Meeting the Iron Needs of Young Children

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Editorial

Most recommended dietary allowances around the world recognize between 25 and 30 essential nutrients and, using a range of terminologies, set recommended nutrient intakes (RNIs) and safe upper levels (ULs) for intake. Among all these nutrients, iron plays a special role arising from its distinct chemical properties. Foremost among these is that the valency change from the ferrous (Fe$^{2+}$) to the ferric (Fe$^{3+}$) state has been adopted by numerous biological processes for electron receipt and donation. Thus, iron acts as a cofactor in a very wide range of enzymes and redox pathways in almost all prokaryotic and eukaryotic organisms. Humans, like many other multicellular animals, have fought an evolutionary battle for iron against a wide spectrum of their potential pathogens. This has led to the evolution of a range of chaperone proteins in the human host that serve to sequester free iron (lactoferrin, transferrin, ferritin, lipocalin-2), heme (hemopexin), and hemoglobin (haptoglobin). In return, bacteria have evolved a spectacular array of over 500 siderophores with very strong binding characteristics that can wrestle iron from the host.

Iron is characterized as a so-called type 1 micronutrient. This means that physical growth of a child continues even if iron is in short supply and hence tissues become depleted. Depletion is gradual and occurs in different tissues at different rates. Hemoglobin levels, which contribute about one-half of body iron stores, tend to be depleted later in the course of deficiency. This means that anemia – the usual clinical indicator of iron deficiency in primary care settings – is in fact a very crude index. Subclinical iron deficiency can exist without anemia. Such deficiency can potentially impair numerous developmental processes including, and perhaps especially, neural development and hence cognition.

The first paper in this series is contributed by Dr. Carla Cerami, a clinically qualified malariologist by training who now specializes in iron and infection at MRC Unit The Gambia. Her paper “Iron Nutriture of the Fetus, Neonate, Infant, and Child” [this issue, pp. 8–14] describes the means by which expert bodies have computed the iron utilization, and hence the dietary iron requirements, at these early life stages. Coverage of the fetus necessarily includes recommendations for the pregnant mother.

The fetus deposits iron slowly until the last trimester of pregnancy when there is a marked acceleration in iron accretion in order to provide the newborn with adequate iron stores. In term neonates, these endowed iron stores are well protected even if the mother is quite iron deficient. However, if a baby is born prematurely it will not have received its full quota of iron and hence iron deficiency is a widely recognised clinical challenge in preterm babies. Dr. Magnus Domellöf, a clinical neonatologist who heads the Department of Clinical Sciences at Umeå University in Sweden, tackles this challenge in the second paper entitled “Meeting the Iron Needs of Low and Very Low Birth Weight Infants” [this issue, pp.16–23]. He cov-
ers both the theoretical basis behind the distinct iron needs of this group of patients as well as summarizing the best available recommendations for clinical practice.

The iron nutriture of the young infant is rather intriguing. Though term babies, as described above, are usually born with a good iron endowment, human breast milk is extremely low in iron and by 6 months of age most fully breastfed babies have depleted all of their iron “birthright” and entered a state of marginal or even moderate iron deficiency. Without a good source of dietary iron, or supplemental iron, deficiency will become worse until at least 12 months of age. Teleologically, we believe that – over evolutionary time – this iron deficiency has protected babies against infectious diseases. However, we no longer live in such hostile times and this begs the question as to whether this primordial adaptation is now harming the brain development and consequent cognition of young children. Dr. Sant-Rayn Pasricha, a clinical hematologist and epidemiologist who leads a research team at the Wal-ter and Eliza Hall Institute for Medical Research in Melbourne, and colleagues have summarized the latest evidence on this question in the third paper “Iron and Cognitive Development: What is the Evidence?” [this issue, pp. 25–38].

In the final paper, Professor Andrew M. Prentice, a nutritionist based at MRC Unit The Gambia, describes how the recent discovery of hepcidin has shed new light on several of the previously intractable puzzles regarding iron metabolism and nutrition. In his paper entitled “Clinical Implications of New Insights into Iron Absorp-tion and Metabolism” [this issue, pp. 40–48], he summarizes current knowledge on hepcidin – the master regulator of iron metabolism. In particular that the dual (and competing) regulation of hepcidin expression by iron status and inflammation help to explain why an estimated 1.25 billion people worldwide suffer from iron deficiency anemia; because, in unhygienic conditions with a high threat of infections they are actively blocking iron absorption. This insight points to a need for a radical rethink about how to combat the global threat of anemia, especially in young children.

Andrew M. Prentice
While iron supplementation can correct anemia at any stage, there is little evidence to support the idea that iron supplementation can correct neurodevelopmental deficits caused by iron deprivation in utero or in early childhood.

Iron Nutriture of the Fetus, Neonate, Infant, and Child
by Carla Cerami

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Key insights
Iron is an essential nutrient throughout the human life cycle, but particularly for the developing fetus, the neonate, and in early childhood. Before birth, iron needs are met through maternal iron transfer, and after birth, through dietary sources. Breast milk is low in iron, and care should be taken to offer additional sources of iron to breastfed infants. Worldwide, iron deficiency remains one of the most common micronutrient deficiencies in children, affecting an estimated 43% of the global population. Although a child with iron deficiency will continue to grow, depletion of tissue iron stores will cause specific clinical symptoms.

Current knowledge
Iron deficiency during fetal development and the first 2 years of life is associated with poor growth and decreased cognitive, motor, and emotional development. Deficiency of iron during this critical window of development can permanently alter the brain and nervous system. The strongest evidence for the effect on neurological outcomes comes from studies in school-aged children and teenagers with iron deficiency and iron deficiency anemia: modest improvements in cognitive outcomes were obtained after iron supplementation. However, the effects of iron supplementation in infants and young children are less clear.

Practical implications
The fetus obtains its iron via the placenta, and 80% of iron is transferred during the final trimester of pregnancy. However, the clinical benefits of maternal iron supplementation on birth outcomes require further study. An alternate strategy to improve the infant's iron stores is delayed cord clamping. Full-term, breastfed infants should begin an iron supplement at 4 months, and formula-fed infants should be given iron-enriched formulas. At weaning and beyond, children should be offered a varied diet that includes foods rich in heme and non-heme iron.

Recommended reading
Iron Nutriture of the Fetus, Neonate, Infant, and Child

Carla Cerami
MRC Unit The Gambia, MRC International Nutrition Group, Fajara, The Gambia

Key Messages
- Iron requirements are high in neonates and infants, particularly in premature babies.
- Breast milk is low in iron, and breastfed infants should be offered additional sources of iron.
- Iron deficiency is the most common micronutrient deficiency in children and causes deficits in exercise capacity and neurodevelopment.

Keywords
Iron · Nutrition · Iron deficiency · Infants · Neonates

Abstract
Iron is a key nutrient and is essential for the developing fetus, neonate, infant, and child. Iron requirements are high during early stages of life because it is critically important for the production of new red blood cells and muscle cells as well as brain development. Neonates, infants, and children obtain iron from dietary sources including breast milk (lactoferrin) and heme- and non-heme-containing foods. Iron deficiency (ID) is the most common micronutrient deficiency in children and causes deficits in exercise capacity and neurodevelopment.

The fetus is completely dependent on maternal iron crossing through the placenta and, although it is generally well protected against deficiency at birth, ID in mothers can increase the risk of ID and IDA in their children as early as 4 months. This review will discuss the uses of iron, iron requirements, and the sources of iron from conception through childhood. In addition, it will describe the prevalence and clinical manifestations of ID and IDA in children and discuss recommendations for iron supplementation of children and pregnant women.

Introduction
Iron is an essential nutrient during all stages of human development. It has particular importance for children because of its critical impact on their development. In the human body, iron is found mainly in (1) hemoglobin in red blood cells (RBCs) and erythroblasts; (2) myoglobin in muscle cells and in other iron-containing proteins such as cytochromes and, catalases (15%); (3) transferrin-bound iron in circulation; (4) storage proteins such as ferritin and hemosiderin. It is essential for DNA replication and many other metabolic processes. By far the biggest use of iron is in the production of new RBCs. However, in infants and children, muscle growth and production of new myoglobin are also important consumers of iron.
Iron Nutriture in Early Life

Iron status is regulated by intestinal absorption and transport, and there is no controlled mechanism for iron excretion. In adults, dietary iron sources provide only 5% of the daily needs and the remainder is obtained by recycling iron released during the breakdown of old RBCs. In contrast, infants and children must obtain 30% of their daily iron from their diet to provide the necessary iron for new muscle cells and RBCs [1].

Iron deficiency (ID) is the most common micronutrient deficiency in children [2, 3]. Anemia, primarily caused by ID, disproportionately impacts young children and pregnant mothers.

In the US, 7–9% of children ages 1–3 years have ID. However, the worldwide burden is much higher with an estimated 43% globally in 2011 and approximately 70% in Central and West Africa [4]. As will be discussed in more detail below, ID during fetal development and the first 2 years of life is associated with poor growth and decreases in cognitive, motor, and social emotional development [5–7]. Both the United States Department of Health and Human Services and the World Health Organization (WHO) have set goals to reduce ID and iron deficiency anemia (IDA). The WHO priorities have now been adopted as Priority Nutrition Indicators for the United Nation’s post-2015 Sustainable Development Goals [8, 9].

**Definitions of ID in Early Life**

Iron is categorized as a Type 1 nutrient. During ID, as in all Type 1 nutrient deficiencies, a child will continue to grow, but tissue depletion of the nutrient occurs and causes specific clinical symptoms [10]. Routine screening for ID and IDA between 6–24 months is recommended, especially for children living in areas with a high prevalence of ID. The minimum laboratory screen for IDA is hemoglobin and the guidelines outlined in Table 1 can be used to define anemia. In upper-income countries, a full blood count is obtained, which will give hemoglobin, hematocrit, mean corpuscular volume, and RBC distribution width. In children with IDA, mean corpuscular volume will be decreased and RBC distribution width will most likely be increased. In some settings, ferritin is also measured as part of the screening process. However, ferritin values can be misleading in children living in areas where the infectious disease burden is high because ferritin is also an acute-phase protein.

<table>
<thead>
<tr>
<th>Population</th>
<th>Hemoglobin, g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>11</td>
</tr>
<tr>
<td>Infants</td>
<td>11</td>
</tr>
<tr>
<td>Children aged 6 months to &lt;5 years</td>
<td>11</td>
</tr>
<tr>
<td>Children aged 5 to &lt;12 years</td>
<td>11.5</td>
</tr>
<tr>
<td>Children aged 12 to &lt;15 years</td>
<td>12</td>
</tr>
</tbody>
</table>

**Effects of ID on Neurodevelopment**

The strongest evidence for an effect on neurological outcomes comes from studies done on cognition in school-aged children and teenagers with ID and IDA [11]. A recent meta-analysis in older children and adults showed evidence of a modest improvement in the cognitive domains of concentration, intelligence, memory, psychomotor skills, and scholastic achievement after iron supplementation [12]. The effects of ID and iron supplementation in young children are less clear. Further details are summarized by Pasricha and colleagues [13] in an accompanying paper in this issue.

Children and infants with ID also have decreased psychomotor and mental development, but the timing, degree, and duration of the deficiency may all have profound effects on how and when these symptoms manifest. The brain is not a homogenous organ and different regions of the brain develop more rapidly at different times in development [14]. For example, in the last trimester of pregnancy there is rapid myelination and development of the striatum and the hippocampus. In children between the ages of 6 months and 3 years there is also rapid myelination and the frontal cortex and basal ganglia (motor control) are both developing most rapidly [15]. Infants with ID can have multiple symptoms that are consistent with impaired hippocampal function, reduced myelination, and altered temperament and altered dopamine metabolism. For example, iron-deficient infants can present with decreased attention and memory [16, 17]; deficits in visual and auditory systems [18, 19]; and altered temperament and social and emotional behaviors [20–22]. Taken together, the evidence implies that ID in early life can permanently alter the brain and nervous system and may explain at least in part why little efficacy has been shown on neurodevelopmental outcomes in iron supplementation trials in young children [23–26].
**Effects of ID on Immunity and Susceptibility to Infection**

Iron has opposing effects on immunity and susceptibility to infection. It can decrease efficacy of and cytokine production by lymphocytes [27, 28], and recent work has shown that it has detrimental effects on the phagocytic activity or macrophages and oxidative burst in neutrophils [29]. On the other hand, ID can be protective against certain infectious diseases including malaria in both children [30–32] and pregnant women [33, 34]. Further information on this is described by Prentice [35] in this issue.

**Effects of ID on Exercise Capacity**

Iron is an essential element for the transportation of oxygen and is a cofactor for enzymes involved in aerobic metabolism. ID and IDA both impair exercise capacity and increase fatigue [36, 37].

