Iron and vitamin D deficiency in children living in Western-Europe
Akkermans, M.D.

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General discussion and future perspectives
1. INTRODUCTION

Iron and vitamin D deficiency are two of the most common micronutrient deficiencies in young children worldwide.\(^1\)\(^2\) Both micronutrient deficiencies are caused largely by dietary deficiency due to poverty, the lack of access to a variety of food products, the lack of knowledge of appropriate dietary practices and a high incidence of infectious diseases in developing countries, and they contribute substantially to the global burden of disease due to their varied negative health consequences.\(^1\)

In Europe, deficiencies in micronutrients are mostly related to the quality of the diet but not the quantity of food consumed.\(^3\) The current lifestyle of most European children with poor nutritional habits (i.a. consumption of highly-processed energy-dense but micronutrient-poor food products), moderate exercise and limited sun exposure (because they spent relatively much time indoors) contributes to the persistence of iron and vitamin D deficiency in resource-rich areas such as Western-Europe. To what extent the iron and vitamin D status of these children is affected, and how to improve this status, was mostly unknown.

The aim of this thesis was to investigate the iron and vitamin D status of children living in Western-Europe. We focused on three aspects of iron and vitamin D status: diagnostic tests for assessing iron status (part I), epidemiological aspects of iron and vitamin D deficiency in young children and different groups of pediatric patients at risk for deficiency (part II), and finally the effect of a micronutrient-fortified young child formula (YCF) as a strategy to prevent iron and vitamin D deficiency in young children living in Western-Europe (part III). In this chapter, the results of our findings are discussed and recommendations for future studies are given.
2. DIAGNOSTIC TESTS FOR ASSESSING IRON STATUS (PART I)

Iron deficiency (ID) exists in two forms: absolute ID (depleted iron stores due to increased demands, insufficient dietary intake, and malabsorption and/or chronic blood loss) and functional ID (iron-restricted erythropoiesis due to chronic infections/inflammation leading to increased hepcidin production that limits iron transfer from enterocytes and macrophages into the systemic circulation).\(^4,5\) Both types of ID can lead to anemia (iron deficiency anemia (IDA) and anemia of chronic disease (ACD), respectively) and are associated with impaired neurocognitive development of infants and young children\(^6-40\) and an impaired immune response\(^2,41\).

In contrast to absolute ID, functional ID should not be treated with iron replacement therapy because iron stores can be adequate whereas iron replacement therapy in children with sufficient iron stores can lead to infections and/or increased disease activity.\(^42,43\) Therefore, it is highly important to adequately assess the iron status of children and to detect those children with absolute ID that should receive iron replacement therapy to prevent neurocognitive deficits. At the same time, clinicians should also be aware of the presence of functional ID as a warning sign of ongoing chronic infection(s)/inflammation or disease activity that can lead to ACD.

There are several iron status biomarkers and each of them represents a different aspect of iron-homeostasis. There is therefore no single standard test to assess ID or to differentiate between absolute and functional ID. Based on the studies described in this thesis, we propose the following sequence for assessing iron status: one should first investigate the presence of absolute ID and its severity, and subsequently, in the case of adequate iron stores, one should investigate the presence of functional ID and its severity. This sequence is illustrated in Figure 1 and will be explained in more detail in the following paragraphs.
Figure 1 Proposed sequence for assessing iron status

1. **Step 1**: Assess the presence of functional ID (on the right side).
   - **No absolute ID**: Assess the presence of functional ID (on the right side).
   - **Serum ferritin**: <5 yr: <12 µg/l ≥5 yr: <15 µg/l
     - **Iron-restricted erythropoiesis?**
       - **Yes**: RDW↑ (depending on analyzer) ZnPP/H↑: <5 yr: >61 µmol/mol heme ≥5 yr: >70 µmol/mol heme
       - **No**: Anemia? Hb ↓ according to WHO criteria
         - **Yes** Iron-depleted erythropoiesis?
           - **Yes**: Absolute ID with iron-depleted erythropoiesis
             - **No**: Absolute ID
               - **No**: IDA
               - **Yes**: Functional ID
       - **No**: Adequate iron status
         - **Yes**: ACD
         - **No**: Functional ID

2. **Step 2**: Assess the presence of iron-depleted erythropoiesis.
   - **RDW↑ (depending on analyzer)** ZnPP/H↑: <5 yr: >61 µmol/mol heme ≥5 yr: >70 µmol/mol heme
     - **Yes**: Anemia? Hb ↓ according to WHO criteria
       - **Yes**: Absolute ID
         - **Yes**: IDA
         - **No**: Functional ID
       - **No**: ACD
     - **No**: Adequate iron status
         - **Yes**: Functional ID
         - **No**: Absolute ID

3. **Step 3**: Assess the presence of absolute ID.
   - **No absolute ID**: Assess the presence of functional ID (on the right side).
   - **Serum ferritin**: ≤5 yr: <12 µg/l ≥5 yr: <15 µg/l
     - **Yes**: Iron-restricted erythropoiesis?
       - **Yes**: RDW↑ (depending on analyzer) ZnPP/H↑: <5 yr: >61 µmol/mol heme ≥5 yr: >70 µmol/mol heme
         - **Yes**: Anemia? Hb ↓ according to WHO criteria
           - **Yes**: Absolute ID
             - **Yes**: IDA
             - **No**: Functional ID
           - **No**: ACD
         - **No**: Adequate iron status
           - **Yes**: Functional ID
           - **No**: Absolute ID
     - **No**: Adequate iron status
         - **Yes**: Functional ID
         - **No**: Absolute ID

**Absolute ID**

**Serum ferritin as indicator of absolute ID**

The first step of assessing iron status is the investigation of the magnitude of iron stores (Figure 1). Low iron stores indicate the presence of absolute ID. An invasive bone marrow aspiration that can directly reflect body iron stores is not feasible for children. Therefore, the measurement of serum ferritin (SF), an indirect estimate of body iron stores, is recommended by the World Health Organization (WHO). If the concentration of another acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable indicator of iron status. However, the WHO advises the concurrent measurement of another acute phase protein to help with the interpretation of SF levels (Figure 1). If the concentration of the additional acute phase protein is higher than the normal threshold, SF should not be considered as a reliable
biomarker reflecting iron stores. In summary, in case of low infection biomarkers (e.g. C-reactive protein (CRP) <10 mg/l) and an adequate SF level (12 µg/l or 15 µg/l for children <5 years and ≥5 years of age, respectively), the presence of absolute ID is ruled out (Figure 1).²

