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# Safety and benefits of antenatal oral iron supplementation in low-income countries: a review

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## Summary

The World Health Organization recommends universal iron supplementation of 30–60 mg/day in pregnancy but coverage is low in most countries. Its efficacy is uncertain, however, and there has been a vigorous debate in the last decade about its safety, particularly in areas with a high burden of malaria and other infectious diseases. We reviewed the evidence on the safety and efficacy of antenatal iron supplementation in low-income countries. We found no evidence that daily supplementation at a dose of 60 mg leads to increased maternal *Plasmodium* infection risk. On the other hand, recent meta-analyses found that antenatal iron supplementation provides benefits for maternal health (severe anaemia at postpartum, blood transfusion). For neonates, there was a reduced prematurity risk, and only a small or no effect on birth weight. A recent trial showed, however, that benefits of antenatal iron supplementation on maternal and neonatal health vary by maternal iron status, with substantial benefits in iron-deficient women. The benefits of universal iron supplementation are likely to vary with the prevalence of iron deficiency. As a consequence, the balance between benefits and risks is probably more favourable in low-income countries than in high-income countries despite the higher exposure to infectious pathogens.

**Keywords:** anaemia, iron supplementation, low-income countries, malaria, pregnancy.

## Introduction

Despite decades of international and national guidelines recommending universal iron supplementation in pregnancy through antenatal care (Table I), coverage remains appallingly low in many countries (Hodgins & D'Agostino, 2014), probably in part due to continued uncertainty about the benefits. Although it is generally accepted that universal antenatal iron supplementation in most settings results in improved maternal iron markers, such as haemoglobin concentration or serum ferritin concentration (Peña-Rosas *et al*, 2015), there has been inadequate and inconsistent evidence about whether it leads to better maternal and neonatal health outcomes, such as maternal mortality, caesarean delivery, preterm delivery, infant mortality or low birth weight. Even within the USA, recommendations for prevention of iron deficiency in pregnancy vary between universal iron supplementation (Centers for Disease Control and Prevention, 2008), various screen-and-treat approaches (American College of Obstetricians and Gynecologists, 2008; Institute of Medicine, 2011), or none of these measures, because the supportive evidence is considered to be insufficient (American Academy of Family Physicians, 2015; Siu, 2015).

Iron interventions in low-income countries present additional challenges. First, there are increased concerns about their safety in populations with a high burden of infections. Particularly unsettling has been evidence from a randomised trial to assess the effect of supplementation with iron and folic acid among young children in Pemba Island, Tanzania. The trial was prematurely stopped because of excess hospitalisation and death (Sazawal *et al*, 2006), reinforcing earlier concerns (Oppenheimer, 2001) that iron supplementation can increase rates of infectious diseases, including malaria. There has since been a vigorous debate about how to safely administer iron to infants, children and pregnant women living in malaria-endemic areas. An expert group convened by the World Health Organization (WHO) recommended restriction of supplementation in children but not in pregnant women (WHO, 2007).

Second, iron interventions in low-income countries often have limited efficacy in reducing anaemia. It is often unclear to what extent this is due to ineffective elimination of iron deficiency or because anaemia is due to other, highly prevalent determinants of anaemia. These factors include

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**Table I.** World Health Organization guidelines for antenatal iron supplementation (WHO, 2012a,b).

| Recommendation* | Suggested scheme |
|---|---|
| Daily oral iron supplementation is recommended as part of the antenatal care to reduce the risk of low birth weight, maternal anaemia and iron deficiency | For prevention, give daily supplementation with 30–60 mg iron throughout pregnancy, starting as early in pregnancy as possible<br>In settings where anaemia in pregnant women is a severe public health problem (40% of higher), a daily dose of 60 mg of elemental iron is preferred over a lower dose<br>Women with anaemia should be daily supplemented with 120 mg iron until haemoglobin concentration become normal, followed by the standard antenatal dose to prevent recurrence of anaemia |
| In settings where the prevalence of anaemia among pregnant women is lower than 20%, intermittent use of iron supplements by non-anaemic pregnant women is recommended to prevent anaemia and improve gestational outcomes | Non-anaemic pregnant women should receive weekly supplementation with 120 mg iron throughout pregnancy, starting as early in pregnancy as possible |

*In malaria-endemic areas, provision of iron and folic acid supplements should be implemented in conjunction with measures to prevent, diagnose and treat malaria.

infections and infection-induced inflammation (intestinal helminths, tuberculosis, human immunodeficiency virus (HIV), possibly *Helicobacter pylori*), other nutritional deficiencies (vitamin A, riboflavin and vitamin $B_{12}$), and haemoglobin disorders (sickle cell, thalassaemia, haemoglobin C, and glucose-6-phosphate dehydrogenase deficiency). Inflammation blocks iron absorption by upregulating hepcidin (Drakesmith & Prentice, 2012) and additionally blocks erythropoiesis.

Third, infections also affect iron markers independently of iron status. This hampers the assessment of iron status, both in individuals and at the population level.

We aim to review the current evidence on the safety and efficacy of antenatal iron supplementation in low-income countries.

## Iron functions and oxidative stress

Iron is the active element of cofactors in many proteins and is thus required for numerous biological processes. These proteins are essential for oxygen transport (haemoglobin), oxygen storage in muscles (myoglobin), mitochondrial electron transport for ATP synthesis, steroidogenesis and detoxification (cytochromes and dehydrogenases), amino acid metabolism (oxygenases), formation of deoxyribonucleotides from ribonucleotides (ribonucleotide reductase), conversion of citrate to citrate in the Krebs cycle (aconitase) and conversion of protoporphyrin IX and iron to haem (ferrochetalase) among numerous other actions.