**Iron during Fetal Development**

Iron is critical for rapidly developing and proliferating cells. During fetal development, iron plays a profound role in organ development, particularly the brain. Evidence suggests that iron is of particular importance to the hippocampus which is rapidly developing during the late stages of gestation [38]. Of course, the region of the brain affected by in utero ID (and therefore subsequent clinical effects observed in the infant) depends on the magnitude of the deficiency and when in pregnancy the deficiency begins. In addition, it is essential for the fetus to acquire adequate iron stores from its mother to sustain growth during the first 6 months of life when the iron intake from breast milk is very low (see below).

The fetus obtains iron from the mother through the placenta and 80% is transferred during the third trimester of pregnancy [39]. Transferrin-bound iron is transferred directly from the maternal blood to the syncytiotrophoblast in the placental villi via transferrin receptor 1 (TFR1). After binding to iron (Fe^{3+}) on the apical side of the syncytiotrophoblast, holotransferrin with its bound iron is internalized and the iron is released into the cytoplasm. The trophoblasts may also take up non-transferrin-bound iron via ZIP8 or ZIP14 [40, 41] and heme iron via LRP1 [42]. No matter which pathway it uses to enter the cell, all non-heme iron is released from the basal side of syncytiotrophoblast via ferroportin (FPN). It remains unclear how iron is transported across the fetal endothelium and then to fetal transferrin [43], and pathways for heme iron are still not fully described.

The regulation of iron transport from the mother to the fetus across the placenta is thought to be controlled by the fetus for the following reasons: (1) infants of anemic women are usually born with normal iron status [44] and (2) fetal signals of iron status and gestational age can influence expression of TFR1 on the placenta [43]. One molecule which may influence placental iron transport is fetal hepcidin [45], but the role of maternal hepcidin remains unclear [46]. Recent evidence indicates that the placenta upregulates iron (and zinc) transporters in response to maternal deficiency [47].

The prevalence of ID is high during pregnancy, and 43% of pregnant women worldwide are anemic. ID accounts for 50–75% of anemia cases and is thought to be largely due to inadequate diet and increased nutritional requirements during pregnancy [48]. However, inflammation also plays a role by downregulating absorption (see Prentice [35] in this issue). It is general policy for pregnant mothers worldwide to be routinely supplemented with iron [49]. The US Center for Disease Control recommends that all pregnant women take a 30 mg/day iron supplement [50] and the WHO recommends supplementation with 30–60 mg/day [51]. However, the UK takes a very different view. Based on evidence that iron absorption is physiologically upregulated in pregnancy and that cessation of menstruation also reduces iron losses, there is no increase in the recommended nutrient intake for iron and supplements are only recommended if there is evidence of anemia [52]. The clinical benefits of maternal iron supplementation on birth outcomes (including preterm delivery, low birth weight, and neonatal morbidity and mortality) are still unclear despite decades of research [49]. A systematic review in 2015 found that routine iron supplementation reduced maternal IDA at birth (relative risk 0.29, 95% CI 0.17–0.49) but did not have consistent benefits on pregnancy outcome [53]. Part of the problem may be that iron is rarely compared against a true placebo control. Notably, a recent trial from Kenya using true controls reported a robust effect on birth weight with a large benefit in women who were iron deficient [54]. In addition, there is no definitive evidence showing that iron supplementation of nonanemic women improves maternal or infant outcomes. An alternate
strategy recommended by the WHO to improve the infant’s body stores of iron is delayed cord clamping after birth [55, 56]. The timing of delayed clamping varies between studies but is generally done between 1 and 5 min after delivery, or at the end of umbilical cord pulsations [57]. This can impart an estimated 80 mL of blood transfer after 1 min and 100 mL by 3 min which will impart an extra 40–500 mg/kg of iron and has been shown to have a significant benefit on ferritin levels in the infant at 6 months [58].

**Iron during the Neonatal Period and Early Childhood**

At birth most full-term infants have high to normal hemoglobin concentrations (15–17 g/dL) and then remain iron replete until 6 months of life. As noted above, babies of mothers with ID and IDA are at increased risk of ID, but this deficiency develops 4–6 months and is not apparent at birth. Premature babies have overall greater nutritional needs and higher iron requirements than healthy full-term babies (Table 2) [59]. Premature infants often also have lower iron stores than full-term infants and are at increased risk of developing ID and IDA [60].

There are 3 main dietary sources of iron: breast milk (where iron is bound to lactoferrin), heme iron, and nonheme iron. For neonates and very young infants, the only sources of iron are breast milk and/or formula. The concentration of iron in breast milk is very low and declines over time from 0.6 mg/L at 2 weeks to 0.3 mg/L at 5 months postpartum [62]. (Note that for a 4-kg baby who is likely to consume around 800 mL breast milk its intake would be about one-fifth of the value recommended in Table 2; but see comments below about the high bioavailability.) Current evidence suggests that if a mother has severe anemia, breast milk concentration decreases further, but not if a mother has mild-moderate anemia [63].

The iron in breast milk is highly bioavailable (50% compared to 3–4% in infant formula), although the precise mechanisms of absorption remain unclear [64]. The iron in breast milk is found in iron-binding proteins, predominantly lactoferrin. Lactoferrin is one of the most abundant proteins in milk [64]. It is a single polypeptide chain (MW 75–90K) that can bind 2 molecules of ferric iron [65] and closely resembles transferrin (the iron-carrying protein in serum) [66]. Like transferrin, lactoferrin functions as both an iron carrier molecule and an iron chelator. For example, transferrin in normal human circulation is only 30–40% saturated with iron, which makes over half of its binding sites available to bind excess iron and accounts for its bacteriostatic activity [67]. The extent to which lactoferrin is saturated with iron in breast milk is uncertain (one estimate suggests 10%); however, it is known to possess bacteriostatic properties which are at least in part attributable to its unsaturated iron-binding capabilities [68]. Lactoferrin is most likely absorbed in the small intestine of infants and neonates [69].

As will be discussed in more detail below, while iron supplementation can correct anemia at any stage, there is little evidence to support the idea that iron supplementation can correct neurodevelopmental deficits caused by iron deprivation in utero or in early childhood. Hence, current evidence suggests that it is important to provide a source of iron for children during the first 2 years of life [7, 70, 71]. Both the WHO [72] and the American Academy of Pediatrics [73] recommend exclusive breastfeeding for 6 months. It is recommended that full-term breastfed infants should start an iron supplement at 4 months (elemental iron 1 mg/kg daily, maximum 15 mg) and the supplement should be continued until the infant is taking sufficient quantities of iron-rich complementary foods [1]. If formula is used, full-term infants should be given iron-fortified formulas [74]. Human milk is also the recommended food for preterm babies, but human milk alone does not supply adequate amounts of iron, protein, calcium, phosphorous, and other micronutrients [75]. Hence, human milk fortifiers, iron supplements, and/or specially formulated preterm formula are often recommended [74]. Breastfed premature infants should start an iron supplement at 2 weeks of age and continue until 1 year of age [59, 71].

Full-term and preterm babies should be taking complementary foods by 6 months of age [76, 77]. Iron-rich complementary foods include meat (lamb, chicken, beef, and pork), baby cereals (including fortified rice), and some vegetables (green beans, peas, and spinach) [76, 78].

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommended dietary amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-term</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Premature</td>
<td>2–4 mg/kg</td>
</tr>
<tr>
<td>1- to 3-year-olds</td>
<td>7 mg</td>
</tr>
<tr>
<td>4- to 8-year-olds</td>
<td>10 mg</td>
</tr>
<tr>
<td>9- to 13-year-olds</td>
<td>8 mg</td>
</tr>
<tr>
<td>14- to 18-year-old boys</td>
<td>11 mg</td>
</tr>
<tr>
<td>14- to 18-year-old girls</td>
<td>15 mg</td>
</tr>
</tbody>
</table>

Table 2. Daily iron requirements during childhood [61]
Iron during Childhood

Iron is obtained entirely from dietary sources; hence, it is important that children are offered a diverse diet with a variety of iron-rich foods in order to provide an adequate intake of iron (Table 2). Children who do not eat at least 3 servings of iron-rich foods/day may benefit from an iron supplement [1]. Heme iron is the most bioavailable form of iron and is readily absorbed from meat, poultry, and fish. Non-heme iron is available from vegetables (especially spinach, lentils, and pumpkin seeds) and fortified cereals. Fortified cereals are the major source of iron for most children in the United States [79]. Other important sources of non-heme iron include beans (kidney, lima, and navy beans) and nuts. Absorption of non-heme iron is increased by foods rich in vitamin C (oranges, grapefruit, broccoli, tomatoes) and decreased by phytate (in bran, oats, and rye fiber), polyphenols (in tea, coffee, and cocoa), dietary calcium, and soy proteins. Calcium inhibits the absorption of iron by as much as 60% and thus there is a risk of ID in children who drink more than 700 mL of cow’s milk per day [50].

Conclusions

Iron requirements are high during all stages of human development. ID and IDA result in deficits in growth, neurological development, exercise capacity, and immune function. The recommended dietary allowances (RDA) are 1 mg/kg for full-term infants, 2–4 mg/kg for premature infants, 7 mg for 1- to 3-year-olds, 10 mg for 4- to 8-year-olds, and 9–13 mg for 9- to 13-year-olds. Iron sources include lactoferrin in breast milk as well as heme and non-heme iron from other dietary sources. Iron supplementation should be implemented if a child has a low hemoglobin level, does not have access to 3–4 servings per day of iron-rich foods, or lives in an area with a high prevalence of ID.

It is important that children are offered a diverse diet with a variety of iron-rich foods in order to provide an adequate intake of iron

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References

Iron Nutriture in Early Life

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In order to have a preventive effect on iron deficiency, iron supplements should be started before iron stores are depleted

Meeting the Iron Needs of Low and Very Low Birth Weight Infants
by Magnus Domellöf

**Key insights**

Around 16% of all newborns worldwide are born with low birth weight (LBW), defined as a birth weight of less than 2,500 g regardless of gestational age. These infants are at high risk of iron deficiency and poor health outcomes. Early nutrition is one of the most important modifiable factors in clinical practice, and ensuring adequate iron status is key for minimizing later morbidity in LBW infants.

**Current knowledge**

A healthy, term newborn has iron stores corresponding to around 25% of total body iron, alongside a high blood hemoglobin concentration (average 170 g/L). They have no need for additional dietary iron during the first months of life. LBW infants, however, have higher iron requirements. First, they have lower iron stores due to their lower birth weight. Second, LBW infants undergo more rapid postnatal growth that depletes internal iron stores earlier compared to term infants. Very low birth weight (VLBW) infants may also experience blood loss and blood transfusions during their time in intensive care, which further impacts their iron status.

**Practical implications**

The recommended amount of iron supplementation depends on the infant’s birth weight. Those who are only marginally LBW (i.e., birth weights of 2,000–2,500 g) should receive 1–2 mg/kg/day iron supplements from 2–6 weeks of age until 6 months. LBW infants (birth weight 1,500–2,000 g) should receive 2 mg/kg/day over the same period, beginning with a human milk fortifier or iron-containing formula. VLBW infants require 2–3 mg/kg/day starting at 2 weeks of age. Due to the individual variability in iron status, these infants need to be closely monitored to ensure that serum ferritin levels are within the acceptable range. Delayed umbilical cord clamping is also recommended for all LBW infants.

**Recommended reading**

Meeting the Iron Needs of Low and Very Low Birth Weight Infants

Magnus Domellöf
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Key Messages
- Infants with low birth weight (<2,500 g) are at high risk of iron deficiency and poor health outcomes.
- Delayed umbilical cord clamping and improved iron intake in low birth weight infants improve long-term health outcomes.

Keywords
Iron · Low birth weight · Preterm

Abstract
Low birth weight (LBW), defined as a birth weight of <2,500 g, affects 16% of all newborns and is a risk factor for impaired neurodevelopment as well as adverse cardiovascular and metabolic outcomes, including hypertension. LBW infants include both term, small for gestational age infants and preterm infants. Most LBW infants have only marginally LBW (2,000–2,500 g). Recent advances in neonatal care have significantly improved the survival of very LBW (VLBW) infants (<1,500 g). LBW infants are at high risk of iron deficiency due to low iron stores at birth and higher iron requirements due to rapid growth. Using a factorial approach, iron requirements of LBW infants have been estimated to be 1–2 mg/kg/day, which is much higher than the requirements of term, normal birth weight infants, who need almost no dietary iron during the first 6 months of life. In VLBW infants, blood losses and blood transfusions related to neonatal intensive care, as well as erythropoietin treatment, will greatly influence iron status and iron requirements. The timing of umbilical cord clamping at birth is of great importance for the amount of blood transfused from the placenta to the newborn and thereby total body iron. Delayed cord clamping of LBW infants is associated with less need for blood transfusion, less intraventricular hemorrhage, and less necrotizing enterocolitis. Randomized controlled trials have shown that an iron intake of 1–3 mg/kg/day (1–2 mg for marginally LBW and 2–3 mg for VLBW) is needed to effectively prevent iron deficiency. There is some recent evidence that these levels of iron intake will prevent some of the negative health consequences associated with LBW, especially behavioral problems and other neurodevelopmental outcomes and possibly even hypertension. However, it is also important to avoid excessive iron intakes which have been associated with adverse effects in LBW infants.