Detecting or excluding absolute ID in children with an acute infection/inflammation is difficult because there are currently no reliable alternative iron status biomarkers that adequately quantify body iron stores in the presence of an acute infection/inflammation. Several “adjustments’’ for SF in patients with an infection and/or inflammation have been proposed and tested in the last decennia but the results are contradicting. For example, in 1985, Witte et al. developed a nomogram describing the relationship between SF and CRP or erythrocyte sedimentation rate (ESR) (another biomarker for infection/inflammation) in an attempt to develop adjusted cut-off levels for SF for certain degrees of infection/inflammation to detect or exclude absolute ID (based on the absence of bone marrow iron stores) in patients with anemia and concurrent infection/inflammation. The authors concluded that a nomogram of SF and CRP or ESR is useful for the confirmation or exclusion of absolute ID in anemic patients with concurrent infection/inflammation.⁴⁴-⁴⁶ In contrast, the later use of this nomogram of Witte et al. by Coenen et al. and a newly developed nomogram by Coenen et al. showed poor performance in adequately detecting or excluding absolute ID in anemic patients with concurrent infection/inflammation.⁴⁷ Besides these contradicting results, the demographic characteristics including age and possible medical condition or the medical history of the investigated patients were not consistently reported thus hampering the extrapolation of these nomograms to children in general and more specifically to certain pediatric patient groups.

A more recent systematic review also reports on the use of higher cut-off levels for SF to a maximum of 200 µg/l for defining absolute ID in both adult and pediatric patients with infection/inflammation.⁴⁸ However, these higher cut-off levels are not evidence-based for the general pediatric population, particularly not in children from a country with a low infection pressure, and they should therefore not be used in the general pediatric population in Western-Europe. Based on the limitations of the aforementioned studies, we do not recommend the use of adjusted values for SF to evaluate absolute ID in children with infection/inflammation living in Western-Europe. As a result, in the studies described in this thesis, we excluded children with an elevated infection biomarker. In theory, this could bias our results (i.e. underestimate ID prevalence) since iron-deficient children are more prone to develop an infection.²,⁴¹,⁴⁹
RDW and ZnPP/H as indicators of absolute ID (iron-depleted erythropoiesis)

Red blood cell distribution width (RDW) and zinc protoporphyrin/heme ratio (ZnPP/H) are two iron status biomarkers reported not to be influenced by infection\textsuperscript{50-52} and could therefore maybe be used as reliable alternatives for SF to indicate absolute ID in children with infections although limited evidence exist for their use in children. RDW reflects the degree of heterogeneity of erythrocyte volume. In case of inadequate iron supply, erythrocytes become smaller and show a larger variation in size and subsequently a higher RDW level. Furthermore, ZnPP/H also increases in periods of less iron availability because zinc replaces the missing iron during formation of the protoporphyrin IX ring in heme. In this thesis, we investigated the diagnostic value of RDW and ZnPP/H to assess iron status in healthy young children (chapter 2), in children with cystic fibrosis (CF) (chapter 3) and in moderately preterm infants (chapter 4).

In chapter 2 of this thesis, we describe the diagnostic capacity of RDW to detect absolute ID and IDA in healthy children aged 0.5-3 years living in the Netherlands. We investigated the presence of absolute ID and IDA in children without an elevated CRP level while using recommended cut-off levels of the WHO for SF and Hb. The results of this study show that RDW can be used in anemic children to discriminate between absolute ID and other causes of anemia, but that it is less useful as predictor of absolute ID (or IDA). This can be explained by the relatively mild absolute ID in the investigated children and a low prevalence of IDA. Severe and long-standing absolute ID affecting the erythropoiesis possibly leading to IDA gives the strongest association between absolute ID and RDW\textsuperscript{53-55} but this is uncommon in the Netherlands. Furthermore, we did not observe a correlation between RDW and CRP in these children (investigated in all children without excluding those with an elevated CRP level) suggesting no influence of an infection on RDW values in otherwise healthy children aged 0.5-3 years living in the Netherlands (chapter 2).

CF, a chronic multi-organ disease characterized by a defective chloride channel, is frequently accompanied by an impaired iron-homeostasis\textsuperscript{56} and its early detection and subsequent treatment is highly important to prevent suppurative lung disease\textsuperscript{57}. Since it has been reported that RDW is not influenced by infection/inflammation (chapter 2) we questioned whether RDW would be useful in detecting absolute ID in pediatric CF patients who are known with frequent respiratory tract infections\textsuperscript{58}. Therefore, in chapter 3 of this thesis, we investigated the diagnostic capacity of RDW to detect absolute ID in a cohort of 53 Dutch children with CF. Absolute ID was defined according to WHO criteria (i.e. a low SF level in patients with a low CRP level).
We observed a positive association between RDW and absolute ID in our pediatric CF patients, but we were not able to calculate an RDW cut-off level indicating absolute ID with useful test statistics (chapter 3). The observed specificity in our pediatric CF patients was much lower (39.4%) (chapter 3) than in the previously mentioned group of healthy young children (64.7%) (chapter 2). This is likely caused by our relatively small sample size of pediatric CF patients (in particular after excluding those with an elevated CRP level). Furthermore, the observed low diagnostic capacity of RDW for absolute ID can also partially be explained by other micronutrient deficiencies such as folate and vitamin B12 deficiency because these deficiencies can also increase RDW values. RDW is therefore, particularly in children with multiple micronutrient deficiencies such as has been reported in pediatric CF patients, not a specific biomarker for absolute ID.