In all of these metabolic processes, iron plays a central role because of its capacity to alternate between reduced (ferrous, $Fe^{2+}$) and oxidised (ferric, $Fe^{3+}$) states. As a powerful oxidant, however, iron is also a potential catalyst of formation of hydroxyl radicals and other reactive oxygen species (ROS). ROS have a variety of biological functions, including in innate and adaptive immunity (Weiss, 2002). For example, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) is a membrane-bound enzyme complex found in phagosomes, the vesicles formed by neutrophils to engulf pathogens as part of the phagocytic process. NADPH oxidase contains two haem groups that play a central role in catalysing oxygen ($O_2$) to the ROS superoxide anion ($O_2^{\bullet -}$), a free radical that is used to help kill invading microorganisms, either directly or through its subsequent conversion to hydrogen peroxide and hypochlorous acid. Iron deficiency is associated with reduced activity of NADPH oxidase and a reduced ability to produce hypochlorous acid, whilst NADPH oxidase activity can be restored by iron supplementation (Kurtoglu *et al*, 2003; Paino *et al*, 2009).

Reactive oxygen species can also have damaging effects. Superoxide, the precursor of most other ROS, is also a frequent by-product of normal cellular metabolism. It is formed intracellularly by 'leakage' (premature outflow) of electrons from the mitochondrial respiratory chain or from microsomal redox-active enzymes, and their subsequent reaction with oxygen (e.g., Bhattacharyya *et al*, 2014). Catalytic conversion of superoxide by superoxide dismutase results in the formation of hydrogen peroxide ($H_2O_2$), which is relatively stable in solution. Both superoxide and $H_2O_2$ are also produced extracellularly by NADPH oxidases on cell plasma membranes of phagocytes, endothelial cells and platelets and by xanthine oxidase that is primarily released into circulation by hepatocytes (Forman *et al*, 2016). Blood $H_2O_2$ concentrations range from a possible low of 0·25 μmol/l to a probable normal range of 1–5 μmol/l, and a high range of 30–50 μmol/l in certain disease states or during chronic inflammation (Forman *et al*, 2016). Reaction of $H_2O_2$ with ferrous iron leads to the hydroxyl radical ($^{\bullet}OH$), an extremely reactive species that can produce substantial damage to DNA, lipids and amino acids.

Thus iron is a double-edged sword: although crucial for many central metabolic pathways and immune mechanisms, its increased accumulation may cause formation of toxic molecules and progressive tissue damage (Weiss, 2002).

Oxidative stress has also been implicated as a mediating factor in the development of self-limiting and dose-dependent adverse effects in the gastrointestinal tract (e.g. constipation, nausea, vomiting, and epigastric discomfort) due to iron supplementation (Kumar *et al*, 2009).

## The role of iron in the struggle between host and pathogens

The metabolic role of iron is not unique to humans: most life forms utilise iron for a variety of metabolic functions. Because iron is extremely insoluble at physiological pH, its resulting scarcity has led to an evolutionary battle for iron between humans and pathogens (Drakesmith & Prentice, 2012). The human host further restricts the availability of iron by ensuring its tight binding to chaperone proteins (e.g., haemoglobin, transferrin, ferritin, lactoferrin), and by modulating the abundance of these proteins as a defence against extracellular pathogens that could otherwise cause a rapidly fatal septicaemia ('iron withholding hypothesis'). Thus, infections are associated with a shift of iron from circulation to intracellular storage, as reflected by decreased serum concentrations of transferrin and iron, and increased serum concentrations of ferritin.

Under normal physiological conditions, the amount of extracellular free iron in serum is too low to sustain propagation of bacteria (Weinberg, 1974). To survive, pathogens have evolved several mechanisms to scavenge host iron (Skaar, 2010). Bacterial toxins damage host cells, leading to the release of ferritin or (in case of erythrocytes) haemoglobin. Most bacteria secrete and resorb small proteins ('siderophores') that bind iron in the environment or competitively remove iron that is contained in host proteins. Other bacterial pathogens and some parasites (e.g., *Trichomonas vaginalis, Trypanosoma brucei, Schistosoma mansoni*) have circumvented the iron-availability issue by acquiring iron through receptor-mediated uptake of transferrin, lactoferrin, haemopexin, haemoglobin, or haemoglobin–haptoglobin complexes. The source of iron for various stages of *Plasmodium* parasites is unclear. Despite access to abundant haemoglobin, the intraerythrocytic parasite appears to lack a haem oxygenase pathway that is required to degrade host haem and release iron for its own utilisation, and it remains unknown whether non-enzymatic degradation of haem occurs inside the parasite (Sigala & Goldberg, 2014).

The iron withholding process is regulated by hepcidin, a small peptide hormone that was discovered in 2000 and that is now known to be the key regulator of body iron homeostasis in many vertebrates (Ganz & Nemeth, 2015). Hepcidin is mostly produced by hepatocytes and acts by binding to ferroportin, the transmembrane iron exporter protein and by inducing its intracellular degradation, thus inhibiting cellular iron efflux. Hepcidin synthesis is greatly reduced by iron deficiency, causing increased absorption in enterocytes of ingested iron. In contrast, hepcidin is greatly increased by inflammation, which causes reduced iron absorption and a blockage of macrophage recycling of iron that would otherwise be available for erythropoiesis and other metabolic processes.

By diverting iron toward macrophages, the host provides an iron-rich intracellular environment that possibly favours the proliferation of pathogens, such as *Mycobacterium tuberculosis* and *Salmonella* species, that are able to survive, proliferate and disseminate throughout the body in macrophages (Drakesmith & Prentice, 2012).