Background
Low birth weight (LBW), defined as a birth weight of <2,500 g at birth, regardless of gestational age, is a major risk factor for poor short- and long-term health outcomes. According to the World Health Organization, the global prevalence of LBW is 15.5%, which means that 20 million LBW...
infants are born each year [1]. The prevalence of LBW is higher in low-income countries and ranges from 4–9% in Europe to 28% in South Asia [2].

LBW is the leading cause of neonatal mortality worldwide and survivors are at high risk of impaired health later in life, including poor neurodevelopment and adverse cardiovascular/metabolic outcomes. Observed adverse health outcomes in children born with LBW are outlined in Table 1.

LBW infants include both term, small for gestational age infants and preterm infants. Most LBW infants have only marginally LBW (2,000–2,500 g). Recent advances in neonatal care have significantly improved the survival of very LBW (VLBW) infants (<1,500 g).

Recent data suggests that early nutrition may be one of the most important factors that can be modified in clinical practice in order to minimize later morbidity in LBW infants and iron is a critical nutrient in this context.

**Iron**

Iron is an essential nutrient which is required for heme synthesis, oxygen transport, and many enzyme functions, especially cellular energy metabolism. LBW infants are at high risk of iron deficiency (ID) due to low iron stores at birth, higher iron requirements due to rapid growth, and in the case of VLBW infants, iron losses due to frequent blood samplings during neonatal intensive care. ID should be avoided since it may have adverse effects on brain development. However, in contrast to most other nutrients, there is no mechanism for iron excretion from the human body and iron is a highly reactive pro-oxidant as well as an important substrate for pathogens, so excessive iron supplementation of infants may have adverse effects [3].

The combination of hemoglobin and ferritin is often used as a measure of iron status in children. Age-specific cutoffs for iron status indicators, including hemoglobin and ferritin, should be used for LBW infants since major physiological changes occur in iron status and red cell morphology during early development (Table 2).

The main public health problem associated with ID in childhood is the risk of poor neurodevelopment. Animal experiments have determined that iron is essential for several aspects of brain development including myelin formation, monoamine synthesis and function, neuronal and glial energy metabolism as well as neuronal growth and arborization [4]. A number of case-control studies in children have shown a consistent association between iron deficiency anemia (IDA) in infancy and long-lasting poor cognitive and behavioral performance up to adolescence [5]. A meta-analysis of 17 randomized controlled clinical trials in children of various ages showed that iron supplementation had a positive effect on mental development indices [6]. However, there is no convincing evidence that iron supplements improve motor or mental development in young children with IDA [7], suggesting that prevention is a more effective strategy. A meta-analysis based on a very small number of studies showed that preventive iron supplements in infancy (starting at 0–6 months of life) had a positive effect, at least on motor development [8].

On the other hand, unnecessary iron supplementation of iron-replete infants may have adverse effects, including increased risk of infections and impaired growth [9]. Iron is an essential nutrient not only for humans but also for almost all living organisms, including pathogenic bacteria and parasites. It has been shown that iron supplementation of young children in malarious regions can increase malaria-related morbidity and mortality if not accompanied by active anti-malaria measures [10, 11]. A possible mechanism for adverse effects of dietary iron may be changes in the gut microbiota. A recent trial in infants showed that iron-fortified porridge resulted in increased abundance of Firmicutes and *Escherichia/Shigella*, while bifidobacteria decreased [12].

Since iron cannot be extracted from the body, intestinal iron absorption is strictly regulated. Preterm VLBW infants with a low postnatal iron intake have a capacity for a relatively high fractional iron absorption: 25–40% from iron supplements given between feedings [13, 14].

<table>
<thead>
<tr>
<th>Category</th>
<th>Adverse outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurodevelopment</td>
<td>Lower IQ [47, 48]</td>
</tr>
<tr>
<td></td>
<td>Behavioral problems [49, 50]</td>
</tr>
<tr>
<td></td>
<td>Autism [51]</td>
</tr>
<tr>
<td></td>
<td>Poor motor function [52]</td>
</tr>
<tr>
<td></td>
<td>Poor visual-motor integration [52, 53]</td>
</tr>
<tr>
<td></td>
<td>Depression [54]</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Hypertension [55, 56]</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular disease [57]</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Obesity [55, 58]</td>
</tr>
<tr>
<td></td>
<td>Diabetes [58]</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Asthma [59]</td>
</tr>
<tr>
<td></td>
<td>Poor renal function [56]</td>
</tr>
<tr>
<td></td>
<td>Shorter telomere length [47]</td>
</tr>
</tbody>
</table>

Table 1. Adverse health outcomes associated with low birth weight
and 11–27% from iron-fortified formula [15, 16], suggesting that they are able to upregulate intestinal iron absorption when needed. It is less clear whether infants are capable of downregulating iron absorption enough when they are iron sufficient, and one study suggested that this might not be the case in infants up to 6 months of age [17]. Studies are lacking on iron bioavailability from multinutrient-fortified human milk but it may be higher than from preterm formula since, in term infants, iron absorption is significantly higher from human milk than from infant formula [18].

**Estimated Iron Requirements**

Total body iron at birth is about 75 mg/kg [19], most of which is found in hemoglobin, and a healthy, term newborn also has some iron stores, corresponding to about 25% of total body iron (Fig. 1). The newborn has a high hemoglobin concentration in blood (average 170 g/L), which is an adaptation to the hypoxic intrauterine environment. Oxygen saturation increases sharply from birth and, consequently, erythropoiesis is downregulated and the hemoglobin level falls to about 120 g/L during the first 6 postnatal weeks [20]. During this period, iron from senescent erythrocytes is transferred from hemoglobin to iron stores, which thereby increase in size. During the following months, as the baby grows and expands its blood volume, iron is gradually transferred back to the hemoglobin compartment until the iron stores are depleted. This means that the normal, term newborn has virtually no need of dietary iron during the first 6 months of life (Fig. 1), which is fortunate since breast milk has a very low concentration of iron (0.3 mg/L) [21, 22].

Two approaches can be used to estimate iron requirements of LBW infants. The first is the factorial approach, i.e., calculating the theoretical iron requirements based on expected growth rate and the iron content of the different body compartments. The second approach is to make recommendations based on interventional trials that have been performed in LBW infants (see Effects of Interventions section).

LBW infants have lower iron stores at birth due to their lower birth weight. As shown in Figure 2, a LBW infant with a birth weight of 2,000 g has higher iron requirements during the first months of life due to more rapid postnatal growth. The factorial approach, assuming an average body weight of 7.5 kg at 6 months, a blood volume of 80 mL/kg and tissue iron of 7 mg/kg indicate that iron stores of an infant with a birth weight of 2,000 g would be depleted within 6–12 weeks after birth and that the requirement of absorbed iron from 6 weeks to 6 months is 0.12 mg/kg/day. Assuming an average bioavailability of 10%, this corresponds to an enteral iron intake of 1.2 mg/kg/day.

Using a factorial approach, the iron requirements of a VLBW infant with a birth weight of 1 kg have been estimated to reach a maximum of 0.37 mg/kg/day at around term age [16]. This corresponds to an enteral intake of 1.4–2 mg/kg/day assuming 20–27% absorption. However, such estimations of iron requirements have not considered blood losses, blood transfusions, or erythropoietin treatment.

---

**Table 2.** Recommended cutoffs for the diagnosis of iron overload, iron deficiency, and anemia in low birth weight infants at different ages [46]

<table>
<thead>
<tr>
<th></th>
<th>Newborn</th>
<th>2 months</th>
<th>4 months</th>
<th>6–24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron overload: S-ferritin, μg/L</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;250</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Iron deficiency: S-ferritin, μg/L</td>
<td>&lt;35</td>
<td>&lt;40</td>
<td>&lt;20</td>
<td>&lt;10–12</td>
</tr>
<tr>
<td>Anemia: hemoglobin, g/L</td>
<td>&lt;135</td>
<td>&lt;90</td>
<td>&lt;105</td>
<td>&lt;105</td>
</tr>
</tbody>
</table>

**Fig. 1.** Body iron compartments and total body iron in a term infant with a birth weight of 3,500 g.
In VLBW infants, blood losses and blood transfusions related to neonatal intensive care, as well as erythropoietin treatment, will greatly influence iron status and iron requirements. Phlebotomy losses commonly amount to 6 mg/kg of iron per week [23] and in some cases much more. Each red blood cell transfusion typically adds 8 mg/kg of iron and hepatic iron stores as well as serum ferritin concentrations in preterm infants are highly correlated to the number of blood transfusions received [24].

Erythropoietin therapy has been advocated as a way to reduce the need for red blood cell transfusions in VLBW infants and is used in some centers. However, this treatment greatly increases iron requirements and high doses of oral or parenteral iron are therefore recommended as an adjunct to this therapy. Factorial calculations suggest that parenteral iron requirements of VLBW infants would be 0.2–0.37 mg/kg/day [16]. However, parenteral iron has been given in much higher doses, up to 3 mg/kg/day, in erythropoietin trials. Some studies showed that 6 mg/kg/day of enteral iron supplements is as effective as parenteral iron in this context [25]. There are insufficient data on safety of high doses of iron in combination with erythropoietin, but a Cochrane meta-analysis showed that early erythropoietin treatment, which includes supplemental iron, increases the risk of retinopathy of prematurity [26].

The timing of umbilical cord clamping at birth is of great importance for the amount of blood transfused from the placenta to the newborn and thereby total body iron. We and others have shown that delayed cord clamping increases iron stores and prevents ID at 3–6 months of age in normal birth weight infants [27, 28]. Delayed cord clamping may be even more important in LBW infants. A Cochrane review concluded that delayed cord clamping of preterm infants is associated with less need for blood transfusion, less intraventricular hemorrhage, and less necrotizing enterocolitis [29].

**Effects of Interventions**

The risk for LBW infants to develop IDA during the first 6 months of life, without additional dietary iron, is as high as 77% [30]. Intervention studies from the 1970s and 1980s showed that prophylactic iron, in most studies at a dose of 2 mg/kg/day, effectively prevents anemia up to 6 months of age in LBW infants with birth weights ranging from 1,500 to 2,500 g [31].

There are relatively few randomized intervention trials in LBW infants comparing the effects of different doses of iron supplements or fortification of human milk or infant formula.

In a study by Friel et al. [32], 58 infants with an average birth weight of 1,500 g were randomized to different infant formulas resulting in iron intakes of 3–6 versus 2–3 mg/kg/day up to 9 months of age. There was no difference in anemia or neurodevelopment at 12 months between the two groups. However, the group that received the higher amount of iron had higher glutathione peroxidase concentrations (a marker of oxidative stress), lower plasma zinc and copper levels, and a higher incidence of respiratory tract infections, suggesting possible adverse effects of the higher iron intake.

Similarly, Barclay et al. [33] found no effect on hemoglobin of an iron intake of 3.6–6.8 mg/kg/day as compared to 1.0–1.6 mg/kg/day from 2 to 30 weeks postnatal age in infants with an average birth weight of 2,000 g. However, there was a lower erythrocyte superoxide dismutase activity in the high iron group, suggesting altered copper metabolism – a possible adverse effect of iron [33].

Taylor and Kennedy [34] randomized 150 VLBW infants to receive 2 mg/kg/day of iron (from fortified breast milk or preterm formula) or an additional 2 mg/kg/day of iron supplements, yielding a total of 4 mg/kg/day, from 2 to 3 weeks of age until 36 weeks postmenstrual age or discharge. No significant effect was observed with regard to the main outcome (hematocrit at 36 weeks) or the number of blood transfusions. Nor was there any significant difference in neonatal morbidity, reticulocyte count, or weight up to 36 weeks. Unfortunately, this study did
not include measurements of ferritin or follow-up after discharge.

Griffin et al. [35] randomized 78 mostly VLBW infants (mean birth weight 1,360 g) to receive infant formulas with differing iron contents from hospital discharge until 6 months’ corrected age. The regimens provided iron intakes ranging from 0.8 to 1.2 mg/kg/day. No significant effects were observed between groups with regard to hemoglobin concentrations, ferritin, or the incidence of ID, and hemoglobin concentrations were similar to those of term infants of the same postmenstrual age.