In contrast to the observed non-influence of CRP on RDW values in the aforementioned group of healthy young children (chapter 2), we did observe a positive correlation between RDW and CRP in our pediatric CF patients that originated from the ‘stable’ patients (no recent pulmonary exacerbation requiring antibiotic treatment) (chapter 3). In conclusion, acute infections in otherwise healthy children or acute exacerbations in pediatric CF patients apparently do not affect RDW values, but chronic infections/inflammation seem to lead to iron-restricted erythropoiesis due to increased hepcidin production in ‘stable’ CF patients. It has to be investigated in larger studies, including the concurrent measurement of hepcidin concentrations, whether increased RDW levels in ‘stable’ CF patients are more associated with functional ID (iron-restricted erythropoiesis) than with severe absolute ID (iron-depleted erythropoiesis).

In summary, RDW is a biomarker that reflects the amount of iron available for the erythropoiesis. RDW values increase when severe absolute ID is affecting the erythropoiesis. Furthermore, chronic infections/inflammation leading to iron-restricted erythropoiesis in children with a chronic condition such as CF and other micronutrient deficiencies such as folate and B12 deficiency can also increase RDW values. Therefore, the assessment of the presence of primarily absolute ID should not be based on the measurement of solely RDW. In our proposed sequence for assessing iron status (Figure 1), we therefore advise to use RDW as a biomarker for severe absolute ID (iron-depleted erythropoiesis) and, in the case of adequate iron stores, a biomarker for functional ID (iron-restricted erythropoiesis). An important note here is that well established reference values in healthy pediatric populations are lacking. Moreover, one should realize that RDW values vary between analyzers depending the technology used for red cell
analysis. Our calculated cut-off levels for RDW in chapter 2 (young children) and chapter 3 (pediatric CF patients) can therefore not be extrapolated to hospitals using different analyzers.

In chapter 4 of this thesis, we describe the natural course of ZnPP/H during the first four months of life in a cohort of Dutch moderately preterm infants who did not receive an erythrocyte transfusion or iron supplementation. The rationale for this study was that the WHO has established only general cut-off levels for ZnPP/H indicating ID in children which we used in this thesis (61 µmol/mol heme or 70 µmol/mol heme for children <5 year and ≥5 years of age, respectively).\(^2\)

The natural course of ZnPP/H during physiological changes in the erythropoiesis and Hb production, for example, in moderately preterm infants, has not been described before thus hampering its use in the diagnosis of ID in these infants. The results of our study show that moderately preterm infants diagnosed with IDA at four months of age (based on Hb and SF in infants with a low CRP level) have higher ZnPP/H levels than infants with an adequate iron status at this age, but we did not observe a difference in ZnPP/H levels between infants with absolute ID and those without (chapter 4). This suggests that an increase in ZnPP/H levels reflects a “deficit” of iron for the erythropoiesis, independent of the iron stores. Future studies should aim to determine reliable cut-off levels for ZnPP/H indicating an impaired erythropoiesis in moderately preterm infants.

In summary, ZnPP/H is another biomarker that reflects the amount of iron available for the erythropoiesis. ZnPP/H is associated with the erythropoiesis and not specific with body iron stores. Therefore, the assessment of the presence of primarily absolute ID should not be based on the measurement of solely ZnPP/H. In our proposed sequence for assessing iron status (Figure 1), we therefore advise to use ZnPP/H as a biomarker for iron-depleted erythropoiesis (severe absolute ID) and, in the case of adequate iron stores, a biomarker for iron-restricted erythropoiesis (functional ID).

In conclusion, elevated RDW and ZnPP/H levels reflect an impaired erythropoiesis, possibly due to severe absolute ID (iron-depleted erythropoiesis) or functional ID (iron-restricted erythropoiesis). In the studies described in this thesis, we did not compare both biomarkers. To the best of our knowledge, studies comparing RDW and ZnPP/H in humans are non-existing. This lack of evidence for superiority of either biomarker hampers the recommendation of the use of one of them over the other. In general, both biomarkers are easily available in most hospitals and can be analyzed in an ethylenediaminetetraacetic acid tube that is frequently
drawn during assessment of iron status to investigate the presence of anemia. The measurement of either biomarker does not require extra blood. We believe that the recommendation for a biomarker should be based on the local laboratory that is present in the hospital since this determines whether RDW is automatically reported during analysis of a complete blood cell count. On the other hand, based on theoretical aspects, we can suggest that ZnPP/H has certain advantages over RDW. For example, ZnPP/H increases only in case of less iron availability for the erythropoiesis or rare circumstances such as lead poisoning that is rare in Dutch children. In contrast, RDW reflects heterogeneity of erythrocyte volume and elevated RDW levels can therefore be caused by several micronutrient deficiencies and not just ID. Elevated ZnPP/H levels are probably more specific for ID than RDW but future studies are necessary to confirm this. Finally, using ZnPP/H instead of RDW will also eliminate the analyzer-dependent results of RDW.

Functional ID

Serum ferritin not a reliable indicator of functional ID (iron-restricted erythropoiesis)
In functional ID, SF levels can be normal or increased as the result of both increased storage and retention of iron in the reticulo-endothelial system (due to hepcidin), and due to the activation of the immune system. SF is therefore not a reliable and specific biomarker for functional ID. Just like for absolute ID, some studies report on the use of higher cut-off levels for SF varying from 30 µg/l to 800 µg/l for diagnosing functional ID in patients with cancer and/or other inflammatory conditions. However, there is no hard evidence for these cut-off levels and we therefore do not recommend their use. Finally, and as mentioned before, SF reflects body iron stores and not an impaired erythropoiesis.

Hepcidin as indicator of functional ID (iron-restricted erythropoiesis)
In case of adequate iron stores (no absolute ID in children without acute infections/inflammation), the presence of functional ID can be investigated by measuring hepcidin levels and/or measuring iron status biomarkers representing iron availability for the erythropoiesis. It is essential to have ruled out absolute ID first, since, as explained before, severe absolute ID can also cause elevated biomarkers representing iron availability for the erythropoiesis.
Hepcidin assays are expensive and available only in research settings whereas standardization ranges for children are also scarce due to limited data on hepcidin levels in healthy children. In contrast, in non-healthy children, hepcidin levels have been reported in preterm\textsuperscript{62} and very low birth weight infants\textsuperscript{63}, and in children with an impaired iron status including anemia\textsuperscript{64-68}, Kawasaki Disease\textsuperscript{69}, obesity\textsuperscript{70-74}, CF\textsuperscript{75}, inflammatory bowel disease (IBD)\textsuperscript{76}, solid tumors\textsuperscript{76} and infections such as Helicobacter Pylori\textsuperscript{77-79} and malaria\textsuperscript{80}.

