

# Iron Supplementation in Predominantly Iron-Replete Populations

## Is there an emerging concern?

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Children outside of a health centre in Dili, Timor-Leste

### The global burden and potential causes of anemia

In 2011, it was estimated that, globally, 496 million non-pregnant women of reproductive age (WRA) were anemic.<sup>1</sup> Defined as a hemoglobin concentration < 120 g/L in non-pregnant WRA,<sup>2</sup> anemia can increase the risk of adverse pregnancy outcomes,<sup>3</sup> impair work capacity and productivity of women,<sup>4</sup> and ultimately hinder social and economic development. There is a tacit assumption that approximately 50% of anemia is due to iron deficiency in low- and middle-income countries (LMIC), which has been the impetus for global WHO recommendations

for blanket (untargeted) iron supplementation among women and adolescents in areas of high anemia prevalence (**Figure 1**).<sup>5,6</sup> However, there are many other potential causes of anemia, including infection/inflammation (e.g., environmental enteric dysfunction, EED), micronutrient deficiencies other than iron (e.g., vitamin B<sub>12</sub> and folate), and genetic factors (e.g., hemoglobinopathies) (**Figure 2**).

“It is often assumed that 50% of anemia is due to iron deficiency, which has been the impetus for global WHO recommendations for blanket iron supplementation”

### Recent surveys have shown a surprisingly low prevalence of iron deficiency among non-pregnant WRA

Recent surveys in Bangladesh,<sup>7</sup> Cambodia,<sup>8–10</sup> the Democratic Republic of the Congo,<sup>11</sup> Nepal,<sup>12</sup> Rwanda,<sup>13</sup> Sierra Leone,<sup>14</sup> and Vietnam<sup>15</sup> have shown a surprisingly low prevalence of iron deficiency ( $\leq 8\%$  based on inflammation-adjusted ferritin <12–15  $\mu\text{g/L}$ ) among non-pregnant WRA (**Table 1**). This is important because if iron deficiency is not a major cause of anemia, then untargeted iron supplementation is at best a waste of resources, and at worst, a potential source of harm.

### Potential reasons for the low prevalence of iron deficiency among women of reproductive age

Probably many factors are contributing to high ferritin concentrations in women, although the exact cause is often difficult to ascertain. Complicating this further is the fact that many of these factors coexist and impact iron metabolism in varying ways.<sup>16</sup>

**FIGURE 1:** Global WHO recommendations for iron supplementation in non-pregnant women of reproductive age in areas of high anemia prevalence (2011 and 2016)



**Left:** Since 2009, the WHO has recommended intermittent IFA supplementation (60 mg elemental iron weekly for three consecutive months (followed by three months of no supplementation, after which the provision of supplements should restart) for menstruating women and adolescent girls in areas of anemia prevalence  $\geq 20\%$ . The guidelines were updated in 2011. **Right:** In 2016, WHO released *complementary* guidelines recommending daily IFA supplementation (30–60 mg elemental iron daily) for three consecutive months of the year for menstruating women and adolescent girls in areas of anemia prevalence  $\geq 40\%$ .

Infection, inflammation, and EED are all potential contributors to high ferritin concentrations. Infectious pathogens, metabolic stress and tissue damage all activate the inflammatory cascade.<sup>17</sup> Cytokines are released, stimulating the production of hepcidin, which functions as the main regulator of iron metabolism.<sup>18</sup> Hepcidin binds to and degrades ferroportin, a transport protein on the surface of the macrophage, sequestering the iron from recycled red blood cells in the macrophage and making it unavailable for erythropoiesis (thus leading to high ferritin concentrations).<sup>19,20</sup> Hepcidin also acts on the gut in a regulatory manner to inhibit iron absorption when hepcidin production is stimulated (as in the presence of inflammation). These are thought to be protective mechanisms to prevent pathogenic organisms from using iron in circulation.<sup>21</sup>