Berglund et al. [36] randomized 285 marginally LBW Swedish infants (2,000–2,500 g) to iron supplements at the following doses: 0 (placebo), 1, or 2 mg/kg/day. The intervention lasted from 6 weeks to 6 months of age. In this population, iron supplements at a dose of 2 mg/kg/day, compared with placebo, significantly reduced the risk of IDA at 6 months [36]. In the placebo group, 36% developed ID and 10% developed IDA, as compared with 4 and 0%, respectively, in the 2-mg group. Iron supplementation at a rate of 1 or 2 mg/kg/day resulted in differences in iron status, but there was no difference between the two groups in the proportion of infants who developed ID or IDA. Iron supplements did not adversely affect infant growth, infections, or other morbidity in this study. Considering compliance to the intervention as well as iron intakes from other sources (about half of the infants were formula-fed), the investigators found that an actual iron intake of 0.25 mg/kg/day was sufficient to prevent IDA and that an intake of 1 mg/kg/day prevented ID [36].

**Timing of Iron Supplementation of LBW Infants**

In order to have a preventive effect on ID, iron supplements should be started before iron stores are depleted, which as a rule of thumb is when the infant has doubled its birth weight, which can be as early as 6 weeks after birth in a moderately LBW infant.

It is generally not recommended to give iron before 2 weeks of age to newborns since there are data suggesting that antioxidant systems are not fully active until that age [37]. In most studies of iron supplementation of moderately or marginally LBW infants, iron supplementation has been initiated at 4–6 weeks of age.

Two randomized controlled studies in VLBW infants have suggested that an early (2 weeks of age) as compared to late (6–8 weeks) start of iron supplementation results in less need for blood transfusions [38, 39].

Effects of Early Iron Intake on Neurodevelopment and Health in LBW Infants

There is a severe paucity of studies investigating the effects of early iron intake on neurodevelopment and other long-term health outcomes in LBW infants.

In the small study by Friel et al. [32] (see above), LBW infants were randomized to a normal or high dose of iron and no effect was observed on neurodevelopment at 12 months.

In 2007, Steinmacher et al. [40] published a follow-up study of the VLBW infants in the study by Franz et al. [38] (see above), who were randomized to early versus late iron supplementation. In this follow-up study, children who received early enteral iron supplementation had a significantly lower risk of having abnormal findings on neurological examination at 5 years of age [40]. However, there were no significant differences between groups in test scores for motor or cognitive development. Furthermore, the fact that the infants in the late iron group received more blood transfusions during the neonatal period makes the groups difficult to compare.

A Cochrane review in 2012 investigated the effects of enteral iron supplementation in preterm and LBW infants and found a total of 26 intervention studies. Of the 21 studies comparing iron supplementation with controls, none evaluated neurodevelopmental status as an outcome [41].

Since the Cochrane review, there has been only one new publication on this topic, providing the best current evidence [42]. This was a follow-up of the randomized controlled trial by Berglund et al. [36], described above, where 385 marginally LBW infants were randomized to receive 0, 1, or 2 mg/kg/day of iron supplements from 6 weeks to 6 months of age. At 3½ years of age, the infants from the original intervention as well as 95 normal birth weight reference children were assessed with a psychometric test (Wechsler Preschool and Primary Scale of Intelligence) and a validated behavioral instrument (the Child Behavior Checklist). There was no difference in cognitive scores between groups, but children in the placebo group had significantly more behavioral problems: 13% compared to 3% in the two iron supplemented groups ($p = 0.027$) and also compared to 3% in the reference group [42].

An interesting result from the follow-up of this intervention study was that LBW children who received early iron supplementation (1 or 2 mg/kg/day) had lower systolic blood pressure at 7 years (mean difference 2.2 mm Hg, 95% CI 0.3–3.2) as well as a lower risk of having systolic blood pressure within the hypertensive range (OR...
This suggests that the increased risk of hypertension that is observed in children and adults who are born with LBW might at least partly be due to ID and that iron supplementation may reduce this risk. There is a well-known association between ID and pulmonary hypertension in adults and iron has been suggested as a therapy for pulmonary hypertension [44]. A possible mechanism for this blood pressure-lowering effect of iron is an effect on nitric oxide synthesis, which has been observed in a rat model [45].

### Conclusions

LBW infants (16% of all newborns globally) are at high risk of ID. Ensuring an adequate iron intake in these infants should be a high priority since it will reduce the risk of ID and IDA and may prevent some of the negative health consequences associated with LBW, especially behavioral problems and other neurodevelopmental outcomes and possibly even hypertension. However, it is also important to avoid excessive iron intakes which have been associated with adverse effects in LBW infants such as an increased risk of infections, impaired zinc and copper status, and increased oxidative stress.

Marginally LBW infants (preterm or term infants with birth weights 2,000–2,500 g) should receive iron supplements of 1–2 mg/kg/day, starting at 2 to 6 weeks of age and continuing to 6 months of age [22]. This dose can safely be recommended for both breastfed and formula-fed infants. In malarious regions, iron supplementation should be combined with anti-malaria measures.

The recommended iron intake of LBW infants with a birth weight of 1,500–2,000 g is 2 mg/kg/day [46] from 2 to 4 weeks of life and this can be achieved initially by using iron-containing human milk fortifier or preterm formula and later (or initially) using iron supplements which should be continued to 6 months of age or possibly longer depending on infant diet.

For VLBW infants, the standard recommendation is a daily iron intake of 2–3 mg/kg/day starting at 2 weeks of age [46]. Infants who receive erythropoietin treatment need a higher dose (up to 6 mg/kg/day) during the treatment period. However, since individual iron status in VLBW infants is highly variable, depending on the number of received blood transfusions and blood losses from phlebotomy, it is recommended to follow these infants with repeated measurements of serum ferritin during the hospital stay. The normal range of ferritin in preterm infants is 35–300 μg/L (Table 2). If ferritin is <35 μg/L, the iron dose should be increased up to 3–4 (or maximum 6) mg/kg/day during a limited period. A prolonged dietary iron intake of >3 mg/kg/day should be avoided in most cases because of possible adverse effects. If ferritin is >300 μg/L, which usually is the result of multiple blood transfusions, iron supplementation and fortification should be discontinued until serum ferritin falls below this level. Iron supplements or intake of iron-fortified formula in the recommended doses should be continued also after discharge, at least until 6–12 months of age, depending on diet. Hemoglobin and serum ferritin should be checked at follow-up visits.

Like all infants, LBW infants should receive iron-rich complementary foods from 6 months of age [22]. In addition, delayed umbilical cord clamping, whenever feasible, is recommended for all LBW infants.

These recommendations are based on data from a relatively small number of intervention studies and very few of them investigated the effect on neurodevelopment or other long-term outcomes. More randomized controlled trials with a long-term follow-up are needed to firmly establish the specific iron intakes which will lead to the best health outcomes in this vulnerable group of infants.

### Disclosure Statement

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### References


A critical rationale for preventing anemia in preschool children, and especially in children under 2 years, is the improvement of short- and long-term cognitive development.


Iron and Cognitive Development: What is the Evidence?
by Leila M. Larson et al.

**Key insights**
The most frequently used iron interventions in clinical and public health practice are oral iron supplements and multiple micronutrient powders. Yet, despite decades of research, there is a lack of conclusive evidence to guide the optimal strategy for addressing iron deficiency in infants and children. The heterogeneous findings from different studies may be due to differences in dosage, duration, and timing of iron treatment, the baseline characteristics of the study population (particularly in terms of anemia and iron status), and compliance with the study interventions.

**Current knowledge**
The first 1,000 days of a child’s life are of critical importance for the developing brain. During this period, iron deficiency has far-reaching consequences, the most important of which is impaired cognitive development. There is thus a keen interest in enhancing the interventions that can prevent or treat iron deficiency anemia in pregnancy and within the first 2 years of life. However, there is a lack of conclusive data from high-quality randomized trials testing the effects of various iron interventions during pregnancy, infancy, and childhood on cognitive development.

**Practical implications**
Although there is clear evidence to support the benefits of iron supplementation on cognitive performance in school-aged children who are anemic, the evidence to support universal iron supplementation in children below 2 years of age remains unclear. Trials in preschool children showed mixed results for visual, cognitive, and psychomotor development, with some studies demonstrating small benefits, whereas others demonstrated no differences compared to placebo. To date, clinical evidence does not appear to advocate the benefits of iron supplementation on child cognitive development during pregnancy. Caution should be exercised to avoid exceeding the recommended doses of iron, as some studies have reported adverse effects of high iron fortification.

**Recommended reading**
Iron and Cognitive Development: What Is the Evidence?

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\textsuperscript{b}School of Public Health and Family Medicine, College of Medicine, University of Malawi, Blantyre, Malawi;  
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\textbf{Abstract}

The theoretical irreversible damage that iron deficiency and iron deficiency anemia can exert on child development makes a compelling argument for action to alleviate the burden. However, a critical analysis of evidence from iron interventions in early life is necessary to determine whether and how iron interventions improve cognitive outcomes. Key iron interventions used in clinical and public health practice include oral iron supplementation and, in young children, iron-containing multiple micronutrient powders. This article examines the evidence to answer 4 main questions. (1) Does antenatal iron supplementation influence long-term child cognitive development? (2) Does oral iron supplementation in preschool children improve short-term cognitive development? (3) Does oral iron supplementation in older children improve cognitive development? And (4), can provision of iron harm cognitive development? Early trials indicated benefit from parenteral iron in young children regardless of anemia status. There also appears to be evidence for benefit using oral iron treatment on cognitive performance in anemic primary school children. However, antenatal and early childhood oral iron intervention studies show inconsistent effects on early and long-term childhood cognitive outcomes.

\textbf{Key Messages}

- Improvement in cognitive development in children is a key rationale for universal iron interventions in pregnancy and in children.
- Data supporting effects of universal iron interventions given either antenatally or in young children on cognitive development remain ambiguous, mainly because high-quality randomized controlled trials have not yet been done.
- Treatment of anemia with oral iron in primary-school-aged children and treatment of iron deficiency anemia with parenteral iron in young children induce marked benefits on cognitive performance, indicating that effects on cognitive performance in young children are plausible.
Introduction

Anemia affects almost 300 million children worldwide [1], and iron deficiency (ID) has been considered the most important cause. Impairments in cognitive development caused by ID comprise perhaps the key rationale for alleviating the burden of anemia in children, and an important justification for preventing anemia in pregnancy. International and national guidelines frequently recognize the importance of anemia in these groups and increasing attention is being paid to identifying resources to address it [2]. An association between impaired cognitive development and ID has long been recognized, and has underpinned the rationale for policy [3]. However, recent evidence synthesis has drawn attention to the limitations in our understanding of this topic.

There is increasing recognition of the critical importance of the first 1,000 days of a child’s existence (from conception to 2 years of age) on brain development. Hypothesized interactions between nutritional status and child development (e.g., Prado and Dewey [83]) may be direct or indirect (Fig. 1). Whether and how these interactions exist for iron status remains uncertain. However, there has been enormous interest in optimizing iron stores during this sensitive period of rapid brain development [4], and interventions which prevent or treat iron deficiency anemia (IDA) in pregnancy or in the first 2 years of life have been considered crucial to improving long-term outcomes in children [5].

Types of Evidence

What Do We Mean by “Evidence”

Information that can be used to inform clinical or public health policy should be derived from high-quality, robust study designs. Evidence to justify an intervention can be classified according to well-established systems. For example, the GRADE (Grading of Recommendations Assessment, Development and Evaluation) working group have established criteria to score the quality of information used to develop clinical recommendations. These systems use an explicit system to score the evidence based on study design limitations, inconsistency of results between studies, indirectness of evidence, imprecision, and reporting bias [6–8]. In most cases, only information from randomized controlled trials or systematic reviews can be considered as sufficient to provide strong recommendations to implement an intervention (Fig. 2) [6].

Animal Studies

Animal studies are appropriate for the determination of mechanisms but inadequate for discerning a clinically relevant effect in humans. As such, animal studies are not considered as “evidence.” Experimental evidence from animal studies suggests that fetal or neonatal ID impairs brain and especially hippocampal development [9]. Early ID has also been associated with defects in myelination and impairments in dopaminergic neurotransmission [10]. Effects of ID on development of these systems have been hypothesized to produce irreversible damage to cognitive performance.

Observational Studies

Observational studies start with a “low”-quality rating in GRADE. Several dozen observational studies have assessed associations between iron status and anemia in...
pregnancy or in infancy and compared these to cognitive outcomes in later childhood or even adulthood [11, 12]. The most important limitation of observational studies is confounding. Anemia often corresponds with impaired socioeconomic status, overall nutrition, and food security [13]; stunting [14]; inflammation and infection (including malaria infection) [15], exposure to indoor smoke [14], birth order, and psychosocial stimulation [16]. ID may also be associated with other confounders such as increased lead absorption [17]. Anemic or iron-deficient mothers may be less able to provide appropriate stimulation to their infants [18]. Anemic mothers may be at higher risk of depression, which may impair mother-child interactions, impairing language and cognitive development. Whilst many studies make great efforts to adjust for these confounders in their analysis, it is implausible that all such confounders are completely removed. In addition, reverse causality in observational studies may also explain some associations: for instance, more developmentally advanced children may engage more in self-feeding, or be provided with a greater iron-rich diet because they appear more mature. Indirect explanations for relationships between ID and cognition include maternal depression, which can lead to poor mother-child interaction and disrupted infant attachment [19, 20].

