175]	Indonesia, adolescent girls (363)	3 months	*Composition 'A':* 750 μg VA 250 μg folic acid 60 mg VC *Composition 'B':* 6000 μg VA 500 μg folic acid 60 mg VC	Placebo 60 mg Fe + 1 'A'/day 60 mg Fe + 1 'B'/week 120 mg Fe + 1 'B'/week	–2.8 3.3** 5.6** 3.7**	Not reported	17.4% of population anaemic at baseline

VA, vitamin A; VC, vitamin C; VD, vitamin D; VE, vitamin E.
* $P < 0.05$ relative to control group.
** $P < 0.01$ relative to control group.

daily doses or higher weekly doses of vitamin A, vitamin C, folate and iron led to significant increases in mean Hb (3.3–5.6 $g\,l^{-1}$) and ferritin levels (14.2–27.2 $\mu g\,l^{-1}$) relative to a placebo group (−2.8 $g\,l^{-1}$ and −4.6 $\mu g\,l^{-1}$, respectively)[175]. A study in New Jersey examined the effects of prenatal multivitamin supplement (containing folic acid, calcium, zinc and iron) use by low income pregnant women on birth outcomes and blood ferritin, zinc and folate levels (Hb and Hct were not assessed)[176]. By 28 weeks' gestation, women who had used the multivitamins during their first or second trimesters had higher serum ferritin values relative to non-users, indicating an improvement in iron stores.

In summary, the prevention and control of nutritional anaemia in many populations will probably require the use of multivitamin supplements. However:

1. Most efficacy studies of multivitamin supplement use to date have not differentiated the haematological effects of vitamins from those achieved with supplemental iron alone.
2. Thus, there is presently insufficient data among pregnant women, adolescent girls and children to assess the haematological benefit of multivitamin supplementation, independent of the iron effect.

Conclusions

A large number of studies have evaluated the effects of single or multivitamin use on various health and nutritional outcomes in different population settings, but most have not been designed to adequately assess vitamin impact on Hb, Hct and other haematological measures. While consistency of effect can sometimes be gleaned for a particular nutrient or group of vitamins across diverse population groups, discrepant findings tend more to be the rule than the exception within and between demographic, life-stage and geographic classes of subjects. Where consistency in effect exists, mechanisms at times can be invoked; discordance in effect between different groups of individuals leaves open a search for mechanisms both to explain the haematological effect and its variability.

The roles of most vitamins in controlling anaemia among high-risk populations have yet to be adequately explored. Among preschool-aged children, there is evidence that vitamins A and C can effectively raise haemoglobin levels, but no studies have been done to assess folic acid, vitamin B_{12}, riboflavin or other vitamins. Among school-aged children, vitamin A has been effective, and there is some evidence that riboflavin can improve haematological status, but no large folic acid, vitamin C, E or B_6 studies have been conducted. Among pregnant women, evidence regarding the effectiveness of folic acid is still conflicting; no studies have isolated the effects of vitamin B_{12}; evidence regarding riboflavin remains insufficient; and studies are needed to demonstrate the effects of vitamins C, E or B_6 in gravid women. Among non-pregnant women of reproductive age, vitamins A and C have been effective in improving haematological status; folic acid and riboflavin have been shown to be effective in small trials, but no studies have assessed the haematological effects of vitamins B_{12}, B_6 or E. Perhaps most importantly, though, is a need for multivitamin supplementation studies among each of these populations that isolate the effects of vitamins from the haematological effects of iron supplementation. Further, there is a need for studies to compare the effectiveness of different vitamin combinations in order to examine possible interactions or enhancement of effects.

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References

1 ACC/SCN. *Second Report on the World Nutrition Situation*. Vol. 1. *Global and Regional Results*. Geneva: Administrative Committee on Coordination/Subcommittee on Nutrition, 1992.
2 WHO/UNICEF/UNU. *Iron Deficiency: Indicators for Assessment and Strategies for Prevention*. Geneva: World Health Organization, 1997.
3 Lee GR. The anemia of chronic disease. *Semin. Hematol.* 1983; **20**: 61–80.
4 Fairbanks VF. Iron in medicine and nutrition. In: Shils ME, Olson JA, Shike M, eds. *Modern Nutrition in Health and Disease*. Philadelphia: Lea and Febiger, 1994; 185–213.
5 Viteri FE. Iron supplementation for the control of iron deficiency in populations at risk. *Nutr. Rev.* 1997; **55**: 195–209.
6 DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *Rapp. Trimest. Statist. Sanit. Mond.* 1985; **38**: 302–16.
7 International Nutritional Anemia Consultative Group (INACG). *Iron Deficiency in Women*. Washington, DC: The Nutrition Foundation, 1981.
8 Rossiter CE. Maternal mortality. *Br. J. Obstet. Gynaecol.* 1985; **5** (Suppl.): 100–15.
9 Dallman PR. Iron. In: Brown ML, ed. *Present Knowledge in Nutrition*. Washington, DC: International Life Sciences Institute/Nutrition Foundation, 1990; 241–50.
10 Christian P. Antenatal iron supplementation as a child survival strategy. *Am. J. Clin. Nutr.* 1998; **68**: 403–4.
11 Sommer A. *Nutritional Blindness*. New York: Oxford University Press, 1982.
12 Humphrey JH, West KP, Sommer A. Vitamin A deficiency and attributable mortality among under-5-year-olds. *Bull. World Health Org.* 1992; **70**: 225–32.
13 Sommer A, West KP. *Vitamin A Deficiency: Health, Survival and Vision*. New York: Oxford University Press, 1996.