Genetic hemoglobinopathies are common in many regions of the world and can result in a decreased or defective hemoglobin production, leading to an increased risk of anemia and other serious health problems.<sup>18,22</sup> Sickle hemoglobin (Hb S) is a variant found in high frequencies across most of sub-Saharan Africa, the Middle East, and India.<sup>23</sup> These hemoglobinopathies tend to have high frequencies in tropical regions because of their conferred resistance against malaria.<sup>23</sup> Some hemoglobinopathies have also been shown to be associated with high ferritin concentrations.<sup>8,24</sup> In Cambodia, we observed that women with the hemoglobin E homozygous genotype (7%,  $n=31/450$ ), which is a disorder caused by a mutation in the  $\beta$ -globin gene from both parents, was associated with 50% (95% CI: 14, 96%) higher mean ferritin concentration, as compared to women with a

**FIGURE 2:** Potential causes of anemia**Infection | Inflammation**

- > Environmental enteric dysfunction (EED)
- > Malaria
- > Helminth (parasites)

**Iron Deficiency**

- > Caused by inadequate dietary iron intake, impaired absorption of iron, or increased loss of iron from the body

**Micronutrient Deficiencies**

- > Vitamins A, B<sub>6</sub>, B<sub>12</sub>, C, riboflavin, folate, copper

**Genetic Factors**

- > Hemoglobinopathies (Hb E, sickle cell, thalassemia)
- > Glucose-6-phosphate dehydrogenase deficiency
- > Mutations in the transmembrane serine protease serine 6 (*TMPRSS6*) gene (causing overproduction of hepcidin and leading to iron-refractory iron deficiency anemia)

# Anemia

normal hemoglobin structure.<sup>25</sup> As such, the diagnostic accuracy of ferritin to estimate iron deficiency in individuals with this genotype is in question. Assessment of genetic hemoglobinopathies is a crucial factor in understanding the potential causes of anemia in a population and in designing and implementing effective anemia reduction strategies and programs.

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Naturally existing iron in groundwater may be a contributing factor to high iron stores. Elevated groundwater iron levels have been reported in Bangladesh and Cambodia.<sup>7,26</sup> However, the potential contribution to body iron stores largely depends on the bioavailability of the iron. Measurement of the iron bioavailability in groundwater is complicated, as both diet composition and an individual’s iron status can influence bioavailability.<sup>27</sup> Another factor related to iron in groundwater is the utilization of point-of-use water filters, which are commonly



Screening women for anemia using the HemoCue® 301+ in rural Cambodia

used in households in some countries including Cambodia.<sup>28</sup> For example, the BioSand filter – a slow sand filter designed to remove arsenic and microbiological contamination<sup>28</sup> – has been shown to remove up to 98% of the iron from groundwater.<sup>26,29</sup> As such, the availability and use of these filter systems at the household level is an important consideration in assessing potential iron intake from groundwater. More rigorous examination of groundwater iron content, chemical form and bioavailability is warranted.

**TABLE 1:** Low prevalence of iron deficiency (based on inflammation-adjusted ferritin) among non-pregnant women of reproductive age in recent surveys