Randomized Controlled Trials

Double-blinded randomized controlled trials are the only way to definitively identify an effect of an intervention on an outcome. These studies provide information about the effect of an intervention on an outcome, rather than the effect of an exposure on an outcome, although analysis of effects of interventions can be substratified by exposure. Given the subjective methods for assessing child development outcomes (e.g., based on direct assessment of a child using a series of standardized tools, assessing milestones, or asking parents about perceptions of their child’s development) [21], adequate blinding of both participants and their parents, as well as outcome assessors, is essential to generate high-quality evidence. Randomized controlled trials can be systematically identified and data combined using a formal approach (“systematic review and meta-analysis”).

From the perspective of making recommendations for interventions, data from clinical trials in humans are essential. Here, we seek to critically evaluate the evidence for effects of iron interventions in (i) pregnancy, (ii) preschool children, and (iii) older children in improving child development, with a view to understanding the potential benefits of these interventions on child development.

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Iron and Cognition

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DOI: 10.1159/000480742
Does Antenatal Iron Supplementation Influence Long-Term Child Cognitive Development?

Several randomized controlled trials have evaluated effects of iron supplementation in pregnant mothers on developmental outcomes in children. Christian et al. [15] followed up a cohort of children who had been born to mothers randomized to iron-folic acid (IFA) supplementation, IFA, and zinc supplementation, iron-containing multiple micronutrients (MMNs), or control (all groups also received vitamin A) during pregnancy, and in whom the prevalence of anemia and ID had been high. At 7–9 years of age, compared to children of mothers who received control, children of mothers randomized to IFA, but not of mothers who received IFA with zinc or MMNs, had higher universal nonverbal intelligence test (UNIT) results (mean in control group 48.2, mean in IFA group 51.7, adjusted mean difference 2.38, p = 0.04), superior executive function tests, and better motor function [15]. The authors had noted an attenuation by zinc of the benefits of IFA on birth weight in the original trial and hypothesized that the addition of zinc may impair iron absorption and hence the effectiveness of iron.

A smaller trial in Australia compared cognitive performance at 4 years of age in children of mothers randomized to iron or placebo during pregnancy, where IDA at delivery was uncommon (11% in the control arm) [22]. Cognitive performance was identical between groups. However, more children in the iron group had an abnormal behavior score (16%) than in the control group (8%) [23]. The authors subsequently followed the children to early school age (6–8 years of age) and again assessed behavior. Whilst there were no differences in average parent- or teacher-reported behavioral or temperament scores, there was a higher incidence of teacher-rated peer problems in the iron group (8%) than in the placebo group (2%) (p = 0.026) [24].

A randomized controlled trial in Western China randomized 5,828 pregnant women to either IFA, MMNs, or folic acid alone, and assessed 1,305 infants using the Bayley Scales at 3, 6, and 12 months of age. There were no differences between children at 3 and 6 months, but at 12 months, children of mothers who had received MMNs had a higher mental development index score by 1.22 (compared with IFA) and 1.00 (compared with folic acid alone) (p = 0.02). There were no effects on motor development [25].

In contrast, a trial of antenatal MMNs compared to either daily or twice-weekly IFA in Vietnamese women found that at the age of 6 months, children born to mothers in the twice-weekly IFA group had a higher Bayley cognitive score (mean difference 1.89) than children born to the daily IFA group (p = 0.03), despite maternal ferritin levels being lower in this group [26]. No significant differences were found compared to the MMN group.

Data from these trials were not included in Cochrane reviews or other key systematic reviews evaluating effects of iron supplementation in pregnancy [27, 28] or the United States Preventative Health Taskforce (USPSTF) review of iron supplementation in pregnancy [29], although data from the Chinese study described above [30] were included in a Cochrane systematic review evaluating effects of antenatal MMNs [31]. Collectively, the clinical evidence does not appear to show that iron supplementation in pregnancy causes improvements in long-term child mental development.

Does Supplementation in Preschool Children Improve Short-Term Cognitive Development?

A critical rationale for preventing anemia in preschool children, and especially in children under 2 years, is the improvement of short- and long-term cognitive development [3, 32]. Several randomized controlled trials have addressed the effect of iron interventions, when compared to control, placebo, or no intervention, on cognitive development in children. Trials in children aged 6 months to 5 years evaluating effects of oral iron supplements or iron-containing multiple micronutrient powders (MNP) on cognitive development are presented in Table 1. Several of the key trials will be discussed here.

Two early studies evaluated effects of parenteral (intramuscular) iron treatment of IDA [33] and nonanemic ID [34] on cognitive development (assessed with the Bayley Mental Development Index [MDI]). Within 8 days of treatment, compared with the placebo group, children treated with IDA showed marked improvements in MDI (mean gain 13.6 points), were found to become more alert and responsive, and had improved gross and fine motor coordination [33]. Among nonanemic ID children, iron improved MDI by 21.6 points, whereas in a control group of non-ID children, there was no benefit from iron [34]. These data provided early evidence that iron interventions improve cognitive performance in iron-deficient infants.

A population-based trial in 6-month-old infants was conducted in Indonesia, where 680 six-month-old infants were randomized to iron, zinc, iron and zinc, or placebo for 6 months. The trial found no benefit on mental development from iron or iron with zinc, compared to either
### Table 1. Randomized controlled trials comparing postnatal oral iron interventions (against control) on cognitive performance in preschool children

<table>
<thead>
<tr>
<th>First author [Ref.], year</th>
<th>Country</th>
<th>Participant age at recruitment</th>
<th>Baseline anemia and iron deficiency status</th>
<th>Intervention</th>
<th>Control</th>
<th>Participants randomized (total, iron, control), n</th>
<th>Duration</th>
<th>Outcomes</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aboud [61], 2011</td>
<td>Bangladesh</td>
<td>8–20 months</td>
<td>Unselected</td>
<td>MNPs with 12.5 mg of iron, 300 μg of vitamin A, 150 μg of folic acid, 50 mg of vitamin C, and 5 mg of zinc daily; also received counseling on responsive feeding and stimulation</td>
<td>Counseling on responsive feeding and stimulation</td>
<td>Total: 192 Iron: 100 Control: 92</td>
<td>7 months</td>
<td>Bayley MDI</td>
<td>Multiple micronutrients did not improve language skills</td>
</tr>
<tr>
<td>Akman [62], 2004</td>
<td>Turkey</td>
<td>6–30 months</td>
<td>Nonanemic; iron deficient</td>
<td>3 mg/kg of ferrous glycine twice daily</td>
<td>No intervention (i.e., no placebo)</td>
<td>Total: 40 Iron: 21 Control: 19</td>
<td>3 months</td>
<td>Bayley MDI and PDI; Denver Development Scales</td>
<td>Iron improved MDI in nonanemic iron-deficient children, restoring lower baseline MDI at baseline in anemic/iron-deficient children to the same levels as controls</td>
</tr>
<tr>
<td>Angulo-Barroso [36], 2016</td>
<td>China</td>
<td>6 weeks</td>
<td>Unselected</td>
<td>1 mg/kg of elemental iron as an iron protein succinylate oral solution</td>
<td>Carrier (placebo)</td>
<td>Total: 1,482 Iron: 746 Placebo: 736</td>
<td>7.5 months</td>
<td>PDMS-2</td>
<td>Children who received iron in infancy reported significantly better PDMS-2 scores than those who did not</td>
</tr>
<tr>
<td>Attanasio [63], 2014</td>
<td>Colombia</td>
<td>12–24 months</td>
<td>Not selected by iron or anemia status</td>
<td>MNPs (including iron 12.5 mg)</td>
<td>No placebo</td>
<td>Control: 318 MNPs: 308</td>
<td>18 months</td>
<td>Bayley scales of infant development-III: cognitive, receptive, and expressive language, and fine and gross motor scores</td>
<td>No benefit from MNPs on developmental outcomes</td>
</tr>
<tr>
<td>Aukett [64], 1986</td>
<td>UK</td>
<td>17–19 months</td>
<td>Anemic (hemoglobin 80–110 g/L); iron status unknown</td>
<td>Iron 24 mg (as ferrous sulfate) + vitamin C 10 mg daily</td>
<td>Vitamin C 10 mg</td>
<td>Total: 110 Iron: 54 Control: 56</td>
<td>2 months</td>
<td>Denver Development Scale (psychomotor)</td>
<td>Iron did not improve psychomotor scores in the overall group</td>
</tr>
<tr>
<td>Deinard [65], 1986</td>
<td>USA</td>
<td>18–60 months</td>
<td>Nonanemic, iron deficient, iron deficiency anemia (all in this group received iron)</td>
<td>Elemental iron 6 mg/kg daily</td>
<td>Placebo</td>
<td>Iron: 22 Control: 23 Total: 45</td>
<td>6 months</td>
<td>Cognitive development (Bayley and Stanford-Binet combined)</td>
<td>No effect from iron on cognitive development</td>
</tr>
<tr>
<td>First author [Ref.], year</td>
<td>Country</td>
<td>Participant age at recruitment</td>
<td>Baseline anemia and iron deficiency status</td>
<td>Intervention</td>
<td>Control</td>
<td>Participants randomized (total, iron, control), n</td>
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<tr>
<td>Idjradinata [66, 67], 1993</td>
<td>Indonesia</td>
<td>12–18 months</td>
<td>Subgroups: iron deficient anemic, nonanemic iron deficient, iron replete</td>
<td>Iron 4 mg/kg (as ferrous sulfate)</td>
<td>Placebo</td>
<td>Total: 129 Iron: 64 Placebo: 65</td>
<td>4 months</td>
<td>Bayley MDI and PDI</td>
<td>Iron improved MDI and PDI in children with baseline iron deficiency anemia but not with baseline nonanemic iron deficiency/normal iron and hemoglobin status</td>
</tr>
<tr>
<td>Lind [35, 68, 69], 2003</td>
<td>Indonesia</td>
<td>6 months</td>
<td>Anemia status unknown; iron status unknown</td>
<td>Iron 10 mg (as ferrous sulfate) + vitamin C, iron 10 mg + zinc 10 mg + vitamin C</td>
<td>Vitamin C alone, zinc + vitamin C alone</td>
<td>Total: 680 Iron + vitamin C alone: 170 Vitamin C alone: 170 Iron + vitamin C + zinc: 170 Zinc + vitamin C: 170</td>
<td>6 months</td>
<td>Bayley MDI and PDI</td>
<td>Iron alone did not improve MDI (nonsignificant difference: 2 points); iron + zinc had no benefit on MDI; iron alone improved PDI vs. placebo; but iron + zinc lowered PDI compared with zinc alone and was identical to placebo</td>
</tr>
<tr>
<td>Lozoff [70], 1982</td>
<td>Guatemala</td>
<td>6–24 months</td>
<td>Subgroups: anemic, nonanemic</td>
<td>Iron 5 mg/kg (as ferrous ascorbate)</td>
<td>Placebo</td>
<td>Total: 68 Iron: 34 Control: 34</td>
<td>1 week</td>
<td>Bayley MDI and PDI</td>
<td>Iron did not improve Bayley MDI or PDI</td>
</tr>
<tr>
<td>Lozoff [71], 1996</td>
<td>Costa Rica</td>
<td>12–23 months</td>
<td>Nonanemic (all anemic infants were treated)</td>
<td>Iron 6 mg/kg/day</td>
<td>Placebo</td>
<td>Total: 50 Iron: 25 Control: 25</td>
<td>6 months</td>
<td>Bayley MDI and PDI; data not extractable as not presented by iron/ control arms</td>
<td>Iron did not affect cognitive performance in nonanemic infants; anemic infants receiving iron (uncontrolled experiment) experienced ongoing diminished baseline cognitive performance, despite recovery in hematology and iron indices</td>
</tr>
<tr>
<td>Metallinos-Katsaras [72], 2004</td>
<td>Greece</td>
<td>3–4 years</td>
<td>Anemic, nonanemic, iron deficient, iron replete</td>
<td>Iron 15 mg (as ferrous sulfate) + multivitamins</td>
<td>Multivitamins</td>
<td>Iron + multivitamins: 31 Multivitamins alone: 17 Total: 48</td>
<td>2 months</td>
<td>Cognitive outcomes</td>
<td>Iron improved in discrimination and selective attention in anemic subjects but had no effect in nonanemic subjects</td>
</tr>
<tr>
<td>Singla [73], 2014</td>
<td>Bangladesh</td>
<td>7–12 months</td>
<td>Not selected by iron status all participants had low birth weight</td>
<td>MNPs including 22 elements (10 mg elemental iron)</td>
<td>Health education only (no placebo)</td>
<td>MNP: 117 No MNP: 114</td>
<td>5 months</td>
<td>Cognitive development; receptive language; expressive language</td>
<td>No effect on cognitive development and expressive language; benefit on receptive language</td>
</tr>
<tr>
<td>First author [Ref., year]</td>
<td>Country</td>
<td>Participant age at recruitment</td>
<td>Baseline anemia and iron deficiency status</td>
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<td>Control</td>
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<tr>
<td>Soewondo [74, 75], 1989</td>
<td>Indonesia</td>
<td>Mean age of iron-replete subjects: 57.5 months, Mean age of iron-deficient subjects: 54.2 months</td>
<td>Anemic, nonanemic, iron deficient, iron replete</td>
<td>50 mg iron daily</td>
<td>Placebo</td>
<td>Iron: 77, Placebo: 99, Total: 176</td>
<td>2 months</td>
<td>Hemoglobin, hematocrit, ferritin, transferrin saturation, cognitive outcomes</td>
<td>Baseline deficits in visual attention and concept acquisition in anemic subjects; receipt of iron (compared with placebo) was a covariate in improvement in these parameters after treatment</td>
</tr>
<tr>
<td>Mebrahtu [76], 2004; Stoltzfus [77, 78], 2001, 2004</td>
<td>Tanzania</td>
<td>12–48 months</td>
<td>Unselected</td>
<td>10 mg ferrous sulfate daily or 10 mg ferrous sulfate + mebendazole daily</td>
<td>Placebo or mebendazole</td>
<td>Iron: 170, Iron + mebendazole: 170, Placebo: 172, Mebendazole alone: 172, Total: 684</td>
<td>12 months</td>
<td>Language and motor milestones</td>
<td>Iron improved language development by 0.8 points</td>
</tr>
<tr>
<td>Surkan [79], 2013</td>
<td>Nepal</td>
<td>4–12 months</td>
<td>Anemia status unknown; iron status unknown</td>
<td>Iron 6.25 mg + folic acid 25 μg ± zinc 5 mg</td>
<td>Placebo ± zinc</td>
<td>Total: 362, Iron + folic acid: 81, Placebo: 100, Iron + folic acid + zinc: 107, Zinc: 74</td>
<td>Variable, 0–37 weeks</td>
<td>Fagan outcomes (novelty, look-away, fixation duration)</td>
<td>Iron did not affect Fagan intelligence scores or attainment of language or motor milestones</td>
</tr>
<tr>
<td>Walter [80], 1989</td>
<td>Chile</td>
<td>12 months</td>
<td>Subgroups: anemic, nonanemic iron deficient, nonanemic iron replete</td>
<td>Iron 15 mg thrice daily (as ferrous sulfate)</td>
<td>Placebo</td>
<td>Total: 196, Iron: 102, Placebo: 94</td>
<td>10 days</td>
<td>Bayley MDI and PDI</td>
<td>Iron did not improve MDI or PDI</td>
</tr>
<tr>
<td>Yalcin [81], 2000</td>
<td>Turkey</td>
<td>6 months</td>
<td>Nonanemic, iron replete</td>
<td>Iron 1 mg/kg (as ferrous sulfate)</td>
<td>No intervention (i.e., no placebo)</td>
<td>Total: 24, Iron: 11, Control: 13</td>
<td>3 months</td>
<td>Bayley MDI and PDI</td>
<td>Iron did not affect MDI or PDI</td>
</tr>
<tr>
<td>First author [Ref., year]</td>
<td>Country</td>
<td>Participant age at recruitment</td>
<td>Baseline anemia and iron deficiency status</td>
<td>Intervention</td>
<td>Control</td>
<td>Participants randomized (total, iron, control), n</td>
<td>Duration</td>
<td>Outcomes</td>
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<tr>
<td>Yousafzai [37, 38], 2014</td>
<td>Pakistan</td>
<td>6–24 months</td>
<td>Unselected</td>
<td>“Enhanced nutrition”: MNP (iron, folic acid, vitamin A, and vitamin C) and nutrition education ± responsive stimulation</td>
<td>No intervention ± responsive stimulation (i.e., no placebo)</td>
<td>Total: 1,493Iron: 742Control: 751</td>
<td>Up to 18 months</td>
<td>Bayley MDI and PDI</td>
<td>Children receiving responsive stimulation had higher development across all domains at 12 and 24 months of age. Children receiving enhanced nutrition had improved mental development index at 12 but not 24 months of age, improved language at 12 and 24 months, and improved socio-emotional scale at 12 and 24 months ($p = 0.06$); motor scale was not different. A follow-up of the cohort to age 4 years showed responsive stimulation had higher cognition, language, and motor skills at 4 years of age than children who did not receive enhanced nutrition and improved motor development but not the other domains.</td>
</tr>
</tbody>
</table>