14 WHO. *Indicators for Assessing Vitamin A Deficiency and their Application in Monitoring and Evaluating Intervention Programmes*. Geneva: World Health Organization, 1996.
15 Beaton GH, Martorell R, L'Abbe KA, *et al*. *Effectiveness of Vitamin A Supplementation in the Control of Young Child Morbidity and Mortality in Developing Countries*. Final Report to CIDA. Toronto: University of Toronto, 1992.
16 Fawzi WW, Chameres TC, Herrera MG, Mosteller F. Vitamin A supplementation and child mortality. *JAMA* 1993; **269**: 898–903.
17 Glasziou PP, Mackerras DEM. Vitamin A supplementation in infectious diseases: a meta-analysis. *BMJ* 1993; **306**: 366–70.
18 Findlay GM, Mackenzie RD. The bone marrow in deficiency diseases. *J. Pathol.* 1922; **25**: 402–3.
19 Wolbach SB, Howe PR. Tissue changes following deprivation of fat-soluble A vitamin. *J. Exp. Med.* 1925; **42**: 753–77.
20 Koessler KK, Maurer S, Loughlin R. The relation of anemia, primary and secondary, to vitamin A deficiency. *JAMA* 1926; **87**: 476–82.
21 Sure B, Kik MC, Walker DJ. The effect of avitaminosis on hematopoietic function. I. Vitamin A deficiency. *J. Biol. Chem.* 1929; **83**: 375–85.
22 Blackfan KD, Wolbach SB. Vitamin A deficiency in infants: a clinical and pathological study. *J. Pediatr.* 1933; **3**: 679–706.
23 Mejia LA, Hodges RE, Arroyave G, Viteri F, Torun B. Vitamin A deficiency and anemia in Central American children. *Am. J. Clin. Nutr.* 1977; **30**: 1175–84.
24 Wolde-Gebriel Z, Gebru H, Fisseha T, West CE. Severe vitamin A deficiency in a rural village in the Hararge region of Ethiopia. *Eur. J. Clin. Nutr.* 1993; **47**: 104–14.
25 Ahmed F, Barua S, Mohiduzzaman M, *et al*. Interactions between growth and nutrient status in school-age children of urban Bangladesh. *Am. J. Clin. Nutr.* 1993; **58**: 334–8.
26 Bloem MW, Wedel M, Egger RJ, *et al*. Iron metabolism and vitamin A deficiency in children in northeast Thailand. *Am. J. Clin. Nutr.* 1989; **50**: 332–8.
27 Mohanram M, Kulkarni KA, Reddy V. Hematological studies in vitamin A deficient children. *Int. J. Vitam. Nutr. Res.* 1977; **47**: 389–93.
28 Hodges RE, Sauberlich HE, Canham JE, *et al*. Hematopoietic studies in vitamin A deficiency. *Am. J. Clin. Nutr.* 1978; **31**: 876–85.
29 Mejia LA, Arroyave G. The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. *Am. J. Clin. Nutr.* 1982; **36**: 87–93.
30 Mejia LA. Vitamin A deficiency as a factor in nutritional anemia. *Int. J. Vitam. Nutr. Res.* 1985; **27**: 75–84.
31 Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. *Am. J. Clin. Nutr.* 1988; **48**: 1271–6.
32 Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *Am. J. Clin. Nutr.* 1988; **48**: 595–600.
33 Smith JC, Makdani D, Hegar A, Rao D, Douglass LW. Vitamin A and zinc supplementation of preschool children. *J. Am. Coll. Nutr.* 1999; **18**: 213–22.
34 Panth M, Shatrugna V, Yasodhara P, Sivakumar B. Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. *Br. J. Nutr.* 1990; **64**: 351–8.
35 Suharno D, West CE, Muhilal, Karyadi D, Hautvast JGAJ. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* 1993; **342**: 1325–8.
36 Shatrugna V, Raman L, Uma K, Sujatha T. Interaction between vitamin A and iron: effects of supplements in pregnancy. *Int. J. Vitam. Nutr. Res.* 1997; **67**: 145–8.
37 Fawzi WW, Msamanga GI, Spiegelman D, *et al*. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet* 1998; **351**: 1477–82.