The following applications of hepcidin have been proposed in children: differentiating between IDA (low hepcidin) and ACD (high hepcidin) in anemic pediatric patients\textsuperscript{64,81}, hepcidin-guided iron replacement therapy, and screening for primary defects in hepcidin regulation\textsuperscript{82}. However, specific cut-off levels for hepcidin that should be used in the aforementioned applications are currently unknown because reference ranges for children are scarce. Normal hepcidin levels in healthy children have only been reported by Uijterschout et al. in Dutch children aged 0.5-3 years\textsuperscript{83} and by Sdogou et al. in Greek children aged 2-12 years\textsuperscript{84}. In the first study, hepcidin-25 concentrations were measured using Weak Cation eXchange Time of Flight mass-spectrometry and a commercial enzyme-linked immunoassay (ELISA). In the second study, hepcidin-25 concentrations were also measured using a commercial ELISA, but from a different manufacturer than in the first study. The different existing assay technologies to assess hepcidin concentrations are not traceable to reference materials and/or reference measurement procedures because these simply currently do not exist. Based on the aforementioned gaps in the literature, we currently do not recommend the measurement of hepcidin as a biomarker for functional ID in children. Before we can recommend measuring hepcidin levels during the assessment of iron status of children, new studies in, mainly healthy, children should be performed to generate reference intervals for hepcidin.

**RDW and ZnPP/H as indicators of functional ID (iron-restricted erythropoiesis)**

In this thesis, we chose RDW and ZnPP/H as biomarkers representing iron availability for the erythropoiesis and hereby indicating the possible presence of functional ID (Figure 1). Both biomarkers are relatively inexpensive and easily available. However, and as mentioned before, future studies are necessary for determining reliable cut-off levels for both biomarkers indicating an impaired iron-homeostasis in different pediatric patient groups and while taking the influence of different analyzers into account on the creation of RDW values. Subsequently, we should investigate whether elevated RDW and/or ZnPP/H
levels occur at the same time as elevated hepcidin levels since this has not been investigated previously, as to the best of our knowledge.

**Hemoglobin levels indicating anemia**

The most severe form of absolute ID and functional ID are IDA and ACD, respectively. The diagnosis of IDA and ACD is based on the combination of the presence of anemia and absolute ID and functional ID, respectively. Investigating their presence is the last step of assessing iron status (Figure 1). Anemia is represented by a low Hb level. The WHO has established cut-off levels for Hb (2 standard deviations below the mean of similarly aged children) indicating anemia that depend on the gender, age and pregnancy status of patients. Furthermore, the WHO sub classifies the severity of anemia as mild, moderate or severe with decreasing Hb levels. In the studies described in this thesis, we used the Hb cut-off levels for ‘non-anemia’ confirming or excluding anemia in general. We did not specify the seriousness of the anemia in our anemic patients because the prevalence of severe anemia is rare in the Netherlands.

**Important notes for assessing iron status: fluctuations in iron status and the possible combination of absolute and functional ID**

It is important to realize that the concurrent measurement of the aforementioned biomarkers (SF, CRP, Hb, RDW and/or ZnPP/H) reflects only a ‘snapshot’ of the current iron status of a child. For example, adequate SF levels and elevated RDW and/or ZnPP/H levels representing decreased iron availability for the erythropoiesis can indicate two situations: the iron stores of a patient with previous severe absolute ID have been restored but the erythropoiesis still needs to normalize or the patients has functional ID. Furthermore, a patient with both low SF levels and elevated RDW and/or ZnPP/H levels can also indicate two situations: a patient has severe absolute ID and subsequent iron-depleted erythropoiesis or the patient has both absolute and functional ID (depleted iron stores and iron-restricted erythropoiesis). This ‘dilemma’ is illustrated in chapter 7 of this thesis.

The study described in this chapter focused on the iron status of Dutch children with IBD. These patients frequently visit the outpatient clinic for close follow-up and a venous blood sample to monitor disease activity, iron status and the presence of anemia. For the purpose of our study, we followed the aforementioned sequence for assessing iron status (Figure 1). The results of this study show that all our
pediatric IBD patients with low SF levels also had elevated biomarkers representing decreased iron availability for the erythropoiesis (RDW and/or ZnPP/H). Following the pathogenesis of IBD, intestinal blood loss and increased inflammation both due to disease activity can lead to absolute and functional ID, respectively. The cross-sectional design of the study limits the complete interpretation of the iron status of these pediatric IBD patients. Future studies should therefore include repeated measurements of iron status biomarkers in children with IBD and aim to clarify the association between disease activity and iron status.