MDI, Mental Development Index; PDI, Psychomotor Development Index; PDMS-2, Peabody Developmental Motor Scale, Second Edition; MNP, multiple micronutrient powders.

Note that the trial protocol mentions cognitive and language development as well.
placebo or zinc alone. There was a 3-point benefit from iron, but not iron with zinc, on motor development [35]. More recently, a large randomized trial in China comparing effects of prolonged iron supplementation to placebo in young infants (from the age of 6 weeks to 9 months) found that iron improved motor development. Effects on cognitive development, although included as an outcome in the trial registration, were not reported in this publication [36].

A 2 × 2 factorial trial in Pakistan evaluated the effectiveness of (i) responsive stimulation (local health worker delivered messages to mothers about responsive care for development, age-appropriate play activities, and problem solving), and (ii) enhanced nutrition, comprising training of health workers to deliver improved nutritional advice to mothers together with MNPs [37]. Importantly, no placebo was used in this trial. Interventions were given to infants enrolled from birth to 23 months of age (MNPs were delivered starting at 6 months of age only). Children receiving enhanced nutrition (education and MNPs) had improved cognitive, language, and socio-emotional development at 12 months and improved language (but not other parameters) at 24 months; by the time the children turned 4 years, prosocial behaviors and motor development (which had not been affected by enhanced nutrition at 24 months) were higher in the enhanced nutrition group, whereas effects on cognitive development were not seen [38]. Importantly, there were no effects from enhanced nutrition on hemoglobin concentration, and adherence to the intervention was limited [37]. Thus, this trial may have identified a transient early improvement in cognitive development which was not sustained throughout the intervention period, and the mechanism may not have been mediated directly via the effect of contents of the MNPs on anemia.

In slightly older children, Stoltzfus et al. [39] randomized 614 Tanzanian preschool children (6–59 months) to 12 months of iron supplementation (10 mg), antihelminthic therapy, a combination of the two, or placebo. The mean age at baseline was about 30 months. Motor and language milestones were assessed by parental report. In this group, nearly all children were anemic at baseline. Iron was found to improve motor scores in children who had been most anemic at baseline, but not overall. Iron improved language scores by 0.8 points on average (p = 0.011) [39]. The placebo-controlled design of the study meant the authors were able to conclude that iron improved child development.

Several trials have attempted to address the effects of iron interventions in children younger than 6 months, either by fortifying infant formula or giving iron supplements to very young children. For example, a small trial gave oral iron supplements or placebo to term breastfed infants from 1 to 6 months of age, and found improvements from iron in visual acuity and psychomotor development but not mental development at 13 months of age [40]. Likewise, no improvements in mental development were reported in a trial comparing iron-fortified with low-iron formula in Canadian children from very low-income households; benefits on motor development were seen at 9 and 12 months of age, but were attenuated by 15 months [41].

Systematic reviews have summarized effects of iron supplementation on child cognitive development through meta-analysis. Pasricha et al. [42] meta-analyzed the effects of daily iron supplementation in children 4–23 months of age and found no clear evidence of benefit from iron on cognitive development at the end of the intervention (mean difference 1.65 [95% CI –0.63, 3.94], p = 0.16; 6 trials). Even when only studies at low risk of bias were included, the effect size was nonsignificant. Likewise, no benefit from iron was observed among children who had been anemic at baseline [42]. Thompson et al. [43] assessed effects of daily iron supplementation in 2- to 5-year-old children, and identified 4 trials including Stoltzfus described above, along with 2 other trials that did not clearly report overall effect sizes from iron on cognitive development. A previous review of the effect of iron interventions in children (delivered through any mechanism) likewise was unable to identify a beneficial effect from iron on cognitive development in children under 27 months of age, although a benefit in older children was seen [44]. Wang et al. [45] undertook a Cochrane review to assess effects of iron supplementation on cognitive development in iron-deficient anemic children under 3 years and did not find evidence that iron supplementation improved cognitive development (1.04 [–1.30, 3.39], p = 0.79) in this group. Larson and Yousafzai [46] meta-analyzed the effects of various nutritional supplementation regimens given postnatally to infants and children on cognitive development (combining trials giving iron, zinc, or MMN combinations of these) in low- and middle-income countries and reported...
that these did not significantly improve child development. Finally, the USPSTF analysis of evidence for iron interventions in high-income countries identified 4 randomized controlled trials, but was unable to find evidence of a benefit from iron on cognitive development [47]. Thus, evidence synthesis approaches have failed to identify evidence of benefit from iron supplementation on cognitive development in children under 2 years of age.

Perhaps more important than short-term cognitive performance are the effects of iron on long-term cognitive development. Two studies have evaluated the effects of iron interventions in the first 2 years of life on long-term cognitive outcomes. Murray-Kolb et al. [48] followed up a cohort of 735 Nepalese children who had been given daily doses of IFA, zinc, IFA with zinc, or placebo at the age of 12–35 months for a variable duration depending on age of enrolment. The children were assessed at the age of 7–9 years using tests of cognitive, executive, and motor function. The authors found that compared with children who had received placebo, there were no differences in children who had received IFA (with or without zinc) either before or after adjustment for confounders. In the adjusted analysis, children who were given IFA supplementation from 12 to 18 months of age (i.e., for the longest duration) performed worse ($p = 0.02$) on tests of executive function and fine motor function compared with children who did not receive IFA [48]. A second trial, in Thailand, followed up 560 of 609 children originally randomized at the age of 6 months to iron (10 mg), zinc, iron and zinc, or placebo given daily for 6 months. Children were followed up at the age of 9 years, when the authors found no differences overall or in any domain of the Wechsler Intelligence Scale for Children-III between the groups, or in the Raven’s Colored Progressive Matrices score. Moreover, no differences in school performance in any subject (Thai, English, Mathematics, or Science) were observed [49]. Collectively, these studies do not support the hypothesis that even quite sustained courses of iron supplementation in young children benefit cognitive performance in the long term.

Failure to convincingly identify a benefit from iron on cognition may be because such a benefit does not exist—perhaps because iron at these doses and via this route does not impact on brain development, or, as hypothesized by some authors, because the effects from ID on early brain development are irreversible [50, 51]. However, the “irreversible damage” hypothesis is not supported by the improvements seen in early trials of parenteral iron in iron-deficient infants [33, 34]. Alternatively, studies have been undertaken in heterogeneous populations or populations where effects from iron may be unlikely to produce benefit. Several of the trials included in Table 1 were very short (<2 weeks) in duration. Several earlier, smaller trials conducted with small samples, using niche cognitive tests, and reported in ways that do not disclose the overall effect size from iron are perhaps better considered as clinical experiments rather than as formal randomized controlled trials designed to generate evidence.

### Interactions between Cognitive and Psychomotor Development

Although meta-analyses do not clearly demonstrate benefits from iron on psychomotor development (including gross and fine motor skills), several of the studies investigating effects of iron in preschool-aged children identified benefits on psychomotor (rather than cognitive) development [35, 36, 52]. It is possible that benefits on psychomotor development may indirectly result in improved cognitive development. Using structural equation modeling, observational studies in Indonesia and Tanzania considered that associations between nutrition and mental development were mediated by motor activity and motor development [53, 54]. Possible effects of benefits of motor development on mental development may be explained by the child having richer experiences, more stimulating situations, and improved interactions with others [55].

### Does Iron Supplementation in Older Children Improve Cognitive Development?

Several trials have evaluated effects of iron supplementation in primary schoolchildren, and Low et al. [56] summarized these studies in a recent systematic review. The authors identified 9 studies conducted in primary school children aged 5–12 years, measuring cognitive testing. Intelligence quotient (IQ) was measured in 5 studies, whilst 3 studies used other author-adapted scales of cognitive performance, and 1 used school performance. Overall, compared with control, iron improved global cognitive scores (standardized mean difference $0.50$ [0.11, 0.90], $p = 0.01$; 9 studies). This benefit was only seen among children who had been anemic at baseline (standardized mean difference $0.29$ [0.07, 0.51], $p = 0.01$; 6 studies), but not in children who had been nonanemic at enrolment. There was no overall benefit from iron supplements in IQ in primary school children (mean difference $5.47$ [−3.24, 4.18], $p = 0.2$; 5 studies), although among anemic children, iron supplements did improve IQ (mean difference $4.55$ [0.16, 8.94], $p = 0.04$; 3 studies). Thus, in contrast to trials undertaken in preschool children, treatment of ane-
mia with iron supplementation in older children demonstrates a benefit on cognitive performance.