38 Mahomed K, Hytten F. Iron and folate supplementation in pregnancy. In: Chalmers I, ed. *Effective Care in Pregnancy and Childbirth*. London: Oxford University Press, 1989.
39 Bloem MW, Wedel M, van Agtmaal EJ, Speek AJ, Saowakontha S, Schreurs WHP. Vitamin A intervention: short-term effects of a single, oral, massive dose on iron metabolism. *Am. J. Clin. Nutr.* 1990; **51**: 76–9.
40 Semba RD, Muhilal, West KP, *et al*. Impact of vitamin A supplementation on hematological indicators of iron metabolism and protein status in children. *Nutr. Res.* 1992; **12**: 469–78.
41 Chawla PK, Puri R. Impact of nutritional supplements on hematological profile of pregnant women. *Indian J. Pediatr.* 1995; **32**: 876–80.
42 Kolsteren P, Rahman SR, Hilderbrand K, Diniz A. Treatment for iron deficiency anaemia with a combined supplementation of iron, vitamin A, and zinc in women of Dinajpur, Bangladesh. *Eur. J. Clin. Nutr.* 1999; **53**: 102–6.
43 Christian P, West KP Jr. Interactions between zinc and vitamin A: an update. *Am. J. Clin. Nutr.* 1998; **68** (Suppl. 2): S435–41.
44 Stoltzfus RJ, Dreyfuss M, Shrestha JB, Khatry SK, Schulze K, West KP. Effect of maternal vitamin A or carotene supplementation on iron-deficiency anemia in Nepalese pregnant women, post-partum mothers, and infants. In: *Report of the XVIII International Vitamin A Consultative Group Meeting, Cairo, Egypt*. Washington, DC: International Life Science Institute Research Foundation, 1997; 86.
45 Koeffler HP, Amatruda TT. The effect of retinoids on haemopoiesis – clinical and laboratory studies. In: Pitman, ed. *Retinoids, Differentiation, and Disease*. London: Ciba Foundation Symposium, 1985; 252–73.
46 Amatruda TT, Koeffler HP. Retinoids and cells of the hematopoietic system. In: Sherman MI, ed. *Retinoids and Cell Differentiation*. Boca Raton, Florida: CRC Press, 1986; 80–103.
47 Jelkmann W, Pagel H, Hellwig T, Fandrey J. Effects of antioxidant vitamins on renal and hepatic erythropoietin production. *Kidney Int.* 1997; **51**: 497–501.
48 Mejia LA, Hodges RE, Rucker RB. Role of vitamin A in the absorption, retention, and distribution of iron in the rat. *J. Nutr.* 1979; **109**: 129–37.
49 Semba RD. Vitamin A, immunity, and infection. *Clin. Infect. Dis.* 1994; **19**: 489–99.
50 Bloem MW. Interdependence of vitamin A and iron: an important association for programmes of anaemia control. *Proc. Nutr. Soc.* 1995; **54**: 501–8.
51 Garcia-Casal MN, Layrisse M, Solano L, *et al*. Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat, and corn by humans. *J. Nutr.* 1997; **128**: 646–50.
52 Layrisse M, Garcia-Casal MN, Solano L, *et al*. Vitamin A reduces the inhibition of iron absorption by phytates and polyphenols. *Food Nutr. Bull.* 1998; **19**: 3–5.
53 Gallagher PG, Ehrenkranz RA. Nutritional anemias in infancy. *Clin. Perinatol.* 1995; **22**: 671–92.
54 Myhre E. Studies on megaloblasts *in vitro*. *Scand. J. Clin. Lab. Invest.* 1964; **16**: 307.
55 Rondanelli EG, Gorini P, Magliulo E, Fiori GP. Differences in proliferative activity between normoblasts and pernicious anemia megaloblasts. *Blood* 1964; **24**: 542.
56 Steinberg SE, Fonda S, Campbell CL, Hillman RS. Cellular abnormalities of folate deficiency. *Br. J. Haematol.* 1983; **54**: 605.
57 Hughes-Jones NC, Wickramasinghe SN. *Lecture Notes on Haematology*. Oxford: Blackwell Science, 1996.
58 Strieff RR, Little AB. Folic acid deficiency in pregnancy. *N. Engl. J. Med.* 1967; **276**: 776.