**Relevance of assessing iron status beyond the classical benefits**

The previously mentioned negative health consequences of ID in children and, furthermore, the therapeutic consequences of a deprived iron status emphasize the importance of adequately assessing iron status in children. Moreover, one should also keep in mind the possible influence of ID and anemia on Hb-related biomarkers such as hemoglobin A1c (HbA1c), obviously important in children with diabetes mellitus (DM). In non-DM and DM adults, contradicting results regarding the influence of iron status on HbA1c levels varying from a negative association (i.e. a deprived iron status leads to a higher HbA1c level) to no influence have been reported. In pediatric DM type 1 patients, the possible association between iron status and HbA1c levels has not been extensively investigated. Therefore, in chapter 8 of this thesis, we determined the prevalence of absolute and functional ID and their association with HbA1c levels in a relatively large sample of children with DM type 1 living in the Netherlands. In this study, we used the previously mentioned sequence for assessing iron status (Figure 1) in children without an elevated high-sensitive CRP (hsCRP). The results of this study show no differences in HbA1c levels between patients with and without a deprived iron status (absolute or functional ID, with or without anemia) (chapter 8). In contrast, two previous studies concerning smaller samples of pediatric DM type 1 patients report higher HbA1c levels in patients with IDA. The difference between our study and the two aforementioned studies can partially be explained by the relatively mildly deprived iron status of our patients as reflected by the low prevalence of anemia. Furthermore, the authors of the two previous studies could have also made a statistical type 1 error (rejecting the H0 hypothesis of no influence of iron status on HbA1c levels) because of their small number of patients. We believe a new study with a comparable design as in our study should be performed to assess whether there is a true influence of iron status on HbA1c levels in pediatric DM type 1 patients, preferably in a population with more severe anemia than in our Dutch population.
The exact mechanism through which ID and anemia can affect HbA1c levels is unclear although several theories have been proposed. Firstly, it has been hypothesized that ID causes an alteration of the quaternary structure of the Hb molecule that facilitates the glycation of the β-globin chain. Secondly, it has been hypothesized that ID leads to prolongation of erythrocyte survival leading to elevated levels of HbA1c. Subsequently, it is speculated that the emergence of young erythrocytes in the systemic circulation with relatively less glycation of Hb after iron therapy could lead to dilution and lowering of the concentration of previously formed glycated Hb. Moreover, the possible association between a deprived iron status and higher HbA1c levels could also be explained by reversed causality: poor eating patients with DM type 1 might also take less care of their diabetes and therefore have both absolute ID and a high HbA1c level. In our study, we did not find an association between dietary iron intake and the presence of absolute ID (chapter 8). This might be due to an overall adequate iron intake as a result of intensive diabetic care with frequent and regular contacts with a dietician. On the other hand, the questionnaire that we used might not have been detailed enough to adequately assess iron intake. Future studies should include a larger sample of pediatric DM type 1 patients because of the low prevalence of absolute ID and anemia in our study sample from the Netherlands. Furthermore, a detailed and validated food questionnaire should be used in attempt to elucidate the aforementioned hypothesis regarding the possible association between iron status and HbA1c levels.

3. EPIDEMIOLOGICAL ASPECTS OF IRON AND VITAMIN D DEFICIENCY IN YOUNG CHILDREN AND DIFFERENT GROUPS OF PEDIATRIC PATIENTS AT RISK FOR DEFICIENCY (PART II)

Iron status and iron deficiency
Up to now, few studies have adequately investigated the epidemiology of ID in children in Europe while differentiating between absolute and functional ID. The prevalence of absolute ID in healthy European children varies from 0.0% to 85.0% depending on the investigated population (different country, age, gender, nutritional habits, etc.) and the definition used (different cut-off levels for SF). The prevalence of IDA in these children varies from 0.0% to 42.0% also depending on the investigated population and definitions used. Functional ID might be more prevalent among children with chronic diseases than healthy children but this has scarcely been studied in Europe. In this thesis,
we investigated the iron status of 4 pediatric populations living in Western-Europe while using international guidelines for assessing iron status and, when possible, differentiating between absolute and functional ID by means of the previously mentioned sequence for assessing iron status as shown in Figure 1. We studied the following populations that will be discussed below: Dutch moderately preterm infants, healthy young children aged 1-3 years living in Western-Europe, Dutch children with IBD and Dutch children with DM type 1.

**Dutch moderately preterm infants**

International recommendations regarding standardized iron supplementation in all preterm infants to prevent absolute ID are mostly based on studies performed in very low birth weight infants (birth weight <1500 gram) and/or infants born <32 weeks of gestation. It is questionable whether these recommendations are applicable to Dutch moderately preterm infants (born ≥32 weeks of gestation). Uijterschout et al. has demonstrated that only 18.9% and 4.9% of these infants develops absolute ID at the age of 4 and 6 months, respectively. Since iron supplementation might be harmful in iron-sufficient infants individualized iron supplementation based on early identification of risk factors leading to absolute ID in moderately preterm infants has been suggested. However, early and specific risk factors for absolute ID in these infants that could be used in an algorithm to guide supplementation have not been reported before.

In chapter 5 of this thesis, we analyzed the iron status of and risk factors for absolute ID at the postnatal age of 6 weeks in a subset of preterm infants that participated in a larger study. The included infants were born between 32 and 35 weeks of gestation in a setting of early cord clamping and without standardized iron supplementation. Absolute ID (based on a low SF level in infants without an elevated CRP) and IDA at 6 weeks occurred in 38.2% and 30.9% of the infants, respectively. To our knowledge, no other studies have investigated the prevalence of absolute ID in these infants at 6 weeks which hampers comparison of study results. An important note here is that the obstetricians did not perform delayed cord clamping during our study (nowadays the standard procedure) which might have led to an overestimation of the prevalence of absolute ID in our infants.

Furthermore, we also showed that a lower birth weight, a lower SF concentration in the first week of life and multiple blood draws during admission were all independently associated with absolute ID at 6 weeks in our infants. More specific, we showed that infants with a birth weight <1830 gram and a SF in the first week <155 µg/l had a 26.4 times higher risk to develop absolute ID (chapter 5).
Future studies are necessary to elucidate whether these findings will still stand when delayed cord clamping is performed at birth.

We did not explicitly investigate the presence of functional ID in our Dutch moderately preterm infants. The immune system of preterm infants is immature predisposing them to serious immune-related conditions like sepsis, meningitis, necrotizing enterocolitis, etc. In theory, these conditions can cause an inflammatory response leading to an increased expression of hepcidin and subsequently functional ID. However, it has been documented that preterm infants have significantly reduced pro-inflammatory cytokine responses (i.a. IL-6) when stimulated with whole micro-organisms in vitro. Moderately preterm infants probably have a more developed immune system than extreme preterm infants and are therefore probably more capable producing cytokines leading to elevated hepcidin concentrations. However, it is currently unknown to what extend moderately preterm infants can develop functional ID during episodes of severe illness.