Country, year of publication	Surveyed population	Definition	Prevalence of iron deficiency	Reference
Bangladesh, 2012	n=209 women in Gaibandha district	Plasma ferritin <12 µg/L	0%	Merrill et al <sup>7</sup>
Cambodia, 2015	n=420 women 18–45 years in Prey Veng province	Serum ferritin <15 µg/L	2.1%	Karakochuk et al <sup>8</sup>
Cambodia, 2015	n=2,112 women 15–39 years in a national survey	Serum ferritin <15 µg/L	8.1%	Wieringa et al <sup>9</sup>
Cambodia, 2015	n=738 mothers 15–49 years with at least one child less than five years of age in a national survey	Serum ferritin <15 µg/L	2.6%	2014 Cambodia Demographic and Health Survey <sup>10</sup>
DR Congo, 2016	n=741 women 18–45 years in South Kivu and Kongo Centrale provinces	Serum ferritin <15 µg/L	5.4% (3.9, 7.3%)	Harvey-Leeson et al <sup>11</sup>
Nepal, 2016	n=500 mothers 15–44 years in Bhaktapur municipality	Plasma ferritin <15 µg/L	5%	Chandyo et al <sup>12</sup>
Rwanda, data not yet published	n=595 women 18–44 years in Northern and Southern provinces	Serum ferritin <15 µg/L	4.8% (3.2, 7.2%)	Angel et al <sup>13</sup>
Sierra Leone, 2016	n=871 women 15–49 years in a national survey	Plasma ferritin <15 µg/L	8.3% (6.2, 11.1%)	Wirth et al <sup>14</sup>
Vietnam, 2015	n=4,986 women planning to become pregnant in next year in Thai Nguyen province	Serum ferritin <15 µg/L	3.5%	Nguyen et al <sup>15</sup>

Values are proportion of women with iron deficiency (%) or iron deficiency prevalence estimates [mean (95% CI)]. Ferritin concentrations were assessed using a combined s-ELISA (with the exception of Chandyo et al (who used an electrochemiluminescence immunoassay), and were adjusted for inflammation using AGP and CRP concentrations based on Thurnham et al methods.<sup>30</sup>

There are a few important caveats to highlight among the studies outlined in **Table 1**. First, the Chandyo et al<sup>12</sup> study and the Cambodia Demographic and Health Survey<sup>10</sup> were conducted among *mothers* who had received iron and folic acid (IFA) supplementation during their pregnancy, which may have been a confounding factor. The findings of low iron deficiency among mothers may not be generalizable to nulliparous women, who had neither been pregnant nor received IFA. Second, all studies reported used Thurnham et al correction factors to adjust ferritin for levels of inflammation, which are based on the stages of inflammation (incubation, early and late convalescence) as assessed by the biomarkers  $\alpha_1$ -acid glycoprotein (AGP) and C-reactive protein (CRP).<sup>30</sup> Recently at the Micronutrient Forum in Cancun, the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project proposed a new linear regression method for the correction of ferritin in the presence of inflammation.<sup>31</sup> Some preliminary unpublished

data have shown that linear regression is a more rigorous method to comprehensively account for inflammation, as compared to the Thurnham method. This is because linear regression relies on AGP and CRP biomarkers as continuous variables, whereas the Thurnham method relies on AGP and CRP as binary variables based on arbitrary cut-offs. It has been reported that using the linear regression method (BRINDA) may result in lower corrected ferritin concentrations and thus a higher prevalence of iron deficiency. This may be of particular importance among populations with a high prevalence of inflammation. As such, the prevalence rates estimated with different inflammation-correction methods should be interpreted with some degree of caution until more evidence is established.

In many countries, numerous iron interventions are often implemented at the same time, with the shared aim of reducing, preventing, and/or treating anemia. For example, according to the National Nutrition Policy in Cambodia,<sup>32</sup> daily IFA (60 mg



Collecting groundwater from a well in rural Cambodia to measure iron levels

iron/day) is recommended for pregnant women for 90 days, for postpartum women for 42 days, and for women who have had a miscarriage or abortion for 42 days. Further, daily IFA (60 mg iron/twice daily [total: 120 mg/day]) is recommended for anemic women for 90 days, after which, if the anemia is not corrected, a second round of the same treatment is advised. The policy also recommends weekly IFA (60 mg iron/week) for all non-pregnant women until they become pregnant (although this program has not yet reached national scale). In addition, the policy also outlines several other strategies to increase iron intake, such as increasing dietary diversity to include more iron-rich sources of food and decreasing the consumption of iron absorption inhibitors (e.g., tea).<sup>32</sup> Recently, the Ministries of Planning and Health in Cambodia mandated the fortification of all fish and soy sauce with iron.<sup>33</sup> Another intervention in Cambodia is the Lucky Iron Fish®, an iron ingot that is placed into the household's cooking pot and slowly releases iron into cooked food.<sup>34</sup> In conclusion, the total iron content consumed by women in these contexts is largely unknown but expected to be high.