## Antenatal iron requirements in low-income countries

The prevalence of iron deficiency in pregnant women often exceeds 50% in low-income countries. In addition to the effects of inflammation in blocking iron uptake, iron deficiency occurs because the diets of poor people are monotonous, low in animal food sources, and primarily based on unrefined cereals, grains and legume seeds. Although these foods have reasonable iron content, they also contain high concentrations of phytate. This phosphate-storage molecule occurs naturally in seeds (including grains, pulses, nuts, seeds in fruits and vegetables) and binds with iron to form insoluble complexes that are not absorbed in the intestine. In addition, phenolic compounds found in tea, coffee and numerous foods (Quideau *et al*, 2011), including the seed coat of many varieties of sorghum, beans and millet, bind iron and inhibit iron absorption. Calcium also restricts iron availability for absorption, an issue that is particularly important in Mesoamerica where calcium-containing lime is added during the process of maize preparation ('nixtamalisation'). Thus the absorption of iron from a simple, monotonous diet as is customary in rural, poor populations is at maximum 5% (Food and Agriculture Organization of the United Nations [FAO]/WHO, 1988). As a consequence, the dietary iron intake levels that are required to meet the physiological iron requirements of the poor in low-income countries is much higher than those with diets that are typical in developed countries.

Iron demands are further increased by losses due to chronic bleeding from intestinal wounds caused by helminth infections (hookworm, *Trichuris trichuria* and *Schistosoma* spp.). Malaria is an important cause of anaemia by inducing haemolysis and supressing erythropoiesis under the influence of inflammation, but it does not normally cause body iron loss: following phagocytosis of erythrocytes, iron is retained in macrophages, whilst iron contained in freely circulating haemoglobin and haem following intravascular haemolysis is recycled by haptoglobin and haemopexin, respectively. However, some iron may be immobilised in haemozoin, an insoluble crystalline pigment formed by *Plasmodium* parasites as a degradation product from digested haemoglobin, and iron may be lost in individuals with glucose-6-phosphate

 

dehydrogenase deficiency who suffer haemoglobinuria following treatment with oxidant drugs.

Due to dietary deficiencies and helminth infections, most women in low-income countries start their pregnancies with absent or marginal iron stores. Because of the cessation of menstruation during pregnancy, iron requirements initially decrease in the first trimester compared to the pre-pregnancy state, but they increase dramatically thereafter to meet the additional demands for iron deposition in the foetus, placenta and umbilicus, as well as the increase in maternal erythrocyte mass (Trumbo *et al*, 2001). Compared to the non-pregnant state, however, iron absorption is markedly suppressed during the first trimester of pregnancy (Svanberg, 1975), perhaps due in part to reduced iron needs, but there is also evidence that erythropoiesis is suppressed under the influence of blunted erythropoietin production and receptivity in the first part of pregnancy (Beguin *et al*, 1991). The functional significance or evolutionary survival benefit of this suppressed iron absorption is unknown, but it has been speculated that a large bolus of supplemental iron may overwhelm the mechanisms to restrict iron absorption, which theoretically causes risks to the fetus because iron may be a weak mutagen (Weinberg, 2010). In any case, it suggests that iron supplementation during the first trimester of pregnancy may have limited efficacy.

With normal pregnancy, maternal plasma volume expands between 6 and 34 weeks of gestation. This plasma volume expansion is followed by an increase in erythrocyte mass, which starts at 8 weeks of pregnancy and continues in the second and third trimesters. Plasma expansion exceeds erythrocyte mass, and the resulting haemodilution is believed to be a physiological adaptation to pregnancy, allowing delivery of nutrients to the foetus, protection of the mother from hypotension, and a reduction of the risks associated with haemorrhage at delivery (Gaiser, 2014).

The amount of iron needed for the expansion of erythrocyte mass is contentious. Iron supplementation will boost this expansion, and thus increase haemoglobin concentrations in the second and third trimester. As a consequence, the estimated iron needs are directly dependent on the cut-off value for haemoglobin concentration (Trumbo *et al*, 2001). Reference values for haemoglobin concentration in pregnancy are not defined by functional criteria, but have been derived from samples of iron-supplemented pregnant women in the developed countries (WHO, 1968). Against this background, it is perhaps not surprising that many studies have found that antenatal iron supplementation produces increased maternal haemoglobin concentrations and a reduced risk of anaemia at term. However, the functional significance of this haematological response has long been questioned (Beaton, 2000; Rush, 2000; Rioux & LeBlanc, 2007). It is notable that optimal pregnancy outcomes in terms of birth weight and pre-term labour occur at a mid-pregnancy haemoglobin of between 95 and 105 g/l (Steer, 2000), which is actually lower than the current definition of anaemia. The issue is even further complicated when considering that the distribution of haemoglobin concentration is known to vary independently of iron status in association with residential altitude, smoking, ethnicity and genetic factors (WHO, 2011; New & Wirth, 2015).

## Safety of antenatal iron interventions in malaria-endemic settings

In 2007, 54·7 million pregnancies occurred in areas with stable *P. falciparum* transmission and a further 70·5 million in areas with low malaria transmission or with *P. vivax* only (Dellicour *et al*, 2010). Even a small increase in malaria risk in pregnant women would probably have major public health implications, given the high prevalence of *Plasmodium* infection in many areas and the deleterious effects of infection on both maternal outcomes (severe anaemia, death) and birth outcomes (reduced birth weight, intrauterine growth retardation, preterm delivery, increased neonatal mortality).

In highly endemic areas, most pregnant women are in a state of premunition, whereby infection occurs without symptoms or signs other than a reduction in haemoglobin concentration by 10–20 g/l. The immunity resulting from frequent exposure to infectious mosquito bites is slowly acquired and it is sufficient to control but not prevent infection in adolescents and adults. In pregnancy, the acquired immunity is transiently reduced, particularly in primiparae and in the first and second trimesters of pregnancy (Brabin, 1983; Desai *et al*, 2007). Left untreated, asymptomatic *Plasmodium* infections may persist for months. The prevalence and density of parasitaemia, as well as the level of infection-induced inflammation, are dependent on previous exposure to infection. To avoid being filtered through the spleen, where it would be cleared from the bloodstream and killed, *P. falciparum* adheres to chondroitin sulfate A in the placental intervillous space (Fried & Duffy, 1996), thus leading to the aggregation of parasitized erythrocytes or haemozoin that can be undetectable in peripheral blood smears. In conditions of low, seasonal transmission, a state of premunition is often not attained.