**Healthy young children aged 1-3 years living in Western-Europe**

The first three years of life are characterized by very rapid growth and development requiring sufficient and high-quality nutrients. In this critical phase, food preferences are formed which carry over into childhood and beyond. It is thought that the maturation of organs is adapted to the nutritional environment in this critical period of development. An excess of energy, imbalances in macronutrient quality, and nutritional deficiencies such as ID may form inappropriate nutritional signals, leading to, for example, metabolic disturbances or the development of obesity. This hypothesis explains why there is an increasingly interest in the nutritional intake and status of infants and young children with regards to future health.

As previously mentioned, the prevalence of absolute ID and IDA in European children varies enormously. One can expect that a low dietary iron intake is an important factor that contributes to the development of absolute ID in these children. However, studies reporting on absolute ID and IDA prevalence did not always provide detailed information on iron intake. Therefore, in chapter 6 of this thesis, we investigated the prevalence of absolute ID (defined by WHO criteria) and IDA and their risk factors including iron intake in healthy young children living in Western-Europe.
Our study population consisted of 325 predominantly Caucasian children that were recruited in Germany, the Netherlands and the United Kingdom. Approximately 50% of the children received primarily formula, whereas a smaller proportion used primarily cow’s milk (CM). Only 1.5% used supplements containing iron (chapter 6). In accordance with other European studies, the average dietary iron intake of the children was found to be lower than the recommended value of 8 mg/day. Furthermore, absolute ID and IDA were present in 11.8% and 3.9% of the children, respectively. Demographic and socioeconomic characteristics did not influence these prevalence rates that could partially be explained by the homogeneity of our study population. In contrast, the nutritional characteristics of the children with and without absolute ID differed on several aspects (chapter 6). Firstly, in accordance with other European studies among young children, the use of CM was associated with a higher prevalence of absolute ID. This could be attributed to the low iron content of CM and its calcium content that inhibits non-heme iron absorption. Furthermore, surprisingly, univariate analyses showed a higher iron intake from food in the children with absolute ID than in those with an adequate iron status, although this association was not seen in our multivariate analyses (chapter 6). We can hypothesize that the composition of the diet of the children with absolute ID may be less in favor of sufficient iron absorption than that of children with an adequate iron status but, unfortunately, we do not have these details. Based on all the aforementioned results we can conclude that many young West-European children do not reach the current recommended dietary iron intake and that the use of CM but also a non-balanced diet (composition not in favor of sufficient iron absorption) can cause absolute ID in these children. The focus on healthy eating habits in young West-European children should be sharpened because balanced nutritional intake in early life may have significant health benefits later on.

Dutch children with Inflammatory Bowel Disease

Anemia is a common extra-intestinal manifestation of IBD (Crohn’s disease (CD), ulcerative colitis (UC) and IBD-unclassified (IBD-U). Studies in pediatric IBD patients report prevalence rates varying from 17% to 78%, depending on the definition of anemia used and the patient population studied. Anemia in these patients may be caused by vitamin B12 deficiency, folate deficiency, or medication (e.g. aminosalicylates, thiopurines). However, the most common cause of anemia in IBD patients is ID, either by a low dietary iron intake due to food aversion and/or severe blood loss (leading to IDA) or chronic inflammation (leading to ACD). Some studies suggest that ID in general is responsible for up to 42% to 88% of the cases of anemia in children with IBD.
However, data on the prevalence of absolute and functional ID in children with IBD are scarce although adequate assessment of iron status and an appropriate indication for iron therapy is highly important in IBD patients. Iron replacement therapy in IBD patients with functional ID and ACD should be avoided since iron can potentially increase intestinal inflammation.\textsuperscript{121,122}

The study described in chapter 7 of this thesis investigated the prevalence of and possible risk factors for absolute and functional ID (with and without anemia) in Dutch pediatric patients with IBD. We investigated the iron status of 59 patients during a regular follow-up visit at our outpatient clinic. We used the flowchart that is shown in Figure 1 to assess the presence of absolute and functional ID. Both absolute and functional ID were highly prevalent in our study population (32.2\% and 80.0\%, respectively) (chapter 7). We hypothesized that disease activity would influence iron status in these patients because increased disease activity is associated with insufficient dietary (iron) intake, intestinal malabsorption (of iron) and chronic blood (hence iron) loss from the gastrointestinal tract.\textsuperscript{123,124} This contributes to the development of absolute ID and subsequently IDA. Moreover, increased disease activity could also lead to the production of inflammatory mediators which may result in the development of functional ID due to increased expression of hepcidin. However, we were not able to confirm these hypotheses in our patients (chapter 7).

The lack of an association between disease activity and iron status in our study is in line with a recently published retrospective study among 90 Swedish children with IBD. In this study, ID (based on SF levels or the transferrin saturation with different cut-off levels for remission and active disease) was present in 85\% of the children in remission (defined as absence of intestinal symptoms and a low CRP or ESR level).\textsuperscript{125} ID seems to be common in every IBD patient; whether a patient is in remission or has an active disease. This can be explained by the limited specificity of the clinical scores (Pediatric Crohn's Disease Activity Index and Pediatric Ulcerative Colitis Activity Index) that we used and the criteria for remission the Swedish research group used to assess disease activity and the overall high prevalence of patients being in remission in both studies. Further studies are needed to determine whether increased disease activity aggravates ID. These studies should be done in a larger population with varying disease activity and subtypes of IBD while using more specific biomarkers of inflammatory activity such as fecal calprotectin, the latter especially in patients with CD.\textsuperscript{126} Finally, future studies should also analyze the iron intake of pediatric IBD patients and investigate whether diminished intake is associated with absolute ID.
**Dutch children with Diabetes Mellitus type 1**

Children with DM type 1 may be at risk to develop functional ID based on their chronic inflammatory state\(^4,^{127}\) although this has not been investigated previously. Furthermore, low dietary iron intakes, as recently observed in Indian patients\(^{128}\), can lead to absolute ID. Moreover, the concurrent presence of celiac disease in these patients can negatively affect iron absorption and subsequently also cause absolute ID. Despite the aforementioned increased risk of a deprived iron status in pediatric DM type 1 patients, studies on this subject are scarce\(^{93,^{129}}\) whereas information on the type of ID in these patients is non-existing. Therefore, in chapter 8 of this thesis, we investigated the iron status of 227 pediatric DM type 1 patients that were recruited in two hospitals in the Southwestern region of the Netherlands. We again used the flowchart that is shown in Figure 1 to assess the presence of absolute and functional ID in these patients.

