General discussion
INTRODUCTION

The World Health Organization (WHO) describes iron deficiency (ID) as a ‘a condition in which there are no mobilizable iron stores and in which signs of compromised supply of iron to tissues, including the erythron, are noted’. ID is the most common nutritional problem in the world, with the highest prevalence in women and young children living in low-resource countries. The WHO estimates that 47% of preschool children worldwide have anemia, in which ID is the underlying cause in about 50%. The main causes for ID in low-resource countries are a low dietary iron intake, high intake of foods that inhibit iron absorption, infectious diseases, and intestinal parasites.

As well as affecting a large number of children in low-resource countries with a high prevalence of malnutrition, ID is also commonly observed in high-resource countries with well-nourished populations. Persisting adverse effects of ID on cognitive and psychomotor development have been observed in children from both low- and high-resource countries.

Aim of this thesis was to establish the prevalence and risk factors of ID in children in a high-income country. We focused on specific groups of children, namely preterm infants, healthy young children, and children with cystic fibrosis (CF). Results described in this thesis showed that the prevalence of ID, defined according to the criteria of the WHO, was 23.8% in 4 to 6-months old preterm infants born between 32+0 and 36+6 weeks gestational age (GA), 18.8% in healthy children aged 0.5 to 3 years, and 13.9% in clinically stable children with CF aged 0 to 18 years. Although the observed prevalence in these studies was lower than reported in low-resource countries, our results illustrate that ID is a major health problem in a high-resource country like the Netherlands. In contrast to the many different causes of ID in low-resource countries, we demonstrated that the prevalence of ID in children in the Netherlands was mainly determined by dietary factors. The relative high prevalence of ID and its risk factors in a generally well-nourished population raises questions about the diagnostic and threshold criteria for ID, health consequences of ID and possible strategies to prevent ID.

In this chapter, the results of our findings are discussed and recommendations for future studies are given. Firstly, considerations with regard to diagnostic criteria for ID and reference ranges for iron status biomarkers in preterm infants,
healthy young children and children with a chronic inflammatory disease will be discussed. Subsequently the effects of ID and iron supplementation on neurodevelopment in preterm infants and healthy young children, and the effects on immunity in children with a chronic inflammatory disease will be debated. Finally the risk factors of ID in preterm infants, healthy young children and children with a chronic inflammatory disease will be discussed, in order to develop strategies to prevent ID in children in a high-income country.

**DIAGNOSTIC CRITERIA FOR IRON DEFICIENCY**

Establishing the iron status in children is complicated by poorly defined age-specific reference ranges for several biomarkers and the lack of consensus on which biomarkers should be used to assess the iron status. Reference ranges for iron status biomarkers in serum and whole blood should ideally be based on body iron stores since the ‘gold standard’ of ID is lack of iron in bone marrow and liver tissue\(^17\). The bone marrow smear has generally been considered the most reliable diagnostic test, but has the limitation of being more invasive than iron status biomarkers in peripheral blood\(^17\). Furthermore, incorrect assessment of iron stores in bone marrow aspirates has been described\(^18\). Concentrations of different biomarkers have been validated against iron concentrations in bone marrow only in children living in low-resource countries with endemic malaria and other infectious diseases\(^19\)-\(^21\). Since the concentration of most iron status biomarkers is influenced by infection and inflammation, reference ranges reported in these studies\(^19,20\) are not applicable to healthy children in high-resource countries like the Netherlands with a much lower infection pressure.

In the absence of bone marrow iron stores as a gold standard, reference values in high resource countries are often defined as SD ±2 or P2.5 – P97.5 values in a ‘healthy’ population\(^22,23\). A healthy population should ideally consist of a large sample of similarly aged, healthy children with no infection or inflammation, and likely to have a low prevalence of ID. Unfortunately reference values for iron status biomarkers that have been published, are often defined in study populations with a relatively small number of children\(^22,24,25\), or without excluding children with a possible infection or inflammation\(^19,26,27\).

Once reference ranges have been established in a healthy population, one should realize that iron status is influenced by genetic factors, growth, demographic factors (e.g. ethnicity and socioeconomic status) and dietary habits (e.g. duration of breastfeeding and use of iron fortified products). Since these factors greatly
differ between and within populations, reference ranges obtained in a certain population may not be applicable for another population. Moreover, different assays are available for many iron status biomarkers. The agreement between these assays is poor for most biomarkers, but especially for soluble transferrin receptor (sTfR) and hepcidin\textsuperscript{28,29}. Therefore, the use of different assays often limits comparison of iron status biomarkers between studies.