In a systematic review of 28 observational studies, iron deficiency (indicated by circulating ferritin concentration) was found to be associated with protection against *Plasmodium* infection in pregnancy (Sangaré *et al*, 2014).

*In vitro* studies have shown that *P. falciparum* is less efficient in parasitizing erythrocytes from iron-deficient donors than from iron-replete donors (Clark *et al*, 2014a,b). The same group has recently shown this to be true in Gambian children and that it applies both to laboratory and local clinical strains of *P. falciparum* (Goheen *et al*, 2016). They have further shown that young erythrocytes are more susceptible to invasion and propagation by *P. falciparum* merozoites than mature erythrocytes (Clark *et al*, 2014a,b; Goheen *et al*, 2016). Seven weeks of iron supplementation completely abrogated the protection offered by iron deficiency anaemia in

 

both Gambian children (Goheen *et al*, 2016) and pregnant Gambian women (Drs Goheen, Bah & Cerami, MRC Unit The Gambia, Banjul, The Gambia, personal communication). These results indicate that the increased erythropoiesis and thus, the greater availability of young erythrocytes in response to iron supplementation leads to a transient increase in *P. falciparum* malaria risk in iron-deficient individuals but not in their iron-replete peers. These findings also corroborate the report of a randomised trial supplementation in Tanzania, which showed that iron-containing multiple micronutrients increased *falciparum* malaria rates by 41% in children with confirmed iron deficiency, whereas there was no evident effect in their iron-replete peers (Veenemans *et al*, 2011). In addition, it was found in this trial that the excess risk of malaria was most pronounced in the first 50 days of supplementation (Veenemans *et al*, 2011). These studies suggest that women are most susceptible to an iron-induced risk of malaria in the second half of pregnancy, when iron requirements and iron absorption are very high, and erythropoiesis is elevated.

Alternatively, it has been postulated that the excess malaria risk due to supplemental iron is mediated by a transient production after each supplemental dose of non-transferrin bound iron (NTBI), i.e. plasma iron species bound to ligands other than transferrin (e.g. citrate, albumin). Because iron binds to these ligands with less affinity than to transferrin, NTBI may be more available to circulating parasites than transferrin-bound iron (Clark *et al*, 2013). This line of evidence would suggest that women in the second half of pregnancy are at increased risk compared to their pre-pregnancy state or in the first half of pregnancy, because iron absorption and thus, the possible production of NTBI due to iron supplementation may be more pronounced in individuals with iron deficiency than in those who are iron replete (Brittenham *et al*, 2014). However, in contrast to the strong evidence cited above for the transient reticulocytosis as the main driver of increased malaria risk, there is no direct evidence in support of the NTBI hypothesis.

In their systematic review Sangaré *et al* (2014) found one single study from Asia that assessed the association between iron supplementation and *P. vivax* infection. In this prospective cohort study, supplementation with iron and folic acid for less than 30 days was associated with an increased risk *P. vixax* parasitaemia. No effect was found for longer supplementation periods. *P. vivax* has a stronger preference than *P. falciparum* for reticulocytes, suggesting a greater susceptibility to iron-induced malaria risk.

The systematic review also identified two randomised trials (Menendez *et al*, 1994; Ndyomugyenyi & Magnussen, 2000) that assessed the effect of iron supplementation on malaria risk in pregnancy. Both were published before the publication of the CONSORT guidelines and were rated as low quality, mostly because allocation concealment was not described (Sangaré *et al*, 2014). No routine medical malaria prevention was used in these trials, and they were conducted before the wide-scale introduction of insecticide-treated nets. In The Gambia (Menendez *et al*, 1994), where malaria is highly seasonal, multigravidae received daily antenatal supplementation with iron (60 mg elemental iron as ferrous sulphate) or its placebo; all women received folic acid (5 mg/day). In Uganda (Ndyomugyenyi & Magnussen, 2000), where malaria transmission is stable, primiparae received oral supplementation with either iron (120 mg elemental iron daily as dextran) and folic acid (5 mg weekly), or control. Both trials failed to find evidence that iron supplementation caused an increased risk of *Plasmodium* infection.

A second systematic review (Peña-Rosas *et al*, 2012) identified and included another trial (Fleming *et al*, 1986) that had been excluded in an earlier version of the same review (Peña-Rosas & Viteri, 2009) because of the high risk of bias due to incomplete outcome data. In this trial, iron supplementation in Nigerian primigravidae produced an elevated nonsignificant risk of infection (Fleming *et al*, 1986).

Two well-conducted randomised trials (Etheredge *et al*, 2015; Mwangi *et al*, 2015) were published after these systematic reviews. Table II compares the key characteristics and findings from these trials. Neither study found an effect of antenatal oral iron supplementation on *Plasmodium* infection at delivery. A limitation of the Tanzanian trial (Etheredge *et al*, 2015) was that women were at low risk of *Plasmodium* infection, because the study was conducted in an urban, low-transmission area; women were iron-replete and non-anaemic at enrolment; and in the intervention period, they attended a monthly clinic, received intermittent preventive treatment of malaria, vouchers for insecticide-treated nets (coverage: 89%), and care for incident malaria. As a consequence, the malaria risk was low (6·7% vs. 6·5% in the iron and placebo groups, respectively) and the 95% confidence interval (CI) of the effect of iron on *Plasmodium* risk was wide (risk ratio: 3%, 95% CI: −35% to 65%). In addition, because women with anaemia, iron deficiency and HIV infection were excluded, the results cannot be extrapolated to populations with a high prevalence in these conditions.