In contrast to the high prevalence rate of absolute ID observed in our Dutch children with IBD (32.2%) (chapter 7), absolute ID was relatively uncommon in our Dutch children with DM type 1 (5.7%) (chapter 8). This can be explained by the different pathophysiology of both diseases. For example, DM type 1 is not accompanied with gastrointestinal blood loss and impaired intestinal absorption of iron leading to absolute ID.

Functional ID occurred far more frequently than absolute ID in our Dutch pediatric DM type 1 patients (46.7%) (chapter 8). However, only a minority of the DM type 1 patients with functional ID also had detectable anemia resulting in a low prevalence of ACD (3.7%). Despite the low overall prevalence of anemia (IDA prevalence 3.1%) one should realize that both types of ID and anemia can lead to impaired neurodevelopment and this should be prevented in all young growing and developing children, including those with DM type 1. Mojs et. al. investigated the iron status of 100 Polish children with DM type 1 aged 6-17 years during a regular follow-up visit. Furthermore, they used the Wechsler Intelligence Scale for Children to assess the efficiency of different cognitive areas such as intelligence, verbal and executive functions, memory and attention and learning processes of their patients. The results of this study show that, although the scores of cognitive functioning presented by all the children are within the standard range for the age, children with lowered ferric parameters had lower scores in non-verbal subtests than those with normal ferric parameters.\(^{130}\) Besides the possible link between iron and neuro-cognitive development in pediatric DM type 1 patients, pediatric endocrinologists should realize that the presence of functional ID indicates ongoing chronic inflammation, probably induced by frequent and...
elongated episodes of hyperglycemia and subsequently high levels of oxidative stress, that can lead to disease complications (such as diabetic nephropathy, retinopathy and neuropathy) and, in the long-run, to anemia.

**Vitamin D status and vitamin D deficiency**
The epidemiology of VDD in (healthy) children in Europe has recently been summarized in a position paper of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition revealing limited data on vitamin D concentrations and VDD prevalence in these children. Unfortunately, some studies in this review included only small numbers of children. Furthermore, no consistent study design and no consistent definition of VDD have been used limiting adequate comparison of study results. Overall, the prevalence rates of VDD vary from 0% to 95% depending the investigated population (different country, age, season, nutritional habits, etc.) and the definition used (different cut-off levels for 25-hydroxyvitamin D (25(OH)D). Factors contributing to VDD, such as demographic characteristics, dietary factors and sun exposure are explained in more detail in the General Introduction of this thesis (chapter 1). Below, we report on the vitamin D status of healthy young children aged 1-3 years living in Western-Europe.

*Healthy young children aged 1-3 years living in Western-Europe*
In chapter 6 of this thesis, we describe the iron and vitamin D status of a group of healthy predominantly Caucasian young children aged 1-3 years living in Western-Europe. The study consisted of a venous blood sample to determine iron and vitamin D status and a questionnaire to assess risk factors for ID and VDD. VDD, defined as 25(OH)D <50 nmol/l, was present in 22.8% of the children. Subsequently, to adjust for seasonal variation in vitamin D status, we calculated subject-specific mean annual 25(OH)D concentrations following the cosinor model of Sachs et al. This method revealed a comparable prevalence of VDD of 22.5% (chapter 6). Both observed prevalence rates of VDD are in line with previous European studies among young children that report prevalence rates varying from 0% to 64%, although performed in pediatric populations with a wider age range, without taking the influence of the season into account and while using different cut-off levels for 25(OH)D.

In accordance with another study among Dutch young children, the use of a food frequency questionnaire in our children revealed an average dietary vitamin D intake below current recommendations. Furthermore, VDD was associated with the use of CM, but not with socioeconomic characteristics or sun exposure.
(chapter 6). Moreover, in accordance with previous studies\textsuperscript{131,134}, the use of vitamin D supplements (recommended in all three countries but only used by 28.6\% of the participating children) was associated with a lower prevalence of VDD.

In a recent publication of the Generation R study, a large multi-ethnic cohort of children living in the Netherlands followed from pregnancy onward, VDD at the age of 6 years (overall prevalence 30\%) was associated with a non-Western ethnicity, the winter, a lower household income, a higher age, more television watching, less biking to school and less playing outside. Furthermore, in a subgroup with dietary data at a median child age of 12.9 months, the authors did not find an association between VDD (at 6 years) and the use of vitamin D supplements (at 12.9 months).\textsuperscript{135} The differences between our study and the Generation R study can partially be explained by the higher age and the higher proportion of non-Western children in the Generation R study. The lack of an association between vitamin D supplements and VDD in the Generation R study is difficult to interpret because the authors examined whether dietary characteristics in early childhood were related to vitamin D status at the age of 6 years. In contrast, we determined the actual vitamin D intake including the use of supplements at the moment of the blood draw.

To achieve an adequate dietary vitamin D intake and subsequent status to prevent rickets and other negative health consequences of VDD in infants and young children, the government of most European countries recommend the use of vitamin D supplements. The reasons for the low compliance to these guidelines (chapter 6 and \textsuperscript{136,137}) should be elucidated in future studies in order to adjust supplementation policies to improve compliance to these recommendations.