In addition to the lack of well-developed reference ranges and assay standardization, there is no agreement on which biomarkers should be used to assess the iron status in children. Many studies defined ID as two or more ‘abnormal concentrations’ in a set of multiple iron status biomarkers\textsuperscript{30,31}. However each biomarker represents a specific aspect of the iron status. Some indicators, such as ferritin, represent iron stores in tissues whereas others are thought to represent iron available for the erythropoiesis (e.g. Hb content in reticulocytes (Ret-Hb)) or cellular iron demands (e.g. soluble transferrin receptor (sTfR)). Since there is no agreement on which set of biomarkers should be used to assess the iron status, many different combinations have been used which hampers comparison of results between studies.

Once agreement is obtained on which biomarkers should be used to establish the iron status, one should take into account that the diagnostic sensitivity of biomarkers in a study population is influenced by the severity of ID in that population and the presence or absence of concomitant infections or inflammation. For example, sTfR has been shown to be valuable in the diagnosis of ID in populations with a high prevalence of infection/inflammation and concomitant severe IDA, since sTfR is less affected by an acute-phase response than ferritin, and provides information on the extent of ID once iron stores are depleted\textsuperscript{32,33}. However the diagnostic value of sTfR and other relatively new iron status biomarkers as Ret-Hb, and hepcidin in populations with a low prevalence of severe IDA as in high-income countries is unclear.

In this thesis we measured ferritin as indicator of iron stores, and mean corpuscular volume (MCV) and Hb as indicators of microcytic anemia. In addition to these conventional iron status biomarkers, we aimed to investigate the value of relatively new biomarkers in children living in a high-income country. Therefore, we measured Ret-Hb as indicator of iron available for erythropoiesis, sTfR as indicator of tissue iron demand, and hepcidin as a promising iron status indicator of which limited data are available in children.

The criteria for ID used in this thesis, and the need to establish appropriate age-specific reference, and uniform population based criteria for ID in children, will be
discussed in more detail in preterm infants, healthy young infants and children with a chronic inflammatory disease such as CF.

**Preterm infants**

In this thesis, ID at the age of 4 and 6 months was defined as a ferritin <20 μg/L and <12 μg/L respectively (chapter 2). IDA was defined as ID in combination with anemia; a Hb <105 g/L (chapter 2). The definition of ID at the age of 6 months was based on recommendations of the WHO and ESPGHAN. Although frequently used, this definition is extrapolated from older age groups, and other reference values for ferritin of 9 μg/L to 19 μg/L have been suggested. Reference values for MCV, Hb, and ferritin in infants aged 4 months were derived from -2 SD values observed in similarly aged Swedish, healthy, exclusively breastfed infants born at term. This population can be considered as ‘iron sufficient’ because it is generally assumed that healthy term infants have sufficient iron stores at birth to prevent ID during at least the first 4 months of life.

Defining reference ranges in preterm infants is complicated because iron status biomarkers derived from postnatal blood samples likely reflect iron supplementation, blood transfusion and phlebotomy practice in the individual infant rather than the physiological range of iron status biomarkers. Moreover, concentrations of iron status biomarkers in preterm infants cannot be considered as ‘normal’ since preterm infants lack the physiological iron accretion during the third trimester of pregnancy. Therefore, reference ranges obtained in healthy, breastfed, term infants are more appropriate. Unfortunately, these are poorly defined for infants younger than 4 months of age. Defining age-specific reference values for iron status biomarkers in healthy, term infants during the first months of life is urgently needed in order to improve the comparability between studies and more importantly, to identify those infants at risk of ID from an early age, and subsequently guide the use of iron supplementation.

There is no consensus on which biomarkers should be used to define ID in infants younger than 6 months of age, whereas interpretation of iron status biomarkers is complicated by age-related changes in erythropoiesis and redistribution of body iron. After birth, iron from senescent erythrocytes is initially transferred from Hb to iron stores in the liver and bone marrow. From the age of about 2 months, iron stores are again mobilized for growth and erythropoiesis. As a consequence, ferritin concentrations rapidly decrease (chapter 2). Ferritin has been shown to closely parallel the size of body iron stores in adults. Although no validation studies have been performed in infants, it is the most frequently used biomarker.
to measure the amount of stored iron. In chapter 2 we showed that a decrease in ferritin was associated with more weight gain in the first 6 months of life. More insight in the physiological changes of ferritin and other iron status biomarkers during the first months of life, and the influences of erythropoietic stimuli and iron status is essential to determine their value in the diagnosis of ID in preterm infants. This is even more important for biomarkers such as Ret-Hb, sTfr and ZPP, since limited data are available on the physiological course and influence of erythropoietic stimuli and iron status on these biomarkers during the first months of life.