By contrast, the risk of *Plasmodium* infection at delivery was high in the Kenyan trial (Mwangi *et al*, 2015) (50·9% vs. 52·1% in the iron and placebo groups, respectively), and the upper limit of 95% CI of the effect of iron excluded an increase in the risk of *Plasmodium* infection beyond 9·5%. Iron deficiency was highly prevalent in the study population (60% prevalence at baseline), whilst few women (16%) possessed insecticide-impregnated mosquito nets. There was no evidence that intervention effects on *Plasmodium* infection risk were modified by gravida status, maternal age, HIV infection, anaemia or iron status at baseline. The baseline characteristics of the study population were typical for pregnant women in many rural settings in low-income countries, so that the results apply to a more heterogeneous population than those from the Tanzanian trial, and to

 

**Table II.** Key characteristics and selected results of two randomised trials to assess the effect of supplementation with iron on maternal *Plasmodium* infection risk at birth.

| | Tanzania trial (Etheredge *et al*, 2015) | Kenya trial (Mwangi *et al*, 2015) |
|---|---|---|
| *n* | 1500 | 470 |
| Study population and design features | | |
| Setting | Urban | Rural, poor |
| Malaria transmission | Low | High |
| Chemoprevention* | As per standard care | As per routine care |
| In possession of insecticide-treated net | 88·5% iron group *versus* 88·9% placebo group | 15·2% iron group *versus* 15·9% placebo group |
| Duration of intervention | From ≤27 weeks of gestational age (by date of last menstrual period) until delivery | From 13 to 23 weeks of gestational age (by ultrasound examination) until 1 month postpartum |
| Iron-deficient, anaemic women | Excluded | Included if haemoglobin concentration >90 g/l |
| HIV-infected women | Excluded | Included |
| Intervention | 60 mg elemental iron as ferrous sulphate or placebo | 60 mg elemental iron as ferrous fumarate or placebo |
| Blinding to intervention | Tablets (do not mask iron taste) | Capsules, opaque |
| Adherence assessment | Monthly tablet counts | Swallowing of supplements was daily observed |
| Outcomes | | |
| *Plasmodium* infection risk at birth† | 6·7% iron group *versus* 6·5% placebo group<br>Risk difference: 0·2%<br>Risk ratio: 3%, 95% CI: −35% to 65% ($P$ = 0·89) | 50·9% iron group *versus* 52·1% placebo group<br>Risk difference: −1·2% (95% CI: −11·8% to 9·5%) |
| Mean birth weight | 3155 g *versus* 3137 g; difference: 26 g ($P$ = 0·89) | Difference: 150 g, 95% CI: 56 g to 244 g ($P$ = 0·002) |
| Preterm birth risk‡ | 15·0% iron group *versus* 16·5% placebo group<br>Risk difference: −1·5%<br>Risk ratio: −9%, 95% CI: −29% to 17% ($P$ = 0·46) | 9·1% iron group *versus* 16·2% placebo group<br>Risk difference: −7·1%, 95% CI: −13·2% to −1·1% ($P$ = 0·02) |

*Intermittent preventive treatment with sulfadoxine-pyrimethamine.
†Primary outcome, defined by histopathological examination and polymerase chain reaction (PCR) analysis of placental biopsies (Etheredge *et al*, 2015) or 1 or more positive results for (i) the presence of parasite lactate dehydrogenase (pLDH) or histidine-rich protein II (HRP2) in plasma, or (ii) by placental histopathology, or (iii) *P. falciparum* DNA in maternal erythrocytes from venous or placental blood by PCR test (Mwangi *et al*, 2015).
‡Preterm birth: gestational age <37 weeks.

conditions with low coverage of insecticide-impregnated mosquito nets.

A limitation in the interpretation of the Kenyan trial was that incidental episodes of malaria or other illnesses during pregnancy were not monitored. Data on the use of intermittent preventive treatment were nonetheless obtained from antenatal health booklets that women kept and handed in after the intervention period. There was no evidence that the effect on *Plasmodium* infection risk was modified by intermittent preventive treatment (*P*-value interaction: 0·91), even in women who had not received a single dose of intermittent preventive treatment (*Plasmodium* infection risk: 41·0% vs. 45·4%; difference −4·3%, 95% CI: −28·5% to 19·8%). This is important because coverage of intermittent preventive treatment in pregnancy remains low in Africa: in 2014, only 17% of pregnant women received the minimum of three recommended doses (Table III).

In summary, the concern that antenatal iron supplementation leads to increased maternal *P. falciparum* infection is not supported by epidemiological evidence, at least with a daily supplemental dose of 60 mg as iron ferrous salts. This finding and the difference from the increased malaria rates in young children is most likely explained by greater levels of acquired immunity in adults than in young children in malaria-endemic settings. More work is needed to evaluate effects on *P. vivax*, which often occurs at lower levels of endemicity, and has a stronger preference for reticulocytes than *P. falciparum*.

## Antenatal iron interventions and bacterial infections

Recent studies indicate that iron interventions can increase the susceptibility to both systemic and enteric infections, even though the health importance of these findings in pregnancy requires further study.