These data are corroborated by other systematic reviews. Sachdev et al. [44] found a beneficial effect from iron interventions (oral or parenteral iron supplementation, fortified formula milk or cereals) on IQ in children aged 8 years or older (standardized mean difference 0.41 [0.20, 0.62], \( p < 0.001 \)) [45]. Likewise, Falkingham et al. [57] summarized 14 trials evaluating the effects of iron supplementation in older children, adolescents, and women on cognitive performance and found that iron improved IQ by 2.5 points (1.24, 3.76), although it had no effect in nonanemic individuals or on other outcomes including academic achievement, memory, or motor development [57].

**Can Provision of Iron Harm Cognitive Development?**

There have been emerging concerns that provision of high concentrations of iron in fortification infant formula may impair long-term cognitive development. Lozoff et al. [58] followed up a cohort of Chilean infants who had been randomized in infancy to receive infant formula fortified with either low (2.3 mg/L) or high (12.7 mg/L) levels of iron. After 6 months, children given the high-iron-fortified formula and/or iron supplements did not exhibit improved cognitive or psychomotor development, but had approximately 5 days earlier age of crawling and had a delayed Fagan looking time, which the authors thought would predict impaired longer-term cognitive performance [58]. However, at the age of 10 years, the authors reassessed the children and found that those who had been randomized to the high-iron formula performed more poorly than their low-formula-receiving counterparts on every outcome (IQ, \( p = 0.06 \); spatial memory, \( p = 0.02 \); arithmetic achievement, \( p = 0.07 \); visual-motor integration, \( p = 0.046 \); visual perception, \( p = 0.06 \)) [59]. A recent update to this cohort indicated a similar pattern of adverse performance in the high-iron-fortified group at the age of 16 years; of 9 cognitive tests performed, 4 showed poorer performance in the high-iron group; baseline hemoglobin did not interact with these effects [60].

**Conclusions**

Improvements in long-term child development are perhaps the most important justification for universal iron (iron supplement or MNPs) interventions in young children and considered an important reason for preventing anemia in pregnancy. Whilst there is clear evidence for benefits of iron treatment on cognitive performance in anemic primary school children, in younger children, and especially in children under 2 years of age, evidence that oral iron interventions (supplements or micronutrient powders) improve short- and long-term cognitive development remains scant. Benefits from parenteral iron on cognition in iron-deficient and anemic infants have been reported in early trials and refute the contention that iron-induced brain damage is intractable, instead suggesting either that oral iron is ineffective or that quality trials have not yet been performed. Likewise, effects of iron in pregnancy on early and longer-term cognitive development remain ambiguous, with failure to demonstrate consistency or dose response. Heterogeneity may be due to differences in dose and duration of iron, timing of commencement of iron, population and epidemiology of anemia and ID, and adherence to interventions.

Although numerous studies purporting to address these questions are available, the issue has still not been addressed in high-quality randomized controlled trials. Several recent trials evaluating effects of iron interventions (including MNPs) were not placebo controlled. Given the potentially subjective nature of cognitive assessments, blinding is essential, and non-placebo-controlled studies do not provide useful evidence for this outcome, regardless of the direction of the finding. We strongly discourage investigators from undertaking non-placebo-controlled trials in the future as they will not add to the literature. High-quality, dedicated, placebo-controlled, adequately powered trials of universal iron interventions on cognitive performance in young children are urgently needed. In the interim, improvements in cognitive development cannot be used as a rationale for implementing universal iron interventions.

**Disclosure Statement**

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Humans have evolved to expend at least as much physiological effort in excluding dietary iron as in acquiring it

Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism
by Andrew M. Prentice

Key insights
A deeper understanding of the pathways involved in iron homeostasis have overthrown the old paradigm that the human gut is poorly designed for iron absorption. Instead, the normal physiological status in humans is one where iron absorption is constantly suppressed in order to maintain stable iron levels in the absence of active mechanisms for iron excretion. To this end, the central role of hepcidin is to balance the physiological need for iron against the threat of infection.

Current knowledge
Hepcidin is a hormone that functions as the master regulator of iron homeostasis. It interacts mainly through the cellular iron exporter ferroportin. Ferroportin transports iron from cellular stores (i.e., enterocytes, macrophages, and hepatocytes) into the blood, thereby raising the levels of circulating iron. Hepcidin induces degradation of ferroportin, resulting in a net lowering of iron concentrations. Upregulation of hepcidin even at very low levels of inflammation underscores the importance of curtailing iron levels when there is danger of infection. Addressing iron deficiency in poor populations therefore needs to combine hygiene and infection control alongside iron supplementation in order to be safe and effective.

Practical implications
Hepcidin, ferroportin, and their regulators are potential targets for the treatment of iron disorders and anemias. Iron overload could be modulated by artificially enhancing hepcidin. To this end, mini-hepcidins that can perpetuate hepcidin action are being explored. Conversely, the use of hepcidin antagonists could be useful for the treatment of anemia related to chronic disease or cancer chemotherapy. However, when designing interventions that affect iron levels, great care should be taken not to exceed safe iron thresholds.

Recommended reading
Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism

Andrew M. Prentice

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Key Messages
- Iron is a paradoxical nutrient with potential for great benefit but also for causing harm if it is not appropriately chaperoned and regulated.
- In the past 20 years advances in molecular biology have elucidated many of the mechanisms by which humans tread the delicate balancing act to derive optimal benefit; key amongst these is the discovery of hepcidin which acts as the master regulator of iron.
- The new insights derived from these basic-science discoveries show that humans exert considerable physiological effort into excluding iron in the face of inflammation and infection, and hence that eliminating these will be at least as important in combatting global iron deficiency as efforts directed towards improving diets.

Keywords
Iron · Hepcidin · Ferroportin · Iron deficiency · Anemia

Abstract
The fact that humans must balance their need for iron against its potential for causing harm has been known for several centuries, but the molecular mechanisms by which we achieve this feat have only been revealed in the last 2 decades. Chief amongst these is the discovery of the master-regulatory liver-derived hormone hepcidin. By switching off ferroportin in enterocytes and macrophages, hepcidin exerts fine control over both iron absorption and its distribution among tissues. Hepcidin expression is downregulated by low iron status and active erythropoiesis and upregulated by iron overload and infection and/or inflammation. The latter mechanism explains the etiology of the anemia of chronic infection. Pharmaceutical companies are actively developing hepcidin agonists and antagonists to combat iron overload and anemia, respectively. In a global health context the discovery of hepcidin shines a new light on the world’s most prevalent micronutrient problem; iron deficiency and its consequent anemia. It is now apparent that humans are not poorly designed to absorb dietary iron, but rather are exerting a tonic downregulation of iron absorption to protect themselves against infection. These new insights suggest that interventions to reduce infections and inflammation will be at least as effective as dietary interventions and that the latter will not succeed without the former.

Introduction
Iron is an intriguing nutrient with many paradoxical characteristics. It is the most abundant element on our planet and yet is hard for living organisms to access due to its very low solubility. Its ability to switch easily be-
between the ferric (Fe^{3+}) and ferrous (Fe^{2+}) state is key to its usefulness in many biological reactions, but managing the electron state also poses a challenge. Iron is an essential and usually highly beneficial nutrient, but at the same time can wreak havoc if it is not appropriately chaperoned to prevent it from causing oxidant damage. In humans these chaperone processes are also critical in withholding iron from bacterial pathogens and maintaining relatively hypoferremic conditions such that microorganisms will not be able to multiply rapidly and can be dealt with by other arms of the innate and adaptive immune systems. This much has been known for many decades with the chemical details of the chaperone mechanisms starting to emerge after Schade’s first descriptions in Science of the iron withholding and bacteriostatic qualities of ovotransferrin and transferrin [1, 2].

Within the past 2 decades advances in molecular biology methods have revealed the finer details of how iron is absorbed, transported, and utilized. Of special relevance has been the discovery of hepcidin: the master regulator of iron. These insights have transformed our understanding of iron metabolism and have immediate clinical relevance as summarized here.

**Twentieth-Century Concepts of Iron Absorption and Regulation**

By the 1930s the legendary scientific duo of McCance and Widdowson [3] had concluded that iron homeostasis in humans is controlled by regulating intestinal absorption. Initial attempts to assess the efficiency of intestinal iron absorption from different foods and diets started with fastidious and very time-consuming balance studies [4, 5]. These were superseded by stable isotopic tracer methods which assessed iron utilization (the aggregate outcome of absorption and ultimate utilization for heme production) in the single isotope mode [6], or true absorption and utilization in the double isotope mode in which one isotope is administered orally and the other intravenously [7].

Arising from these and related studies came the “textbook” knowledge of iron absorption [8] which, simply put, was: (a) that iron absorption is a dynamic process with auto-regulatory feedback (hence, for instance, pregnant women with greater iron needs upregulate their absorption); (b) that heme iron is better absorbed and utilized than non-heme iron; (c) that iron absorption is inhibited by various binding agents in foods (such as phytates and phenolic compounds); (d) that iron absorption is enhanced by co-ingestion of vitamin C; (e) that there is competition for absorption between iron and zinc; and (f) that there is no active process to excrete iron from the body. These were the basic tenets around which most iron interventions were designed and most recommendations were set. Of particular impact was an underlying assumption that the human gut was poorly designed to absorb iron. This prompted extreme practices in which, for instance, young children were (still are) deemed to require 2 mg/kg iron (when their actual daily need is a very small fraction of this), were given highly absorbable iron, often with vitamin C, and dosing was advised to occur between meals to avoid interference by phytates or related “anti-nutrients.” These recommendations for highly nonphysiological dosing regimens have probably caused considerable iatrogenic harm judging from the adverse outcomes of many randomized trials of iron supplementation to children [9]. With the modern insights summarized below, and viewed from a more nuanced understanding of the origins and “intentions” of the finely evolved systems for iron handling, these failures in public health practice are unsurprising. We now know that, far from being ill-designed to absorb iron, children in unhygienic environments are working hard to exclude iron from their systems; and the processes mediating this iron blockade are now well understood.

**Molecular Mechanisms of Iron Absorption at the Intestinal Lumen**

It seems remarkable that the mammalian divalent cation transporter 1 (DCT1; now termed DMT1) was only discovered and cloned in the rat 20 years ago by Gunshin et al. [10] at Harvard. In the original description in the rat the investigators described the 12 transmembrane loops, its tissue distribution (very high in enterocytes lining the villi of the duodenum but with some expression in most tissues studied), and the fact that mRNA levels were highly upregulated by dietary iron deficiency [10]. They also correctly predicted that the hereditary hemochromatosis gene (HFE) was likely to be part of the iron-sensing regulation of DCT1. These first discoveries led the way to our current understanding of the molecular mechanism of iron absorption [11]. The absorption of non-heme iron requires that it be reduced to Fe^{2+} by the action of duodenal cytochrome B (Dcytb) which co-localizes with DMT1 in the brush border membrane [12]. Once inside the enterocyte, iron can either be stored in ferritin (and later lost when the cells are sloughed) or can traverse the cell and enter the circulation via ferroportin. Ferroportin

Hepcidin-Mediated Regulation of Iron Absorption and Metabolism

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Fig. 1. Iron absorption, recycling, and organ distribution. Values for an adult human male. Reproduced with permission from Hentze et al. [16].

Fig. 2. Hepcidin is the dominant regulator of iron absorption and incorporation into erythrocytes. Data from young Gambian children with postmalarial anemia and control anemic subjects without recent malaria. Reproduced with permission from Doherty and colleagues [22].

Fig. 3. Current knowledge of the key pathways in the regulation of hepcidin expression. Three main molecular pathways have been identified that modulate hepcidin transcription: JAK/STAT3, BMP/SMAD, and HFE/TIR2 signaling pathways. IL6-induced inflammatory stimuli activate the JAK/STAT3 pathway which in turn triggers hepcidin production. Increased hepatic cellular iron induces BMP6 expression which then interacts with BMPR and HJV, forming a complex, thereby activating the SMAD pathway. The SMAD pathway involves phosphorylation of SMAD1, 5, and 8 (pSMADs), formation of the pSMADs/SMAD4 complex, and subsequent translocation of this complex to the nucleus to activate hepcidin gene expression. Extracellular Tf-Fe^{2+} mediates a second iron signal. When transferrin saturation increases, Tf-Fe^{2+} displaces HFE from TIR1. HFE then interacts with TIR2 to form the HFE/TIR2 complex. Consequently, this complex activates hepcidin transcription via the HJV/BMP/SMAD and/or ERK/MAPK signaling pathways. Furthermore, TMPRSS6 and furin cleave HJV to form a soluble HJV, which can selectively inhibit BMP-induced hepcidin expression. Furin and TMPRSS6 can be regulated by hypoxia and via HIF1. Erythropoietic drive may also control hepcidin expression by EPO production. EPO subsequently stimulates erythroferrone expression, which acts together with TWSG1 to inhibit hepatic hepcidin expression by suppressing the BMP/SMAD pathway. JAK, Janus-associated kinase; STAT, signal transducer and activator of transcription; HFE, human hemochromatosis protein; TIR, transferrin receptor; SMAD, sons of mothers against decapentaplegic; BMP, bone morphogenetic protein; HJV, hemajuvelin; HIF, hypoxia inducible factor; MAPK, mitogen-associated activated protein kinase; ERK, extracellular signal-regulated kinase. Reproduced with permission from Wallace [11].