Furthermore, it is important to realize that whatever biomarker is used to assess iron status, its determination adds to iatrogenic blood and, hence, iron loss and consequently further predisposes the preterm infant to ID\textsuperscript{39}. Every milliliter of blood loss represents a loss of 0.35-0.50 mg iron (assuming an Hb concentration of 100-150 g/L). In order to minimize the amount of blood loss, iron status should be measured with as less biomarkers as possible, that provide as much as possible information. Future studies are needed to identify the biomarker or combination of biomarkers that meets these criteria.

Interestingly, hepcidin can be measured in urine, and hepcidin concentrations in serum and urine correlate in preterm infants\textsuperscript{40,41}. We showed in chapter 3 that serum hepcidin concentrations were more strongly associated with iron stores than with erythropoiesis. Furthermore, serum hepcidin was significantly lower in infants with ID (ferritin <20 μg/L) compared those without ID at the age of 4 months (chapter 3). These results suggest that serum hepcidin might be a useful indicator of iron stores in preterm infants with no concomitant infection/inflammation. Considering these results, we may hypothesize that urinary hepcidin might be used as a non-invasive iron status biomarker. However, only 5% of hepcidin filtered by the kidneys is found in urine, and the very low glomerular filtration rate (GFR) of preterm infants may impair renal filtration of hepcidin\textsuperscript{40}. The potential influence of the low GFR, and other factors such as infection/inflammation on urinary hepcidin concentrations in preterm infants warrants further investigation. Furthermore, studies on standardization of hepcidin assays in general, and definition of reference values in serum and urine are needed in order to investigate the potential diagnostic value of hepcidin in the diagnosis of ID in preterm infants.

**Children aged 0.5 to 3 years**
In this thesis we defined ID in children aged 0.5 to 3 years as a ferritin <12 μg/L.
(chapter 4), according to the criteria of the WHO\(^1\). However, a lower reference value of <10 \(\mu\)g/L that was based on the 5\(^\text{th}\) percentile of ferritin concentrations in a large population of children in the US, has been suggested\(^42\). In accordance with these results, we observed an effect on iron available for erythropoiesis only when ferritin was <10 \(\mu\)g/L, suggesting this might be a more appropriate cut-off value for these children (chapter 5).

Reported reference ranges for Ret-Hb\(^{37,43,44}\) and sTfR\(^{22,45}\) in young children vary widely. These differences may be attributed to differences in methods that have been used to calculate reference ranges, and differences in characteristics of populations being studied. As previously described, reference values for these biomarkers should be defined in a large population of similarly aged, healthy children with no infection/inflammation, and likely to have a low prevalence of ID. Pediatric age-specific reference ranges for hepcidin have been reported only in a small group of Kenyan infants\(^46\). In chapter 6 we described reference ranges for hepcidin in healthy young children in a high-resource country, measured by mass-spectrometry (MS) and an immunochemical (IC) assay. The reference ranges reported in this study are helpfull for future studies investigating the diagnostic and therapeutic potentials of hepcidin.

Many different biomarkers, and even more combinations of biomarkers have been used to define ID in young children\(^{31,47}\). Each biomarker represents a specific aspect of the iron status. Furthermore, the diagnostic sensitivity of biomarkers in a study population is influenced by the severity of ID in that population, and the presence or absence of concomitant infection/inflammation. In chapter 5 we showed that the discriminative value of Ret-Hb and sTfR for the detection of iron depletion in a population of young, healthy children with a low prevalence of severe IDA is very limited. This should be considered when deciding which biomarkers to use in the assessment of iron status. By using a multiple criteria model, only children with severe, longstanding IDA will be classified as ID. We therefore conclude that this approach has no additive value in high resource populations with a low prevalence of severe IDA.