59 Blot I, Papierhik F, Kaltwasser JP, *et al*. Influence of routine administration of folic acid and iron during pregnancy. *Gynecol. Obstet. Invest.* 1981; **12**: 294.
60 Herbert V, Das KC. Folic acid and vitamin B12. In: Shils ME, Olson JA, Shike M, eds. *Modern Nutrition in Health and Disease*. Philadelphia: Lea and Febiger, 1994; 402–25.
61 Burland WL, Simpson K, Lord J. Response of low birthweight infants to treatment with folic acid. *Arch. Dis. Child.* 1971; **46**: 189–94.
62 Strelling MK, Blackledge DG, Goodall HB. Diagnosis and management of folate deficiency in low birthweight infants. *Arch. Dis. Child.* 1979; **54**: 271–7.
63 Ehrenkranz RA. Iron, folic acid, and vitamin B12. In: Tsang RC, Lucas A, Uauy R, *et al*., eds. *Nutritional Needs of the Preterm Infant: Scientific Basis and Practical Guidelines*. Baltimore: Williams and Williams, 1993; 177–94.
64 Fleming AF. The aetiology of severe anaemia in pregnancy in Ndola, Zambia. *Ann. Trop. Med. Parasitol.* 1989; **83**: 37–49.
65 Batu AT, Toe T, Pe H, Nyunt KK. A prophylactic trial of iron and folic acid supplements in pregnant Burmese women. *Isr. J. Med. Sci.* 1976; **12**: 1410–17.
66 Srisupandit S, Pootrakul P, Areekul S, *et al*. A prophylactic supplementation of iron and folate in pregnancy. *Southeast Asian J. Trop. Med. Public Health* 1983; **14**: 317–23.
67 Charoenlarp P, Dhanamitta S, Kaewvichit R, *et al*. A WHO collaborative study on iron supplementation in Burma and Thailand. *Am. J. Clin. Nutr.* 1988; **47**: 280–97.
68 Basu RN, Sood SK, Ramachandran K, Mathur M, Ramalingaswami V. Etiopathogenesis of nutritional anemia in pregnancy: a therapeutic approach. *Am. J. Clin. Nutr.* 1973; **26**: 591–4.
69 Sood SK, Ramachandran K, Mathur M, *et al*. WHO sponsored collaborative studies on nutritional anaemia in India: 1. The effects of supplemental oral iron administration to pregnant women. *Q. J. Med.* 1975; **44**: 241–58.
70 Iyengar L, Rajalakshmi K. Effect of folic acid supplement on birth weights of infants. *Am. J. Obstet. Gynaecol.* 1975; **122**: 332–6.
71 Iyengar L, Apte SV. Prophylaxis of anemia of pregnancy. *Am. J. Clin. Nutr.* 1970; **23**: 725–30.
72 Osifo B. The effect of folic acid and iron in the prevention of nutritional anaemias in pregnancy in Nigeria. *Br. J. Nutr.* 1970; **24**: 689–94.
73 Jackson RT, Latham MC. Anemia of pregnancy in Liberia, West Africa: a therapeutic trial. *Am. J. Clin. Nutr.* 1982; **35**: 710–14.
74 Fleming AF, Martin JD, Hahnel R, Westlake AJ. Effects of iron and folic acid antenatal supplements on maternal haematology and fetal wellbeing. *Med. J. Aust.* 1974; **2**: 429–36.
75 Colman N, Larsen JV, Barker M, Barker EA, Green R, Metz J. Prevention of folate deficiency by food fortification. III. Effect in pregnant subjects of varying amounts of added folic acid. *Am. J. Clin. Nutr.* 1975; **28**: 465–70.
76 Fleming AF, Ghatoura GBS, Harrison KA, Briggs ND, Dunn DT. The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria. *Ann. Trop. Med. Parasitol.* 1986; **80**: 211–33.
77 Mackey AD, Picciano MF. Maternal folate status during extended lactation and the effect of supplemental folic acid. *Am. J. Clin. Nutr.* 1999; **69**: 285–92.
78 Tee ES, Kandiah M, Awin N, *et al*. School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls. *Am. J. Clin. Nutr.* 1999; **69**: 1249–56.
79 Areekul S, Subcharoen A, Cheeramakara C, Srisukawat K, Limsuwan S. Studies on the effect of folic acid supplement on folate and vitamin B12 status in children. *Southeast Asian J. Trop. Med. Public Health* 1980; **11**: 81–6.