(For figure see next page.)
is the only known mammalian iron exporter that facilitates and modulates cellular iron efflux [13]. The exchange of ferrous iron from ferroportin requires its reoxidation to ferric by the action of ceruloplasmin [14] or (in the intestine) its membrane-bound counterpart hephaestin [15]. Excellent reviews with greater detail are available for readers with a deeper interest [11, 16].

**Net Iron Absorption and Iron Recycling**

An average adult human loses about 1 mg of iron daily largely through the sloughing of epithelial cells in the intestine. This is a notably small amount when compared to the recommended doses for prevention and treatment of iron deficiency anemia (often 200 times as much or more). In contrast to this very small net intake, the amount of iron internally recycled in the body is considerably more; estimated to be 25–30 mg/day (Fig. 1) [16]. In biology, cycles with a high flux such as this offer the opportunity for exquisite control; if there is a regulatory gateway in the cycle, then closing this gateway has an immediate and profound effect on the concentration of the substrate concerned. As we will see below, this is how the acute phase reaction can elicit such a very rapid hypoferremia. For many years the details of the sensors, regulators, and effectors of these processes remained obscure though it was assumed that there must be one or more hormonal regulators that can transmit information from iron-utilizing end organs (primarily the bone marrow) to the intestine.

**Hepcidin: The Master Regulator of Iron Metabolism**

Although many pathways and molecular mechanisms are involved in regulating iron metabolism, the current view is that these are all coordinated by the hepatic-derived peptide hepcidin which acts as the master regulator of iron. Hepcidin integrates diverse signals about iron need with counter-regulatory signals designed to suppress iron intake and recycling when there is a threat of infection [17]. The mature 25 amino acid bioactive form of hepcidin contains 8 cysteines linked by 4 disulfide bridges. Hepcidin–like molecules with high homology to human hepcidin are found in mammals, fish, reptiles, and amphibians, but not in birds or invertebrates.

Hepcidin was independently discovered in the early 2000s by 3 groups who were searching variously for novel antimicrobial peptides and for a liver-expressed iron-responsive gene [18–20]. Ganz and colleagues [19] in Los Angeles coined the name hepcidin to describe a hepatic-derived peptide with microbicidal properties. (Note that although the hepcidin molecule does itself possess some antimicrobial activity, this is rather weak compared to peptides such as defensins, and its primary contribution to innate immunity is via regulation of iron.) Experiments manipulating the hepcidin gene *Hamp1* soon demonstrated that *Hamp1* knockouts became iron loaded and *Hamp1* overexpression led to congenitally fatal iron deficiency [17]. Nemeth et al. [21] in the Ganz lab then showed that the molecular mechanism of hepcidin’s action was via binding to, and causing the internalization and degradation of, ferroportin.

Ferroportin is heavily expressed in 2 sites (on the basolateral membrane of enterocytes and on macrophages) and thereby regulates both iron absorption and tissue redistribution [17, 21] (Fig. 1). Hepcidin-mediated down-regulation of ferroportin on the enterocytes blocks iron egress causing a build-up of intracellular iron which in turn blocks further uptake by DMT1. Note that by exerting its control on the basolateral side of the cell it also blocks the uptake of iron released from heme by the action of heme oxygenase. Figure 2 shows that iron utilization by young anemic children is very efficient at low levels of hepcidin but is almost entirely blocked when hepcidin levels are high [22]. Ferroportin blockade in macrophages prevents iron recycling [21].
Fig. 5. Hepcidin, iron partitioning, and infectious pathogens. By inhibiting ferroportin, hepcidin controls the total amount of iron entering the circulation as well as the compartments and cell types to which the iron is directed. The usual amounts of iron in serum (bound to transferrin), hemoglobin, macrophages, and hepatocytes are given (represented by light gray columns). In conditions of high hepcidin (dark gray columns), iron recycling through macrophages is inhibited while consumption of iron by the bone marrow continues, leading to a rapid decline in serum iron levels. Over time, persistently high hepcidin reduces iron absorption and locks iron in the reticuloendothelial system, so that hemoglobin iron levels wane, causing anemia. Iron redistribution also leads to less iron in hepatocytes, which express low levels of ferroportin. Conversely, when hepcidin is low (black columns), iron is highly absorbed and readily released from macrophages, leading to elevated levels in serum and moderately increased hemoglobin. The excess iron in the system accumulates in parenchymal tissues, most frequently in hepatocytes. Note that the changes in iron levels represented by the columns are intended to represent trends rather than quantitative measures. Iron-requiring pathogens often inhabit specific anatomical compartments or target particular cell types. Examples include some types of bacteria that thrive and become pathogenic in the bloodstream; others, such as M. tuberculosis, are macrophage-tropic. Plasmodium replication in hepatocytes and erythrocytes is highly iron-dependent (many other pathogen types also target hepatocytes because they are energy- and nutrient-rich). Fluctuations in hepcidin – directly consequent to infection as part of the innate immune response, or due to the underlying iron status of the infected individual, or caused by experimental or therapeutic intervention – alter iron availability to the infectious organism, with likely downstream effects on pathogenesis. Iron is also required for host immune mechanisms to generate microbicidal effectors (reactive oxygen species, nitric oxide) and for antigen-specific lymphocyte proliferation. Image credits: Wikimedia, CDC, NIH; bacteria images copyright Dennis Kunkel Microscopy Inc. Reproduced with permission from Drakesmith and Prentice [23].

Competing Control of Iron by Iron Needs and Infection/Inflammation

Hepcidin represents an exquisite example of evolution in response to the 2 opposing aspects of iron in biology; its obligate need for many physiological processes versus the threat it poses by encouraging pathogen growth. Existing data suggest that hepcidin balances the need for iron against the threat of infection [23]. Figure 3 (reproduced from Wallace [11]) and its accompanying legend gives a simplified summary of the mechanisms by which hepcidin achieves this balancing act. The figure summarizes current knowledge and numerous further details are sure to emerge.

In brief, the iron-responsive modulation derives signals from hepatic iron and transferrin saturation and pos-
sibly oxygen saturation through HIF1. The recently described bone marrow-derived hormone, erythroferrone, produced by young erythroblasts also downregulates hepcidin expression in response to active erythropoiesis [24]. Conversely, inflammation, mostly mediated through IL-6 but with alternative stimulation by IL-22 and type 1 interferon, upregulates hepcidin and thus blocks iron absorption and recycling [25]. Figure 4 illustrates the impressive speed and extent by which hepcidin can elicit an immunoprotective innate response [26].

Figure 5, reproduced from Drakesmith and Prentice [23], illustrates how differential levels of hepcidin expression change the iron concentration in various body compartments with likely consequences for the susceptibility to infection. Hepcidin-induced hypferremia in circulating plasma protects against the acute threat of bacterial and yeast sepsis. Prolonged upregulation of hepcidin leads to the anemia of inflammation or of chronic infection [27], which in turn protects against the blood stage of malarial infection [28]. Similarly, hypferremia in hepatocytes protects against the hepatic stages of malaria infection [29]. Intriguingly, though as yet unproven, we propose that iron lock-down in macrophages may be the reason that intracellular pathogens have selected macrophage phagosomes as their niche of choice [23].

**New Insights into the Causes of Iron Deficiency in Low-Income Countries**

Prior to this century, almost all research into the etiology of iron deficiency anemia concentrated on dietary factors and was based around the premise, as mentioned above, that humans are inherently badly designed to absorb iron especially from cereal-based diets. The discovery of the hepcidin-ferroportin axis effectively turns this assumption on its head and requires a thorough re-examination of the key etiological factors driving deficiency. Key to this conclusion is the finding that most known gene defects affecting iron status lead to iron overload, not deficiency, even in populations on a low iron intake [11]. The one exception relates to defects in the TMPRSS6 gene which normally suppresses hepcidin gene expression. Rare defects in this gene result in hepcidin overexpression and so-called iron-refractory iron deficiency anemia (IRIDA) [30]. These insights reveal that instead of struggling to absorb sufficient iron, the normal physiological state in humans is one of tonic suppression of iron absorption in order to maintain body iron homeostasis in the absence of any pathways for secretion of an excess.

We have extensively studied populations with high levels of anemia (pregnant women and young children in rural areas of Gambia and Kenya) and have been forced to re-evaluate many of our preconceived notions. First, in cross-sectional studies in both populations and using structural equation modelling we showed that hepcidin levels were, as predicted on the basis of the known biology of hepcidin, predicted by a combination of iron status, inflammation, and infection [31, 32].

We then used receiver-operator characteristic analysis to determine hepcidin cutoffs that best define iron deficiency anemia and the anemia of inflammation [33, 34]. In children the defined threshold (5.5 ng/mL, using the Bachem ELISA) also distinguished iron absorbers from nonabsorbers [33]. With support from the Bill & Melinda Gates Foundation, we have recently completed 2 randomized controlled trials in Gambian pregnant women and children under the umbrella of the HIGH Consortium (Hepcidin and Iron in Global Health) [35, 36]. These trials are testing whether it would be possible to develop a point-of-care diagnostic for hepcidin-guided iron administration on the principle that a hepcidin level below 5.5 ng/mL in children (or 2.5 ng/mL in pregnant women [34]) would signal that the subject was “safe and ready to receive iron.” These trials will shortly report full results.

Using longitudinal hepcidin measurements (weekly for 12 weeks) in over 400 participants from the children’s trial [35] we have shown that each child maintains a remarkably consistent level of hepcidin over the 3 months of study (albeit gradually rising in response to the iron supplementation). Fifty percent of the children consistently maintain their hepcidin above the 5.5 ng/mL, indicating that they are blocking iron absorption (Prentice et al., manuscript in preparation). Unsurprisingly, these high hepcidin levels are associated with raised CRP and AGP (markers of inflammation), but remarkably we found that hepcidin was upregulated even at very low levels of inflammation. This has important implications for the prevention and treatment of iron deficiency and its associated anemia as we discuss in our conclusions below.
**Prospects and Possible Applications for Hepcidin Agonists and Antagonists**

Pharmaceutical companies are investing considerable resources in the potential therapeutic applications that could be derived from manipulating hepcidin [37]. For instance, iron overload could potentially be modulated by artificially enhancing hepcidin. To this end, Ganz and his team have synthesized a series of mini-hepcidins; short peptides that maintain hepcidin action, are re-engineered to be stable, and could potentially be orally active [38, 39]. In the global health context mini-hepcidin might be able to prevent the iron overload that occurs even in untransfused beta-thalassemia (due to the hepcidin-suppressing action of excess erythrophere caused by the ineffective erythropoiesis that generates excess erythroblasts) [40]. Conversely, there is great interest in the possibility that hepcidin antagonists could be used to prevent or treat the anemia of chronic disease and anemias associated with treatments such as cancer chemotherapy [37]. Clinical trials are underway for both hepcidin agonists and antagonists and it is likely that these will become commonly used therapeutic agents in the mid-term future.

**Conclusions: Implications of the New Molecular Insights**

It goes without saying that the design and implementation of preventative or therapeutic interventions to combat disorders of iron metabolism (most commonly iron deficiency anemia in a global context) are greatly aided by a clearer understanding of the precise mechanisms regulating iron metabolism. As described above, the most important implication of these discoveries is the realization that humans have evolved to expend at least as much physiological effort in excluding dietary iron as in acquiring it. This has been driven by the hazardous nature of iron; both in causing oxidative damage and in promoting the growth of microorganisms. There is clear evidence that a moderate degree of iron deficiency anemia is highly protective against malaria [28] and likely protective against a range of bacterial and fungal pathogens [23] and possibly viruses [41]. This has resulted in the evolution of exquisite systems for chaperoning iron and regulating its intake and organ distribution. Against this background, our former attempts to counteract iron deficiency by using very high nonphysiological doses of highly absorbable iron given without food seem clumsy to say the least and have almost certainly caused a great deal of iatrogenic harm. An analogy would be that we have been trying to use a sledgehammer to break down a closed door rather than learning how to pick the lock. Our new understanding that even low level inflammation blocks iron absorption via the hepcidin/ferroportin axis throws the spotlight on the need to eliminate infections and the consequent inflammation. This will require so-called nutrition-sensitive interventions around improvements in hygiene and infection control and it is quite possible that such interventions could be more important in poor populations than nutrition-specific interventions around iron.

**Disclosure Statement**

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