Although hepcidin has been described as a promising biomarker in the assessment of iron status in adults\(^{48}\), limited data are available on the diagnostic potentials of hepcidin in children\(^19\). Hepcidin has been suggested as a biomarker that may contribute to the diagnosis and therapy of hereditary defects in iron metabolism, and as a biomarker that could be used to guide iron supplementation therapy\(^{48,50,51}\). The latter hypothesis was recently rejected by
the authors of a study on hepcidin in severely anemic African children\textsuperscript{21}. In that study, hepcidin was a poor predictor of bone marrow ID and iron incorporation\textsuperscript{21}. However, in contrast to hepcidin concentrations observed in our population of healthy young children (chapter 6), hepcidin concentrations in these severely anemic children were largely undetectable, probably as a result of the high erythropoietin levels that may have suppressed hepcidin production\textsuperscript{21}. Although erythropoietin levels were not assessed in our study, concentrations are likely to be lower compared to those observed in these African children. The results reported in that study may therefore not be applicable to our population of children living in high-resource countries with a much lower prevalence of severe IDA. The diagnostic potentials of hepcidin measurements in children living in high-resource countries warrant further investigation.

In order to investigate these diagnostic potentials of hepcidin and other iron status biomarkers such as sTfR, studies on assay standardization are urgently needed. In chapter 6 we showed that hepcidin concentrations measured with mass-spectrometry (MS) were consistently lower than hepcidin concentrations measured with an immunochemical (IC) assay. These results are in accordance with those reported in adults\textsuperscript{29}. We attribute the differences between the assays to the fact that the respective companies assigned a lower value to the Bachem standard (used in the IC assay) than to the PI standard (used in the MS method), resulting in the higher measurement results in the Bachem assay. These results illustrate the need for harmonization of different methods\textsuperscript{29}.

**Children with a chronic inflammatory disease**

We defined absolute ID in children with CF as a ferritin $<12\ \mu g/L$ or $<15\ \mu g/L$ in children younger or older than 5 years respectively (chapter 9). However, ferritin acts as an acute phase reactant, and the use of this restrictive cut-off value might therefore underestimate the prevalence of absolute ID in this population. Various methods have been proposed to interpret iron status biomarkers in the presence of infection/inflammation, including the use of a higher cut-off value for ferritin\textsuperscript{1,32}, and methods that mathematically adjust individual ferritin concentrations by using conversion factors based on acute-phase proteins such as C-reactive protein (CRP)\textsuperscript{53}. However, the latter method is not appropriate in children with CF, since CRP is not helpful for identifying infections in these patients\textsuperscript{54}. Furthermore, the use of a higher cut-off value for ferritin as suggested by the WHO\textsuperscript{1}, appears to underestimate the prevalence of ID in children with inflammation and overestimate the prevalence of ID in children without inflammation\textsuperscript{33}. Moreover, it is still unclear to what extent adjustment of the cut-off value of ferritin is required. Therefore, we used the more restrictive criteria of ID in our carefully selected
population of relatively healthy children with CF. However, this is not ideal, and there is a need for studies investigating appropriate reference ranges of iron status biomarkers in populations with a high prevalence of infection/inflammation.

Considering these diagnostic limitations of ferritin in the presence of infection/inflammation, measurement of other iron status biomarkers, such as sTfR have been suggested to be more informative. sTfR is considered to be less affected by the acute-phase response than ferritin\(^{33}\). However, in chapter 10 we have demonstrated that sTfR concentrations were similar in children with and those without ID, defined as a ferritin <12 μg/L or <15 μg/L in children younger or older than 5 years respectively. Furthermore, sTfR concentrations were within the normal range in all children (chapter 10). Since sTfR starts to increase only after severe depletion of iron stores\(^{55}\), we suggest that ID in our population was not severe enough to cause a significant increase in sTfR concentration. We therefore conclude that sTfR is not useful to determine the iron status this population of relatively healthy children with CF and a low prevalence of severe IDA (chapter 10).

No data were available on hepcidin concentrations in children with CF. We hypothesized that hepcidin concentrations would be increased in children with CF, which may contribute to the development of ID. However, in contrast to our expectations, hepcidin concentrations in children with CF were low, and concentrations below the limit of detection were observed in 25% of the clinically stable children (chapter 10). It has been reported that regulation of hepcidin synthesis is more dynamic compared with ferritin, and varies more slowly in response with erythropoietic iron demands\(^{56}\). Although hepcidin acts as an acute phase reactant similar to ferritin, hepcidin declines more rapidly after an infection or inflammatory signal has been cleared\(^{51}\). We may hypothesize that in children with ID recovering from inflammation or an infection, hepcidin is already low to maximize iron absorption for erythropoiesis, whereas ferritin is still above the suggested cut-off values for ID. Hepcidin might therefore be a more sensitive indicator than ferritin to detect ID in children with a high burden of inflammation or infections. However, in order to investigate the clinical use of hepcidin in children with a chronic inflammatory disease, age-specific reference ranges for hepcidin concentrations in children older than 3 years are required.