80 Hoffbrand AV. Folate deficiency in premature infants. *Arch. Dis. Child.* 1970; **45**: 441–4.
81 Kendall AC, Jones EE, Wilson CID, Shinton NK, Elwood PC. Folic acid in low birthweight infants. *Arch. Dis. Child.* 1974; **49**: 736–8.
82 Roberts PMM, Arrowsmith DE, Lloyd AVC, Monk-Jones ME. Effect of folic acid treatment on premature infants. *Arch. Dis. Child.* 1972; **47**: 631–4.
83 Stevens D, Burman D, Strelling MK, Morris A. Folic acid supplementation in low birth weight infants. *Pediatrics* 1979; **64**: 333–5.
84 Worthington-White DA, Behnke M, Gross S. Premature infants require additional folate and vitamin B-12 to reduce the severity of the anemia of prematurity. *Am. J. Clin. Nutr.* 1994; **60**: 930–5.
85 Herbert V. Vitamin B12. In: Olsen RE, Broquist HP, Chichester CO, *et al*., eds *Nutrition Reviews' Present Knowledge in Nutrition*, 5th edn. Washington, DC: The Nutrition Foundation, 1984; 347–64.
86 Herbert V. Vitamin B12. In: Brown ML, ed. *Present Knowledge in Nutrition*. 6th edn. Washington, DC: International Life Sciences Institute/Nutrition Foundation, 1990; 170–8.
87 Antony AC. Megaloblastic anemias. In: Hoffman R, Benz EJ, Shattil SJ, *et al*., eds. *Hematology: Basic Principles and Practice*. New York: Churchill Livingstone, 1991; 392–422.
88 Allen LH, Backstrand JR, Chavez A, Pelto GH. *Functional Implications of Malnutrition. Mexico Project Final Report*. Storrs, CT: University of Connecticut, 1992.
89 Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. In: Allen LH, Lonnerdal B, eds. *Nutrient Regulation During Pregnancy, Lactation, and Infant Growth*. New York: Plenum Press, 1994; 173–86.
90 Levy S, Rachmilewitz EZ, Izak G, Salomonowitz A, Rachmilewitz M. A therapeutic trial in anemia of pregnancy. *Isr. J. Med. Sci.* 1968; **4**: 218–22.
91 Lane M, Alfrey P. The anemia of human riboflavin deficiency. *Blood* 1965; **25**: 432–42.
92 Alfrey CP, Lane M. The effect of riboflavin deficiency on erythropoiesis. *Semin. Hematol.* 1970; **7**: 49–54.
93 Bates CJ. Human riboflavin requirements and metabolic consequences of deficiency in man and animals. *World Rev. Nutr. Diet.* 1987; **50**: 215–65.
94 Sirivech S, Driskell J, Frieden E. NADH-FMN oxidoreductase activity and iron content of organs from riboflavin and iron-deficient rats. *J. Nutr.* 1977; **107**: 739–45.
95 Ulvik RJ. Ferritin iron as a substrate for synthesis of protoheme in intact rat liver mitochondria. *FEBS Lett.* 1981; **132**: 281–4.
96 Perry GM, Anderson BB, Dodd N. The effect of riboflavin on red-cell vitamin B6 metabolism and globin synthesis. *Biomedicine* 1980; **33**: 36–8.
97 Fairweather-Tait SJ, Powers HJ, Minski MJ, Whitehead J, Downes R. Riboflavin deficiency and iron absorption in adult Gambian men. *Ann. Nutr. Metab.* 1992; **36**: 34–40.
98 Powers HJ. Riboflavin–iron interactions with particular emphasis on the gastrointestinal tract. *Proc. Nutr. Soc.* 1995; **54**: 509–17.
99 Decker K, Dotis B, Glatzle D, Hinselmann M. Riboflavin status and anaemia in pregnant women. *Nutr. Metab.* 1977; **21** (Suppl. 1): 17–19.
100 Powers HJ, Bates CJ, Lamb WH. Haematological response to supplements of iron and riboflavin to pregnant and lactating women in rural Gambia. *Hum. Nutr. Clin. Nutr.* 1985; **39C**: 117–29.
101 Buzina R, Jusic M, Milanovic N, Sapunar J, Brubacher G. The effects of riboflavin administration on iron metabolism parameters in a school-going population. *Int. J. Vitam. Nutr. Res.* 1979; **49**: 136–43.