*Ex vivo* studies have illustrated that oral supplementation at doses recommended for prevention of iron deficiency can potentially undermine innate immunity that is associated with iron withholding. Adult male volunteers were given oral supplementation with iron (2 mg/kg iron as ferrous sulfate), and *ex vivo* growth of sentinel bacteria species was assessed

 

**Table III.** Efficacy and coverage of key interventions recommended by the World Health Organization (WHO) to prevent malaria* in African pregnant women.

| Intervention | Policy | Protective efficacy against malaria | Coverage |
|---|---|---|---|
| Insecticide-treated mosquito nets | In endemic areas with intense malaria transmission, all pregnant women should receive, as early as possible in pregnancy, one long-lasting insecticidal net through immunisation and antenatal care visits† | Compared with no nets (Gamble *et al*, 2006):<br>• Risk of peripheral parasitaemia at delivery reduced by 23% (95% CI: 14–48%; $I^2$: 0%)<br>• Parasite density reduced by 7% (95% CI: −11% to 23%, $I^2$: 42%)<br>• Placental parasitaemia reduced by 21% (95% CI: 2–37%, $I^2$: 35%) | In 2015, 55% of the population of sub-Saharan Africa was sleeping under an impregnated mosquito net (WHO, 2015) |
| Intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine | In areas with moderate to high malaria transmission in Africa, delivery of sulfadoxine-pyrimethamine is recommended at each of the three recommended antenatal care visits after the first trimester, with a minimum of three doses received during each pregnancy (WHO, 2014) | Reduction in risk compared to placebo/no intervention (Radeva-Petrova *et al*, 2014):†<br>• Maternal parasitaemia (i.e. presence of asexual stage parasites in thick smears in peripheral, placental, or cord blood): 62% (95% CI: 41–76%, $I^2$: 86%)<br>• Malarial illness (history of fever episodes prior to delivery): 76% (95% CI: −12% to 95%, 1 study only)<br>• Placental parasitaemia: 55% (95% CI: 39–67%, $I^2$: 54%)<br>• Cord blood parasitaemia: 53% (95% CI: −1% to 78%, $I^2$: 40%) | Only 52% of eligible pregnant women received at least one dose of IPT in pregnancy in 2014, while 40% received two or more doses and 17% received three or more doses (WHO, 2015) |

*Effects on malaria-associated outcome such as maternal anaemia, birth weight and perinatal mortality are not listed because they were outside the scope of the current review.

†Universal access to and use of long-lasting insecticidal nets remains the goal for all people at risk of malaria.

in sera collected before and at various time points after supplementation (Cross *et al*, 2015). Growth of *Staphylococcus aureus*, which preferentially scavenges haem iron, was unaffected. By contrast, *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella enterica* serovar Typhimurium, and *Staphylococcus epidermidis* showed markedly elevated growth in response to iron supplementation. Growth rates were strongly correlated with transferrin saturation, which transiently increased from 42·1% to 75·7%, but the effects may also have been mediated at least in part by transient production of NTBI.

Iron supplementation and iron fortification have also been shown to adversely modify the abundance and virulence of pathogenic enteric bacteria (Zimmermann *et al*, 2010; Kortman *et al*, 2012; Dostal *et al*, 2014; Jaeggi *et al*, 2015).

## Safety of antenatal iron interventions in women with high haemoglobin concentration

There are concerns that antenatal iron supplementation in women with high haemoglobin concentration (above 130–135 g/l) in the second and third trimesters can lead to an increased risk of adverse pregnancy outcome, such as premature delivery and low birth weight (Peña-Rosas & Viteri, 2009). Most reports on this association concerned observational studies e.g. (Murphy *et al*, 1986; Zhou *et al*, 1998; Steer, 2000; Xiong *et al*, 2000; Casanueva & Viteri, 2003), which may be confounded because high haemoglobin concentrations may also be due to other pregnancy complications (e.g., a failure of the normal pregnancy-induced physiological plasma volume expansion, pre-eclampsia, hypertension) that can cause maternal and perinatal deaths.

In a randomised trial in Iran, antenatal supplementation with iron (50 mg as ferrous sulphate) in women with haemoglobin concentration ≥132 g/l in the early stage of the second trimester resulted in an increased risk of women with hypertension disorder [2·7% vs. 0·8%, difference: 1·9% (95% CI: −0·2% to 4·1%)] and small-for-gestational age neonates [15·4% vs. 10·1%, difference: 5·3% (95% CI: 0·5–10·2%)] (Ziaei *et al*, 2007). However, in a recent large trial among Chinese pregnant women with haemoglobin concentration >145 g/l at enrolment (gestational age <20 weeks), supplementation with iron (30 mg as ferrous fumarate) *increased* birth weight by 91 g (95% CI: 3–180 g) (Wang *et al*, 2016). It should also be noted that in most low-income countries, women with haemoglobin concentrations >130 g/l constitute a small proportion of the total number of pregnancies.

 

**Table IV.** Effect of daily antenatal iron supplementation on selected outcomes, meta-analysis of randomised controlled trials (Peña-Rosas *et al*, 2012).*