The plethora of definitions of ID used in literature, with a wide variation in used biomarkers, and even more differences in applied reference ranges, hinders the comparison between studies. Development of age-specific laboratory
criteria for ID in infants and children and standardization of assay methods are urgently required. In this thesis we suggest reference values for ferritin and hepcidin in children aged 0.5 to 3 years (chapter 5 and chapter 6). Furthermore, we discourage the use of Ret-Hb, sTfR, or a ‘multiple criteria model’ as screening measures for ID in a high resource country with a low prevalence of severe IDA (chapter 5). However, the choice of a particular cut-off point to define ID is essentially arbitrary as there is no clear evidence to determine at which level ID begins to have significant deleterious effects. Ideally the definition of ID should be based on studies showing that children with biomarkers outside reference ranges have poor neurodevelopmental outcomes. Future studies should therefore focus on long-term consequences of different stages of ID.

At this time, ferritin is the most useful biomarker in the screening of ID in healthy children living in high-income countries. The results described in this thesis suggest that in the absence of infection/inflammation, a ferritin concentration <10 μg/L might be a suitable cutoff value to define ID in children aged 0.5 – 3 years. For infants younger than 6 months, age-specific reference ranges are poorly defined. However, we found an increased risk of later ID in infants with ferritin concentrations <150 μg/L and <80 μg/L at the age of 1 and 6 weeks, respectively. Suggested lower reference values for ferritin that may be used to define ID in clinical practice are presented in Table 1. In addition to ferritin, assessment of another acute-phase is necessary to rule out any influence of infection/inflammation, especially in children with a chronic inflammatory disease. Assessment of sTfR is not useful to determine the iron status in children with a chronic inflammatory disease, living in a high-income country with a low prevalence of severe IDA.

**Table 1:** Suggested age-specific lower reference values for serum ferritin (μg/L).

<table>
<thead>
<tr>
<th>Age</th>
<th>Lower reference values for serum ferritin (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>150</td>
</tr>
<tr>
<td>1.5 months</td>
<td>80</td>
</tr>
<tr>
<td>4 months</td>
<td>20</td>
</tr>
<tr>
<td>0.5 – 3 years</td>
<td>10</td>
</tr>
<tr>
<td>3 – 5 years</td>
<td>12</td>
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<tr>
<td>&gt;5 years</td>
<td>15</td>
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</table>
Iron deficiency and neurodevelopment

The main health problem associated with ID in infancy and childhood is the risk of poor neurodevelopment. The human brain almost triples its weight from birth to 3 years of age and reaches at that age 85% of its adult size. The average concentration of iron in brain tissue is far higher than that of all other metals, except zinc. Animal studies have shown that iron is essential for several aspects of brain development and function such as myelination, monoamine neurotransmitter function, neuronal and glial energy metabolism and hippocampal dendritogenesis. However, a causal linkage between individual ID and brain function has been difficult to confirm due to large sample sizes and long follow-up time, that are required, and many confounding factors, that need to be taken into account. Sensitivity to iron-restrictive conditions depends on the severity and duration of ID, and the stage of development during which ID occurs. Unfortunately, the threshold levels of ID that will cause adverse effects on neurodevelopmental outcome are unknown.

Since most follow-up studies used a multiple criteria model to define ID, only the effects of severe, longstanding IDA have been studied. There is a well-verified association between IDA and impaired cognitive, motor and behavioral performance in infants and children. Whether these negative neurodevelopmental consequences described in children with IDA also apply to children with ID without anemia needs further evidence. Results of studies performed in animals suggest that during ID, available iron is prioritized to red blood cells over other organs, including the brain, suggesting that negative neurodevelopmental effects may develop before anemia occurs. Moreover, limited evidence suggests that children with ID and no anemia tend to have lower developmental scores, verbal competency, comprehension and intelligence and more behavioral problems compared with children who are iron-replete. Although ID is associated with adverse effects on neurodevelopmental outcome, one should realize that iron is a potent pro-oxidant. Non-protein bound iron has been suggested to cause formation of reactive oxygen species. Furthermore, it has been demonstrated that iron overload may have adverse effects, e.g. increased risk of infection and impaired growth. Therefore, it is important to identify those infants who may benefit from iron supplementation, but also to avoid iron supplementation in cases where it is not necessary.
IRON SUPPLEMENTATION

Preterm infants
In chapter 2 we have showed that ID and IDA at the age of 4 or 6 months are common in preterm infants born between 32+0 and 36+6 weeks of GA, supporting the need of preventive iron supplementation. Iron supplementations during the first six months of life at a dose of 1 – 2 mg/kg per day and 2 – 3 mg/kg per day for preterm infants with birth weights <1800 and ≥1800 g respectively, are recommended by the ESPGHAN. However, the prevalence of ID in our population of predominantly formula fed infants was relatively low compared to prevalence rates previously observed in Swedish infants who were mainly breastfed during the first six months of life. Since dietary iron intake in these predominantly breastfed Swedish infants was lower, and the prevalence of ID was higher compared to our population of predominantly formula fed infants, more Swedish infants might have advantage of iron supplementation.