102 Charoenlarp P, Pholpothi T, Chatpunyaporn P, Schelp FP. Effect of riboflavin on the hematologic changes in iron supplementation of schoolchildren. *Southeast Asian J. Trop. Med. Public Health* 1980; **11**: 97–103.

103 Powers HJ, Bates CJ, Prentice AM, Lamb WH, Jepson M, Bowman H. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. *Hum. Nutr. Clin. Nutr.* 1983; **37C**: 413–25.

104 Suboticanec K, Stavljenic A, Schalch W, Buzina R. Effects of pyridoxine and riboflavin supplementation on physical fitness in young adolescents. *Int. J. Vitam. Nutr. Res.* 1990; **60**: 81–8.

105 Ajayi OA, Okike OC, Yusuf Y. Haematological response to supplements of riboflavin and ascorbic acid in Nigerian young adults. *Eur. J. Haematol.* 1990; **44**: 209–12.

106 Oski FA. Anemia due to other nutritional deficiencies. In: Williams WJ, Beutler E, eds. *Hematology*. New York: McGraw-Hill, 1995; 511–15.

107 Bothwell TH, Bradlow BA, Jacobs P, *et al*. Iron metabolism in scurvy with special reference to erythropoiesis. *Br. J. Haematol.* 1964; **10**: 50–8.

108 Lynch SR, Cook JD. Interaction of vitamin C and iron. *Ann. NY Acad. Sci.* 1980; **355**: 32–44.

109 Hallberg L, Brune M, Rossander-Hulthen L. Is there a physiological role of vitamin C in iron absorption? *Ann. NY Acad. Sci.* 1987; **498**: 324–32.

110 Clark NG, Sheard NF, Kelleher JF. Treatment of iron-deficiency anemia complicated by scurvy and folic acid deficiency. *Nutr. Rev.* 1992; **50**: 134–7.

111 Cook JD, Monsen ER. Vitamin C, the common cold, and iron absorption. *Am. J. Clin. Nutr.* 1977; **30**: 235–41.

112 Stokes PL, Melikian V, Leeming RL, Portman-Graham H, Blair JA, Cooke WT. Folate metabolism in scurvy. *Am. J. Clin. Nutr.* 1975; **28**: 126–9.

113 Chow CK. Nutritional influence on cellular antioxidant defense systems. *Am. J. Clin. Nutr.* 1979; **32**: 1066.

114 Hodges RE, Baker EM, Hood J, Sauberlich HE, March SC. Experimental scurvy in man. *Am. J. Clin. Nutr.* 1969; **22**: 535–48.

115 Hodges RE, Hood J, Canham JE, Sauberlich HE, Baker EM. Clinical manifestations of ascorbic acid deficiency in man. *Am. J. Clin. Nutr.* 1971; **24**: 432–43.

116 Sauberlich HE. Ascorbic acid. In: Brown ML, ed. *Present Knowledge in Nutrition*, 6th edn. Washington, DC: International Life Sciences Institute/Nutrition Foundation, 1990; 132–41.

117 Garry PJ, Vanderjagt DJ, Hunt WC. Ascorbic acid intakes and plasma levels in healthy elderly. *Ann. NY Acad. Sci.* 1987; **498**: 90–9.

118 National Research Council (NRC). *Recommended Dietary Allowances*. Washington, DC: National Academy Press, 1989.

119 Seshadri S, Shah A, Bhade S. Haematologic response of anaemic preschool children to ascorbic acid supplementation. *Hum. Nutr. Appl. Nutr.* 1985; **39**: 151–4.

120 Mao X, Yao G. Effect of vitamin C supplementation on iron deficiency anemia in Chinese children. *Biomed. Environ. Sci.* 1992; **5**: 125–9.

121 Angeles IT, Schultink WJ, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *Am. J. Clin. Nutr.* 1993; **58**: 339–42.

122 Suboticanec-Buzina K, Buzina R, Brubacher G, Sapunar J, Christeller S. Vitamin C status and physical working capacity in adolescents. *Int. J. Vitam. Nutr. Res.* 1984; **54**: 55–60.

123 Ajayi OA, Nnaji UR. Effect of ascorbic acid supplementation on haematological response and ascorbic acid status of young female adults. *Ann. Nutr. Metab.* 1990; **34**: 32–6.

124 Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH. Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *Am. J. Clin. Nutr.* 1990; **51**: 649–55.

125 Garcia OP, Diaz M, Rosado JL, Allen LH. Community trial of the efficacy of lime juice for improving iron status of iron deficient Mexican women. *FASEB J.* 1998; **12**: A647 (abstract).

126 Kuizon MD, Platon TP, Ancheta LP, Angeles JC, Nunez CB, Macapinlac MP. Iron supplementation studies among pregnant women. *Southeast Asian J. Trop. Med. Public Health* 1979; **10**: 520–7.

127 Sharma DC, Mathur R. Correction of anemia and iron deficiency in vegetarians by administration of ascorbic acid. *Indian J. Physiol. Pharmacol.* 1995; **39**: 403–6.

128 Pekiner B, Nebioglu S. Effect of vitamin C on copper and iron status in men and guinea pigs. *J. Nutr. Sci. Vitaminol.* 1994; **40**: 401–10.

129 Farrell PM, Roberts RJ. Vitamin E. In: Shils ME, Olson JA, Shike M, eds. *Modern Nutrition in Health and Disease*. Philadelphia: Lea and Febiger, 1994; 326–41.