### Emerging concerns regarding iron supplementation in children

Although there is strong evidence that iron supplementation and/or at-home fortification reduces the risk of anemia and/or iron deficiency in young children,<sup>35–37</sup> there have been some emerging concerns of risk of adverse outcomes with the provision of iron, specifically among children with infections such as malaria, pneumonia, tuberculosis, or diarrhea.

A trial by Soofi et al<sup>38</sup> in Pakistan showed that iron-containing micronutrient powders were effective at reducing iron deficiency anemia but increased the incidence of diarrhea and the risk

of bloody diarrhea and respiratory illness among children six to 18 months of age. A recent Cochrane review did not find an increased risk of non-malaria infectious diseases associated with iron supplementation among children < 18 years of age, although the authors concluded with the caveat that iron supplementation was safe “when malaria prevention or management services are provided efficiently.”<sup>39</sup> This poses a challenge to interpretation of the findings in this review, as there is currently a lack of well-established criteria and/or indicators used to measure the efficiency of malaria surveillance and treatment programs.

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A study by Zimmermann et al<sup>40</sup> in Cote d'Ivoire examined the effect of iron-fortified biscuit consumption on the gut microbiota profile in Ivorian children six to 14 years of age. After six months of intervention, there was a significant increase in the number of pathogenic enterobacteria, a decrease in beneficial lactobacilli bacteria, and an increase in mean fecal calprotectin concentration (a marker of intestinal inflammation) in the children receiving iron-fortified biscuits, as compared to non-fortified biscuits.<sup>40</sup> Iron is a growth-limiting nutrient for many pathogen-



Fish sauce and soy sauce are now fortified with iron in Cambodia

ic gut bacteria, thus it is plausible that increasing the colonic iron content of the gut could cause adverse changes to the gut microbiota profile.

At a recent symposium on “*Iron Screening and Supplementation of Iron-Replete Pregnant Women and Children*” at the National Institutes of Health (Bethesda, Maryland, USA), Zimmermann proposed potential solutions to minimize these risks associated with iron supplementation and/or at-home fortification, such as decreasing the dose of iron, the use of a more absorbable form of iron (e.g., chelated iron), or the addition of ascorbic acid or endogenous phytase (to increase iron absorption and decrease the load of colonic iron that reaches the gut), or adding a prebiotic non-digestible source of fiber (e.g., galacto-oligosaccharides, GOS) to enhance the proliferation of beneficial gut bacteria. These promising approaches require further evaluation.

### The dilemma of iron supplementation in predominantly iron-replete non-pregnant women of reproductive age

There is evidence that iron supplementation reduces the risk of anemia and/or iron deficiency among menstruating women. A recent systematic review by Low et al concluded that there is evidence from ten trials (including 3,273 non-pregnant menstruating women 12–50 years) that suggests daily oral iron supplementation reduces the prevalence of anemia (relative risk [RR]: 0.39 [95% CI: 0.25, 0.60]).<sup>41</sup> These ten trials included all women of menstruating age (irrespective of anemia or iron status) and at varying doses and durations of iron therapy (between 4 and 12 weeks).<sup>41</sup> However, the authors of this review did not examine the potential risks of iron supplementation (e.g., oxidative stress, lipid peroxidation, or iron overload), as no studies included in the review measured risk-related outcomes other than the adverse gastrointestinal side effects of iron supplementation (e.g., constipation) and the studies were not of sufficient duration to investigate chronic morbidities.<sup>41</sup>