Recently updated guidelines in the Netherlands recommend iron supplementation at a dose of 2 – 3 mg/kg per day for all preterm infants with a birth weight <2000 g from the age of 2 – 6 weeks until discharge. After discharge, iron supplementation is continued only in those infants receiving breast milk and/or standard infant formula, but not in infants receiving post-discharge formula.

Iron supplementation, either as iron drops or as iron-fortified formula, improves the iron status in preterm and low-birth weight infants (GA <35 weeks or birth weight <2500 g). Limited evidence suggests that preventive iron supplementation may have positive effects on neurodevelopmental outcome. Long-term effects of preventive iron supplementation in preterm and low-birth weight infants have been described in only three studies. Two of these studies focused on very low-birth weight infants (mean birth weight <1500 and <1300 g) and one study described the effects of iron supplementation in marginally low-birth weight infants (preterm and term infant with a birth weight of <2500 g). In the latter study, Berglund et al. reported a lower risk of behavioral problems at the age of 3 years in infants who received iron supplementation (1 – 2 mg/kg per day) compared to those who received no iron supplementation. However, no beneficial effects were observed on cognitive development or auditory brainstem response in the iron supplemented group. Furthermore, although adverse effects of iron supplementation, such as increased oxidative injury, a slower rate of weight gain and length growth, have
not been observed in low birth weight infants⁴⁷,⁷⁸, this must be considered also in preterm infants, and the benefits of iron supplementation should be compared to the possible risks. Considering the observation that the majority (>75%) of our predominantly formula fed late preterm infants received no iron supplementation and were iron-replete at the age of 6 months, individualized iron supplementation, with consideration of local feeding practices and personal needs of an infant, might be an alternative to standardized iron supplementation for all infants with a birth weight of <2500 g. We have shown that infants with a ferritin <150 μg/L at the age of 1 week had a 6-fold increased risk of ID (chapter 2). Therefore, we suggest that preterm infants born >32 weeks GA in a high-income country should receive iron supplementation only if they are exclusively breastfed, and/or when ferritin concentrations are <150 μg/L at the age of 1 week. Ferritin concentrations should be monitored during the first months of life (e.g. at the age of 1.5 and 4 months, respectively), especially in those infants with a high weight gain. It is important that future studies investigate the efficacy and safety of such individualized iron supplementation, and not only measure the effects on iron status, but also investigate the effects on long-term neurodevelopmental outcome.

Healthy children aged 0.5 to 3 years

In chapter 4 we showed that the prevalence of ID and IDA were present in 18.8% and 8.5% of healthy children aged 0.5 to 3 years. In accordance with results described in preterm and low-birth weight infant, iron supplementation improves iron status in young children with ID and IDA⁸⁴,⁸⁵. However, there is no convincing evidence that iron supplementation improves neurodevelopmental outcome. Iron supplementation in young children with IDA had no effect on psychomotor development or cognitive function within 6 to 11 days after commencement of therapy⁸⁶,⁸⁷. Effects of iron supplementation on neurodevelopment in infants with ID and no anemia were reported in only two randomized controlled trials. One study demonstrated a significant difference on cognitive development (Bayleys mental developmental index) after iron treatment in infants aged 6 – 30 months⁸⁸, whereas no effects were observed on psychomotor development (Bayleys psychomotor developmental index)⁸³,⁸⁸. However, neither study adjusted for pre-treatment development scores⁸³,⁸⁸. The lack of effect of iron supplementation in young children with ID and IDA may be attributed to the duration of iron treatment, that may have been too short, or to the outcome measure (changes in Bayley Scale scores), that may be an insensitive measure of real changes, with behavioral effects in particular. Furthermore, structural brain changes caused by ID and IDA may be already
irreversible in children younger than three years of age\(^6\). The latter implies that instead of detection and treatment of existing ID, prevention might be necessary to protect children against neurodevelopmental consequences of IDA and/or ID. Given the relatively high prevalence of ID observed in late preterm infants and healthy young children in a high-resource country, more studies on the long-term effects of ID and IDA are required. Since possible developmental consequences of ID are likely to be smaller than the effects of severe IDA, the sample size of future studies need to be large enough to detect small differences between groups. Furthermore, there is an urgent need for research examining the effectiveness and possible adverse effects of standardized iron supplementation.