130 Dinning JS, Day PL. Vitamin E deficiency in the monkey. I. Muscular dystrophy, hematologic changes and the excretion of urinary nitrogenous constituents. *J. Exp. Med.* 1957; **105**: 395.

131 Marvin HN, Dinning JS, Day PL. Erythrocyte survival in vitamins E-deficient monkeys. *Proc. Soc. Exp. Biol. Med.* 1960; **105**: 473–5.

132 Porter FS, Fitch CD, Dinning JS. Vitamin E deficiency in the monkey. IV. Further studies of the anemia with emphasis on bone marrow morphology. *Blood* 1962; **20**: 471.

133 Marvin HN. Erythrocyte survival of rats deficient in vitamin E on vitamin B_6. *J. Nutr.* 1963; **80**: 185–90.

134 Chou AC, Broun GO, Fitch CD. Abnormalities of iron metabolism and erythropoiesis in vitamin E-deficient rabbits. *Blood* 1978; **52**: 187–95.

135 Horwitt MK, Century B, Zeman AA. Erythrocyte survival time and reticulocyte levels after tocopherol depletion in man. *Am. J. Clin. Nutr.* 1963; **12**: 99–106.

136 Ehrenkranz RA. Vitamin E and the neonate. *Am. J. Dis. Child.* 1980; **134**: 1157–66.

137 Oski FA, Barness LA. Vitamin E deficiency: a previously unrecognized cause of hemolytic anemia in the premature infant. *J. Pediatr.* 1967; **70**: 211–20.

138 Ritchie JH, Fish MB, McMasters V, Grossman M. Edema and hemolytic anemia in premature infants. *N. Engl. J. Med.* 1968; **279**: 1185–90.

139 Lo SS, Frank D, Hitzig WH. Vitamin E and haemolytic anaemia in premature infants. *Arch. Dis. Child.* 1973; **48**: 360–5.

140 Williams ML, Shott RJ, O'Neal PL, Oski FA. Role of dietary iron and fat on vitamin E deficiency anemia of infancy. *N. Engl. J. Med.* 1975; **292**: 887–90.

141 Gross SJ, Landaw SA, Oski FA. Vitamin E and neonatal hemolysis. *Pediatrics* 1977; **59**: 995–7.

142 Melhorn DK, Gross S. Vitamin E-dependent anemia in the premature infant. I. Effects of large doses of medicinal iron. *J. Pediatr.* 1971; **79**: 569–80.

143 Gross S, Melhorn DK. Vitamin E-dependent anemia in the premature infant. *J. Pediatr.* 1974; **85**: 753–9.

144 Fermanian J, Salmon D, Olive G, Zambrowski S, Rossier A, Caldera R. Double blind study of vitamin E compared to placebo in the prevention of anemia in the low birth weight infant during the 7th week of life: therapeutic study [in French]. *Nouv. Rev. Fr. Hematol. Blood Cells* 1976; **16**: 245–54.

145 Blanchette V, Doyle J, Schmidt B, Zipursky A. Hematology. In: Avery GB, Fletcher MA, MacDonald MG, eds. *Neonatology: Pathophysiology and Management of the Newborn*. Philadelphia: JB Lipincott, 1994; 952–99.