There are potential risks of iron supplementation in iron-replete populations (linked to iron overload), such as increased oxidative stress and reactive oxygen species,<sup>42</sup> which in turn have been associated with the pathogenesis of chronic conditions such as diabetes and its resulting complications (e.g., diabetic nephropathy and cardiovascular disease),<sup>43,44</sup> DNA damage leading to cancer,<sup>45</sup> and neurodegenerative diseases (e.g., Parkinson’s disease).<sup>46</sup> It is suspected that an even higher risk from iron supplementation may exist among iron-replete individuals with inflammation and certain genetic hemoglobinopathies, as these individuals are already at risk of high iron stores.<sup>47</sup>

Further to the aforementioned risks described for children, the impact of adverse changes to the gut microbiota in women (especially pregnant women) are not known but could potentially impact future offspring through epigenetic mechanisms.<sup>48</sup> More research in this field of work is urgently needed to ascer-



Nutrition education session outside of a health centre in Southern Ethiopia

tain if risks of iron supplementation in iron-replete populations (especially among those individuals with hemoglobinopathies and inflammation) translate to adverse outcomes of biological or clinical significance.

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**“If iron deficiency is not a major cause of anemia, then national policies and programs for anemia reduction may need to be re-evaluated”**  
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### Possible solutions and future directions

- > It is crucial to comprehensively assess and understand the multifactorial causes of anemia in each country-specific setting or population, and when possible, this should include a genetic (e.g., hemoglobinopathies) and biochemical assessment of indicators related to hemoglobin and iron status. This is fundamental to designing and implementing effective anemia reduction strategies and programs.
- > It may be warranted to reduce the iron content in IFA supplements from 60 mg to 30 mg in regions where women of reproductive age have shown to have a low prevalence of iron deficiency and/or where anemia is probably caused by reasons other than iron deficiency. The recent 2016 WHO guidelines that recommend daily IFA supplementation for three consecutive months of the year among menstruating women and adolescent girls in areas of anemia prevalence  $\geq 40\%$ , suggest a dose between 30 mg and 60 mg elemental iron daily.<sup>6</sup> These guidelines were based upon the evidence

generated in a recent systematic review by Low et al.<sup>41</sup> Further, the UNICEF/WHO/UNU multiple micronutrient formulation for pregnant and lactating women (UNIMMAP) contains only 30 mg iron (rather than 60 mg). This dose was chosen based on the rationale that adherence would probably be improved with lower dose (due to increased adverse side effects with a higher dose), and that a lower dose of iron would have less adverse impact on zinc absorption.<sup>49</sup> In the recently published WHO Antenatal Care Guidelines, daily IFA (30-60 mg iron) is recommended for pregnant women, however, the guideline also provides an alternative context-specific recommendation for intermittent IFA (120 mg iron weekly) in populations with an anemia prevalence among pregnant women <20%, or when the daily IFA is not accepted due to adverse side effects.<sup>50</sup>

- > Future studies should include outcomes that measure both the risks and benefits of iron supplementation in order to better inform policy. The potential risk-related outcomes could include indicators of oxidative stress, lipid peroxidation, DNA or cell damage, gut microbiome or metabolites related to iron overload, in addition to the adverse gastrointestinal side effects of iron supplementation (e.g., constipation, diarrhea, and abdominal pain).

### Conclusion

The efficacy and safety of iron supplementation probably varies by population and context, and also depending on the proportion of anemia that is due to iron deficiency rather than other causes. Recent surveys showing a low prevalence of iron deficiency among non-pregnant WRA warrants further attention to the potential risks of iron supplementation in predominantly iron-replete populations.

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**“Further attention to the potential risks of iron supplementation in predominantly iron-replete populations is warranted”**

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*Presentations from the recent symposium on “Iron Screening and Supplementation of Iron-Replete Pregnant Women and Children” at the National Institutes of Health (Bethesda, MD, USA) are available online via:*  
[ods.od.nih.gov/Research/Iron.aspx#workshop](https://ods.od.nih.gov/Research/Iron.aspx#workshop)

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