**EFFECTS OF IRON DEFICIENCY AND IRON SUPPLEMENTATION ON IMMUNITY IN CHILDREN WITH A CHRONIC INFLAMMATORY DISEASE**

In chapter 9 and 10 we showed that ID is common in children with CF. In addition to the previously described neurodevelopmental effects, ID is associated with abnormalities of cellular immune function, characterized by decreased T-lymphocyte, reduced proliferative capacity of T cells and altered T cell subsets\(^8\)\(^9\)\(^2\). The effects of ID on humoral immunity remain controversial\(^9\)\(^3\), but most studies reported no effects on serum immunoglobulins in children with ID\(^8\)\(^9\)\(^3\)\(^4\). The high prevalence of ID that we observed in children with CF might theoretically increase the risk of infections in these children, with severe viral infection and intracellular bacteria in particular. However, despite proven effects of ID on cellular immunity, the clinical effects of ID on the susceptibility to infections remain controversial\(^9\)\(^4\), and no studies are available on the immunological or clinical effects of ID in children with CF. Conversely, iron is an essential nutrient for pathogenic microorganisms. Bacteria in the CF lung require iron for growth and possess mechanisms to obtain iron from human tissues\(^9\)\(^5\)\(^6\). Elevated concentrations of sputum iron concentrations were associated with inflammation in adult CF patients\(^9\)\(^7\). Furthermore, iron enhances the formation of pseudomonas aeruginosa (PA) biofilm communities\(^9\)\(^8\), which can be visualized in the sputum of patients\(^9\)\(^9\). Iron supplementation in patients with a chronic inflammatory disease, such as CF, is therefore associated with several concerns. Intravenous iron supplementation in adult CF patients colonized with PA resulted in an increase in inflammatory markers and worsening of clinical symptoms\(^1\)\(^0\), whereas no clinical deterioration was observed in CF patients with an absolute ID\(^1\)\(^0\)\(^1\)\(^,\)\(^1\)\(^0\). The potential negative effects of iron
supplementation in CF patients likely depend upon the underlying cause; in absolute ID patients might benefit from iron supplementation whereas in functional ID the underlying inflammation should be treated. The absolute ID that we observed in our population suggests that these children might benefit from iron supplementation. However, there are no data available on the effect of iron supplementation in children with CF. More insight in the underlying mechanism of ID in CF patients, and the association between ID and immune function, PA colonization, and sputum iron is warranted. We suggest that iron supplementation should be considered only in those CF patients with proven absolute ID and no current infection or PA colonization.

**PREVENTION OF IRON DEFICIENCY**

Since negative neurodevelopmental effects of IDA may be irreversible despite iron treatment\(^3,103\), prevention of ID in early life is important to achieve optimal development. We will discuss the risk factors of ID that we identified in preterm infants, healthy young children and children with CF, in order to develop possible strategies to prevent ID in these children.

**Preterm infants**

ID in preterm infants born between 32+0 and 36+6 weeks of GA was associated with a lower birth weight, a shorter duration of formula feeding and more weight gain in the first six months of life (chapter 2). These results are in accordance with those described in other studies\(^104\), and illustrate the increased iron requirements in preterm infants as result of their lower iron stores at birth, and higher iron demands needed for growth and erythropoiesis compared to infants born at term. However, it remains difficult to estimate the exact iron intake that is necessary to prevent ID in all preterm infants. A total iron intake of 1 mg/kg per day has been suggested to prevent ID in marginally low birth weight infants (preterm and term infants with a birth weight <2500 g)\(^47\). This intake theoretically could be achieved with a daily intake of 150 mL/kg formula fortified with 7 mg/L iron. However, we observed a lower mean iron intake in infants with no ID, whereas higher iron intakes were found in both infants with and those without ID (chapter 2). These results illustrate that higher iron intakes from formula compared with breast milk reduce the prevalence of ID, but do not necessarily protect infants against ID. To ensure an adequate iron intake in all preterm infants, including those who are exclusively breastfed, general iron supplementation...
is currently recommended. However, we hypothesized that individualized iron supplementation may be more appropriate in a population of late preterm infants who were predominantly formula fed (chapter 2). Theoretically, an individualized follow-up schedule based on the iron status in the first week of life, growth velocity, type of milk feeding and dose of iron supplementation would be ideal. However, it remains to determine how such individualized supplementation could be performed in clinical practice. Furthermore, it should be emphasized that limitation of the amount of iatrogenic blood loss though phlebotomy\textsuperscript{105}, and implementation of delayed cord clamping\textsuperscript{106-108} will improve iron status, and reduce the risk of ID in preterm infants.