146 Blanchette V, Bell E, Nahmias C, Garnett S, Milner R,

Zipursky A. A randomized control trial of vitamin E therapy in the prevention of anemia in low birth weight (LBW) infants. *Pediatr. Res.* 1980; **14**: 591.
147 Zipursky A. Vitamin E deficiency anemia in newborn infants. *Clin. Perinatol.* 1984; **11**: 393–402.
148 Zipursky A, Brown EJ, Watts J, *et al.* Oral vitamin E supplementation for the prevention of anemia in premature infants: a controlled trial. *Pediatrics* 1987; **79**: 61–8.
149 Conway SP, Rawson I, Dear PRF, Shires SE, Kelleher J. The early anaemia of the premature infant: is there a place for vitamin E supplementation? *Br. J. Nutr.* 1986; **56**: 105–14.
150 Ferlin MLS, Chuan LS, Jorge SM, Vannucchi H. Early anemia of prematurity. *Nutr. Res.* 1998; **18**: 1161–73.
151 Majaj AS. Vitamin E-responsive megaloblastic anemia in infants with protein-calorie malnutrition. *Am. J. Clin. Nutr.* 1963; **12**: 374–9.
152 Whitaker JA, Fort EG, Vimokesant S, Dinning JS. Hematologic response to vitamin E in the anemia associated with protein-calorie malnutrition. *Am. J. Clin. Nutr.* 1967; **20**: 783–9.
153 Asfour RY, Firzli S. Hematologic studies in undernourished children with low serum vitamin E levels. *Am. J. Clin. Nutr.* 1965; **17**: 158–63.
154 Baker SJ, Pereira SM, Begum A. Failure of vitamin E therapy in the treatment of anemia of protein-calorie malnutrition. *Blood* 1968; **32**: 717–25.
155 Kulapongs P. The effect of vitamin E on the anemia of protein-calorie malnutrition in northern Thai children. In: Olsen RE, ed. *Protein-Calorie Malnutrition*. New York: Academic Press, 1975; 263–8.
156 Neufeld EJ, Mandel H, Raz T, *et al.* Localization of the gene for thiamine-responsive megaloblastic anemia syndrome, on the long arm of chromosome 1, by homozygosity mapping. *Am. J. Hum. Genet.* 1997; **61**: 1335–41.
157 Handler P, Featherston WP. The biochemical defect in nicotinic acid deficiency. *J. Biol. Chem.* 1943; **151**: 395.
158 Spivak JL, Jackson DL. Pellagra: an analysis of 18 patients and a review of the literature. *Johns Hopkins Med. J.* 1977; **140**: 295–309.
159 Wintrobe MM, Follis RH Jr, Alcayaga R, Paulson M, Humphreys S. Pantothenic acid deficiency in swine. *Bull. Johns Hopkins Hosp.* 1943; **73**: 313.
160 Richert DA, Schulman MP. Vitamin interrelationships in heme synthesis. *Am. J. Clin. Nutr.* 1959; **7**: 416–25.
161 McCurdy PR. Is there an anemia responsive to pantothenic acid? *J. Am. Geriatr. Soc.* 1973; **21**: 88–91.
162 Ofori-Nkansah N, Weissenfels I, Pribilla W. Vitamin B6 deficiency anemia [in German]. *Schweiz. Med. Wochenschr.* 1975; **105**: 1319–24.
163 Meier PJ, Giger U, Brandli O, Fehr J. Acquired, vitamin B6-responsive, primary sideroblastic anemia, an enzyme deficiency in heme synthesis [in German]. *Schweiz. Med. Wochenschr.* 1981; **111**: 1533–5.
164 Reinken L, Kurz R. Activity studies of an iron–vitamin B_6 preparation for euteral treatment of iron deficiency anemia [in German]. *Int. J. Vitam. Nutr. Res.* 1975; **44**: 411–18.
165 Natta CL, Reynolds RD. Apparent vitamin B6 deficiency in sickle cell anemia. *Am. J. Clin. Nutr.* 1984; **40**: 235–9.
166 Bradfield RB, Jensen MV, Gonzales L, Garrayar C. Effect of low-level iron and vitamin supplementation on a tropical anemia. *Am. J. Clin. Nutr.* 1968; **21**: 57–67.
167 Reinken L, Kurz R. The treatment of anaemia due to iron-deficiency with iron combined with vitamins [in German]. *Klin. Padiatr.* 1978; **190**: 163–7.
168 Tell'mann Akh, Saava ME, Aasvee KE. Effects of prophylactic vitamin administration to Tallin's schoolchildren on their health and physical development [in Russian]. *Vopr. Pitan.* 1992; May–June: 36–40.
169 Buzina R, Grgic Z, Jusic M, Sapunar J, Milanovic N, Brubacher G. Nutritional status and physical working capacity. *Hum. Nutr. Clin. Nutr.* 1982; **36C**: 429–38.
170 Das BK, Bal MS, Tripathi AM, Singla PN, Agarwal DK, Agarwal KN. Evaluation of frequency and dose of iron and other hematinics – an alternative strategy for anemia prophylaxis in rural preschoolers. *Indian J. Pediatr.* 1984; **21**: 933–8.
171 Powers HJ, Bates CJ, Lamb WH, Singh J, Gelman W, Webb E. Effects of a multivitamin and iron supplement on running performance in Gambian children. *Hum. Nutr. Clin. Nutr.* 1985; **39**: 427–37.
172 Liu DS, Bates CJ, Yin TA, Wang XB, Lu CQ. Nutritional efficacy of a fortified weaning rusk in a rural area near Beijing. *Am. J. Clin. Nutr.* 1993; **57**: 506–11.
173 Van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJ. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. *Am. J. Clin. Nutr.* 1999; **69**: 497–503.
174 Thu BD, Schultink W, Dillon D, Gross R, Leswara ND, Khoi HH. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. *Am. J. Clin. Nutr.* 1999; **69**: 80–6.
175 Angeles-Agdeppa I, Schultink W, Sastroamidjojo S, Gross R, Karyadi D. Weekly micronutrient supplementation to build iron stores in female Indonesian adolescents. *Am. J. Clin. Nutr.* 1997; **66**: 177–83.
176 Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, Krueger PM. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. *Am. J. Epidemiol.* 1997; **146**: 134–41.