| Outcome | Participants, *n* (trials, *n*) | Summary measure | Effect (95% CI) | $I^2$ |
|---|---|---|---|---|
| Maternal haemoglobin concentration at or near term (at 34 weeks gestation or more) | 3704 (19) | Mean difference | 8·9 g/l (7·0–10·8 g/l) | 0·87 |
| Maternal haemoglobin concentration within 6 weeks postpartum, g/l | 956 (7) | Mean difference | 7·6 g/l (5·5–9·7 g/l) | 0·4 |
| Maternal anaemia at term (haemoglobin concentration <110 g/l at 37 weeks of gestation or later) | 2199 (14) | Risk ratio | 0·30 (0·19–0·46) | 0·8 |
| Maternal severe anaemia at postpartum (haemoglobin concentration <80 g/l) | 1339 (8) | Risk ratio | 0·04 (0·01–0·28) | 0 |
| Maternal iron deficiency at term (as defined by researchers, based on any indicator of iron status at 37 weeks gestation or more) | 1256 (7) | Risk ratio | 0·43 (0·27–0·66) | 0·85 |
| Maternal high haemoglobin concentrations at or near term (haemoglobin concentration >130 g/l at 34 weeks gestation or later) | 4850 (9) | Risk ratio | 3·08 (1·28–7·41) | 0·96 |
| Transfusion provided to the mother | 3453 (3) | Risk ratio | 0·61 (0·38–0·96) | 0 |
| Birth weight | 9385 (14) | Mean difference | 30·8 g (5·9–55·7 g) | 0·23 |
| Low birth weight (<2·5 kg) | 8480 (11) | Risk ratio | 0·81 (0·68–0·97) | 0·16 |
| Premature birth (<37 weeks of gestation) | 10 148 (13) | Risk ratio | 0·88 (0·77–1·01) | 0 |
| Infant haemoglobin concentration within the first 6 months; counting the last reported measure after birth within this period | 533 (2) | Mean difference | −1·3 g/l (−8·1 to 5·6 g/l) | 0·89 |
| Infant serum ferritin concentration in the first 6 months; counting the last reported measure after birth within this period | 197 (1) | Mean difference | 11·0 μg/l (4·37–17·63 μg/l) | NA |

NA, not applicable; 95% CI, 95% confidence interval.
*Any supplements containing iron *versus* same supplements without iron or no treatment.

## Benefits of antenatal iron interventions

Iron deficiency is the primary cause of anaemia in all regions of the world (Kassebaum *et al*, 2014), and iron interventions can prevent 20–50% of the prevalence of anaemia in pregnant women (Black *et al*, 2013; Black, 2014; WHO, 2015). Anaemia is a moderate-to-severe public health problem in pregnancy in virtually all countries worldwide, affecting 46%, 49%, 39% and 25% of pregnant women in WHO regions of Africa, Southeast Asia, the Eastern Mediterranean and Americas, respectively (WHO, 2015).

Based on observational studies on the association between anaemia and mortality, iron deficiency in pregnancy has been estimated to cause 115 000 maternal deaths and 591 000 perinatal deaths worldwide. The associated loss of healthy life years amounts to almost 20 million disability-adjusted life years (DALYs) from perinatal causes and almost 3·5 million from maternal causes (Stoltzfus *et al*, 2004). Observational studies on the relationship between anaemia and maternal mortality typically suffer from severe methodological shortcomings (reviewed by Rush, 2000) that preclude solid conclusions about causality.

A recent meta-analysis of randomised trials (Peña-Rosas *et al*, 2012) was undertaken to close the knowledge gaps on the causal effects of antenatal iron supplementation on maternal and neonatal health outcomes. Key results are summarised in Table IV.

First, antenatal iron supplementation was found to reduce the risk of a transfusion being required by the mother by 39% and the risk of severe anaemia at postpartum (haemoglobin concentration <80 g/l) by 96%. We hypothesize that effects on these outcomes are mediated by two possible mechanisms. First, in women with iron deficiency, iron supplementation can reduce the severity of blood loss at or after delivery, as indicated by a study showing that the severity of anaemia before a spontaneous vaginal delivery was associated with the amount of blood loss at delivery and the immediate postpartum period (Kavle *et al*, 2008). Second, in women with prenatal iron deficiency anaemia, iron supplementation is likely to raise pre-delivery haemoglobin concentrations, thus reducing the risk of severe anaemia following perinatal blood loss. In both cases, iron supplementation would provide women with an increased chance of surviving maternal haemorrhage, which is the leading cause of maternal deaths during pregnancy (GBD 2013 Mortality and Causes of Death Collaborators, 2015). Unfortunately, in the meta-analysis referenced above (Peña-Rosas *et al*, 2012), the effect on maternal deaths was not estimable because of a lack of data.

Second, the effect of antenatal iron supplementation on pre-partum and post-partum haemoglobin concentrations was generally positive but varied greatly in size between trials (as shown by the high values for $I^2$, the percentage of total variation across studies that is due to heterogeneity). Subgroup analysis suggested that the effect of intervention on maternal anaemia at term was less pronounced in settings with malaria than in those without malaria (risk reduction: 39% vs. 82%, *P*-interaction: 0·0006). This finding can be explained at least in part by evidence that malaria-induced inflammation reduces iron absorption under the influence of upregulated production of hepcidin (Verhoef, 2010; Spottiswoode *et al*, 2012).

Third, the meta-analysis found antenatal iron supplementation increased birth weight by 31 g, and it decreased the risk of low birth weight by 19%. This effect on birth weight is small compared to the normal variability in birth weight (the usual standard deviation is ~450 g) and corroborates findings from other recent meta-analyses that found no effect on birth weight (Vucic *et al*, 2013) or an increase of only 41 g (Haider *et al*, 2013).

A major limitation of these meta-analyses is that they were based on randomised trials that excluded women with anaemia or iron deficiency; or women received iron as rescue therapy during intervention (including those in the placebo group); or initial anaemia status and iron status had not been specified. As a consequence, the results do not apply to iron-deficient women, and they cannot be extrapolated to populations with a high prevalence of iron deficiency or anaemia.

To our knowledge, the study in Kenyan women cited previously (Mwangi *et al*, 2015) is the only placebo-controlled trial conducted so far that enrolled women with iron deficiency and anaemia at baseline. In that trial, antenatal iron supplementation increased birth weight by 150 g (95% CI: 56–244 g). It was anticipated that iron absorption and thus effects of administered iron would depend on baseline iron status. Subgroup analysis confirmed that the effect of iron on birth weight was larger in women with iron deficiency at baseline than in those who were initially iron-replete (234 g vs. 39 g; difference, 195 g; 95% CI, −3 to 393 g; $P = 0{\cdot}05$). The risk of low birth weight decreased by 58%. The absolute risk reduction was 6·0% (95% CI, 0·8–11·1%), indicating that, on average, 16·8 women (95% CI, 9·0–61·3%) needed to receive supplementation to prevent 1 case of low birth weight.