**Children aged 0.5 to 3 years**

In healthy young children a higher iron intake from follow-on formula, and the visit of preschool/daycare were associated with a lower prevalence of ID, whereas consumption of >400 mL cows’ milk was associated with a higher prevalence of ID (chapter 4).

After approximately 6 months of age, children become increasingly dependent on iron from complementary foods. Since young children typically consume relatively small amounts of foods, complementary foods require high nutrient density including much higher iron contents than is needed for adult diets. For example, per 100 kcal of food, a 6 to 8 months old infant needs nine times as much iron as an adult male (WHO needs 0.5 mg iron/100 kcal based on 2700 kcal per day and recommended intakes of iron)\textsuperscript{1}. However, we found that the mean iron content in the diet of many young children is too low to meet the high iron requirements (chapter 8) as was also showed by a large food consumption survey performed in the Netherlands in 2005 – 2006\textsuperscript{109}. Moreover, the contribution of meat to the total iron intake was relatively low, whereas the consumption of cows’ milk was relatively high compared with other European countries\textsuperscript{110,111}. We suggest that the composition of the diet as observed in our study increases the risk of ID in young infants.

In order to decrease the prevalence of ID, preventive strategies should therefore focus on improvement of the dietary composition in young children, with special attention for the stimulation of foods with a more favorable iron bioavailability and limitation of the amount of cows’ milk products. A recent randomized trial in the US compared the effects of weaning infants using pureed meat compared with iron-fortified cereals\textsuperscript{112}. The meat intervention resulted in lower iron intake but a similar iron status at 9 months of age, suggesting that the higher bioavailability of heme-iron improves iron status more effectively\textsuperscript{112}. Consumption of foods with a more favorable iron bioavailability might be sufficient to meet
physiological requirements at a lower dietary iron intake. However, when consumption of these foods is problematic, the use of iron-fortified products might be necessary to prevent ID. Furthermore, the observed lower prevalence of ID in children visiting preschool/daycare compared to those who stay at home, suggests that factors such as using meals collectively with other children, structural moments when meals are used and more time and attention for eating might have a favorable effect on dietary iron intake (chapter 4). More studies are required to investigate whether the dietary intake and habits in children visiting daycare differ from those who stay at home.

**Children with a chronic inflammatory disease**

In chapter 10 we showed that in contrast to adult CF patients, ID in our population of young children with CF could be solely attributed to an absolute ID (chapter 10). We suggest that similar to healthy children, an insufficient dietary iron intake is the most likely cause for ID in this population. However, since we did not measure sputum iron content we cannot exclude sputum iron loss as a possible cause of ID, as has been suggested in adolescent and adult CF patients\(^{113,114}\). More insight in the underlying mechanism of ID in CF patients and the association between PA colonization, serum and sputum iron is warranted in order to develop effective preventive and therapeutic strategies. In general, data on prevalence and causes of ID in children with other chronic inflammatory disease are scarce. More studies are required to increase the insight in the disease specific pathophysiology of ID in children with a chronic inflammatory disease, such as rheumatoid arthritis and diabetes mellitus.

In conclusion, results of the studies described in this thesis showed that ID is common in late-preterm infants, healthy young children and children with cystic fibrosis. Since negative neurodevelopmental effects may be irreversible despite iron treatment, prevention of ID is important to achieve optimal development. For preterm infants, delayed cord clamping, limiting the amount of iatrogenic blood loss, and iron supplementation are necessary to reduce the risk of ID. Furthermore, the efficacy and safety of individualized iron supplementation as an alternative to general supplementation needs to be further investigated. We suggest that preventive strategies for healthy young children should focus on improvement of the dietary composition, with special attention for the stimulation of foods with a more favorable iron bioavailability and limitation of the amount of cows’ milk products. In order to develop preventive strategies in children with a chronic inflammatory disease, more studies are required to
increase the insight in the disease specific pathophysiology of ID. Subsequently, the effectiveness of preventive strategies should be measured in long-term follow-up studies, with special attention for neurodevelopmental outcome.

REFERENCE LIST