Additional results indicated that the improvements in birth weight were achieved at least in part through an increased gestational duration, a decreased risk of prematurity and an increased neonatal length (Mwangi *et al*, 2015). Lastly, antenatal iron supplementation improved neonatal iron stores at 1 month postpartum, thus probably delaying the age at which iron deficiency will develop during infancy. Consistent with the effect on birth weight, the effect on plasma ferritin concentrations in neonates varies by initial iron status of the mother (Mwangi *et al*, 2015), with larger effects in children with mothers who were iron deficient at baseline (unpublished observations).

## Screen and treat: an alternative to universal iron supplementation in pregnancy?

As an alternative to universal supplementation, iron supplementation could be restricted to pregnant women who are screened and diagnosed with iron deficiency during antenatal clinics. As minimum requirements, these tests should be low-cost, practicable in field conditions and provide immediate results at the point of care. At present, the only two diagnostic tests that could possibly meet these criteria are haemoglobin concentration and whole blood zinc protoporphyrin content (commonly expressed as its molar ratio to haem). Both markers have the disadvantage that they are influenced by inflammation independently of iron status. Their diagnostic utility was recently evaluated in Kenyan pregnant women without inflammation, *Plasmodium* infection or HIV infection (Mwangi *et al*, 2014). When these markers are used for screening purposes in settings with a moderate or high prevalence of iron deficiency, however, their ability to discriminate between individuals with and without iron deficiency was found to be inadequate, whether used alone or in combination. When applied to all women (i.e. including inflammation and infections), their diagnostic performance would presumably have been even worse.

There is no universally accepted method for accounting for inflammation when estimating iron status using ferritin values. In low-income countries, the WHO recommends measuring inflammation markers, such as C-reactive protein (CRP) and/or α-1-acid glycoprotein (AGP). These are used together with ferritin to exclude inflamed individuals from analysis or to alter the cut-off of ferritin used to define deficiency. Recently, the Biomarkers Reflecting Inflammation and Nutrition Determinants of Anaemia (BRINDA) project proposed possible methods to address inflammation and other potential confounders of ferritin (Suchdev *et al*, 2016). The quantitative assessment of circulating concentrations of CRP and AGP allows for adjustment of ferritin concentration for the effect of inflammation (Suchdev *et al*, 2016; Thurnham & Northrop-Clewes, 2016). In addition, point-of-care tests are rapidly developing, with quantitative tests already being commercially available for circulating concentrations of ferritin and C-reactive protein. Access to such tests to allow diagnosis of iron deficiency in low-income countries is highly desirable because screening based on plasma ferritin concentration has been shown to better predict beneficial responses to iron interventions than screening based on anaemia (Mwangi *et al*, 2015).

## Discussion and conclusions

As with any intervention, uncertainty about the safety of antenatal iron supplementation is inevitable, because

 

conclusive proof of safety would require that randomised controlled trials in a wide variety of conditions consistently produce 95% CIs with upper limits that exclude an increased risk of adverse effects. In other words, trials would need to show that antenatal iron supplementation actually *protects* against *Plasmodium* infection.

In the present review, we found no evidence that antenatal iron supplementation at a daily dose of 60 mg/day leads to an increased risk of maternal *Plasmodium* infection. This finding should not be extrapolated to young children, who in endemic areas have lower levels of acquired immunity, and for whom there is substantial evidence that iron supplementation can increase the incidence of malaria.

The trial in Kenyan women (Mwangi *et al*, 2015) provides unique and compelling evidence, indicated by multiple markers, that antenatal supplementation with iron provides maternal and neonatal health benefits in iron-deficient pregnant women that vary from those in their iron-replete counterparts. Thus, the benefits of universal iron supplementation are likely to vary with the prevalence of iron deficiency, and also probably exceed the relatively small benefits found in earlier meta-analyses (Peña-Rosas *et al*, 2012; Haider *et al*, 2013). In settings where health personnel make decisions for individual women without diagnostic tests, they need to consider the *a priori* probability of iron deficiency. In most conditions in low-income countries, this probably is high and may exceed 50%.

Thus, the balance between benefits and risks is probably more favourable in low-income countries than in high-income countries. It should be noted that even in women with iron deficiency in their second pregnancy trimester, antenatal iron supplementation increased birth weight by 234 g without evidence of an increased risk of malaria (Mwangi *et al*, 2015).

Considering the continued efficacy of intermittent preventive treatment and insecticide-impregnated mosquito nets (Table III), it is prudent that iron supplementation is provided to pregnant women in conjunction with measures to prevent, diagnose and treat malaria, as recommended by the WHO. The study by Mwangi *et al* (2015) suggests, however, that the benefit outweighed the risk even in conditions of poor coverage of preventive measures.

Uncertainties remain about the safety of antenatal iron supplementation in conditions with *P. vivax* transmission, about the health importance of studies suggesting that iron supplementation can (in *ex vivo* experiments) stimulate the growth of potential pathogens in serum, and about the balance of benefits and risks of antenatal supplementation in malaria-endemic areas at doses of iron exceeding 60 mg/day. This is particularly important in view of the WHO recommendations of using a daily treatment dose of 120 mg in women with anaemia, and a weekly dose of 120 mg in non-anaemic pregnant women (Table I).

## Conflict of interest

London School of Hygiene and Tropical Medicine and Wageningen University have jointly applied for a patent for the invention relating to an iron supplement for use in the treatment and/or prevention of infant low birth weight, which lists Hans Verhoef as the inventor. No other disclosures were reported.

## Author contributions

MNM and HV prepared a first draft of the paper. All authors critiqued and approved the final manuscript.

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