\textbf{Iron and vitamin D status: co-existence of ID and VDD}

In the previously mentioned group of healthy young children aged 1-3 years living in Western-Europe, we also investigated the co-existence of absolute ID and VDD. We observed that a total of 5.9\% of the children had both micronutrient deficiencies (chapter 6). This co-existence of absolute ID and VDD has not been described previously in European children. However, several theories for this phenomenon have been reported in studies that simultaneously analyzed the iron and vitamin D status of Asian (anemic) children (which we explained in chapter 1 of this thesis).\textsuperscript{138-142}

A recent systematic review by Azizi-Soleiman et al. describes 3 observational studies and 7 intervention studies among children and adults that investigated
the development of VDD due to ID. Most studies had limitations such as small sample sizes, inadequate definitions for ID and VDD, possible selection bias and being the result of secondary analyses. However, overall, the studies show a possible association between iron and vitamin D status in humans. On the other hand, the intervention studies did not reveal a statistically significant effect of iron supplementation on the vitamin D status of the study participants. Future studies taking the aforementioned limitations into account should be conducted to elucidate the co-existence of absolute ID and VDD in children. For example, a new larger study among young children may investigate whether combined supplementation of iron and vitamin D reveals a better iron and vitamin D status than single supplementation with iron or vitamin D while using international guidelines for defining absolute ID and VDD (which we used in this thesis).

4. PREVENTION OF IRON AND VITAMIN D DEFICIENCY IN YOUNG CHILDREN LIVING IN WESTERN-EUROPE (PART III)

As previously showed, the iron and vitamin D intake of young children in Western-Europe is below the current recommendations. Subsequently, the prevalence of absolute ID and VDD is rather high in this vulnerable population (chapter 6). There are several ways to improve the micronutrient status of young children. These include an even more strict focus on current nutritional recommendations, attempts to change some of the current dietary habits that do not contribute to an adequate iron and vitamin D intake (e.g. discouraging the consumption of highly-processed energy-dense but micronutrient-poor food products and stimulating a balanced composition of their diet, for example, to enhance iron absorption), consuming fortified food products and drinks and/or taking more or different supplements. In general, the choice will depend on local habits, the cost and accessibility of products, and the quality and quantity of commonly used food products and drinks. Furthermore, one should take into account the individual acceptability of toddlers. Toddlers are known to be difficult eaters and forcing the consumption of certain products such as fruit and vegetables will not always positively contribute to a healthy eating pattern. Repeated dishing up, possibly with a different preparation, complimenting children on eating even a bit, but also parents giving a good example by eating the same dish can stimulate toddlers to eat.
Micronutrient-fortified YCF

The strategy of fortification of frequently consumed food products such as milk, bread and margarine can aid in achieving an adequate intake of certain micronutrients in children but this has not been adequately studied in the European setting. Therefore, in chapter 9 of this thesis, we explored the use and effect of a micronutrient-fortified YCF on the iron and vitamin D status of West-European toddlers. The tested YCF was a commercially available formula that contained, next to iron and vitamin D, several other micronutrients and less calcium and more vitamin C than CM to enhance iron absorption. The results of this intervention show that daily consumption of the before described YCF for 20 weeks preserves the iron status and improves the vitamin D status of West-European toddlers (chapter 9). Subgroup analyses based on type of milk before start of the study, showing a higher increase in SF in original CM users than in original formula users, can explain the preservation of iron status in the total YCF group. Furthermore, we can also state that adding iron to a young child’s diet can counteract the expected decrease in SF over time due to rapid growth requiring the use of stored iron. Moreover, regarding vitamin D status, the use of the aforementioned fortified YCF during 20 weeks effectuated an increase in 25(OH)D concentration with 9 nmol/l and a nearly 50% reduction in VDD prevalence in West-European toddlers. These effects were reached without causing serious adverse events (for example, infections), an affected stool pattern or an impaired growth (chapter 9). In conclusion, the use of a micronutrient-fortified YCF safely and effectively improves the iron and vitamin D status of young children living in Western-Europe and thus reduces the prevalence of absolute ID and VDD in these children.

Additional analyses of vitamin D supplement users (only 30.5% of the included children) and non-users separately suggest a different effect of YCF on VDD prevalence in both groups. In vitamin D supplement users, we did not observe a significant lower odds of having VDD after the intervention. This can be explained by the already lower prevalence of VDD at baseline of these users compared to the VDD prevalence of non-users at baseline. In summary, young children not consuming vitamin D supplements will benefit the most from YCF (chapter 10). In addition, these analyses show that different strategies for different populations are probably best to improve vitamin D status in the whole population.

In the case of iron fortification, one should realize that the absolute amount of iron does not necessarily reflect the fraction absorbed because iron absorption depends on the complete food matrix. Therefore, ensuring that iron in YCF is
delivered in a bioavailable form would improve the nutritional benefits of YCF on iron status. Previous studies have shown that the addition of vitamin C, as in our formula, and prebiotics, to YCF improves iron bioavailability.\textsuperscript{159} Furthermore, it has also been shown that multi-micronutrient fortification, also in our formula, results in more positive effects on biochemical indicators of micronutrient status than single micronutrient fortification.\textsuperscript{160}

**Future recommendations: nutritional advices and new studies**

Most European guidelines state that CM is accepted after the age of 1 year, although we observed that this nutritional habit is associated with a higher prevalence of absolute ID and VDD (chapter 6). YCF, instead of CM, as part of a toddler’s diet, could play a role in ensuring sufficient intake of iron and vitamin D in European children >1 year of age as we showed that its use has favorable effects on iron and vitamin D status in these children (chapter 9). The exact dosage and timing of YCF are unclear but it is likely that approximately 300ml per day (the amount of milk recommended by most European countries for children this age), preferably at least until the age of 2 years, will aid in improving iron and vitamin D intake in toddlers. Furthermore, the concurrent supplementation of vitamin D should also be promoted since no strategy will reach compliance of 100%. This will not result in toxic vitamin D levels as we showed in our study (chapter 9).

In chapter 9 and chapter 10, we report on biochemical changes in iron and vitamin D status but not on functional outcome parameters such as cognitive tests and bone health. The long-term benefits of YCF, for example, on neuro-cognitive development and the immune system remain to be elucidated. Unfortunately, our study was not powered for investigating the long-term benefits of the use of YCF in West-European toddlers. Ideally, a future larger study should focus on functional outcome parameters and also aim to determine in what time frame young children would benefit the most from YCF.

To prevent iron and vitamin D deficiency in West-European toddlers one may also consider the general fortification of regular CM. In the United States of America and Canada the prevalence of VDD is much lower than in Europe, probably due to the practice of general fortification of regular CM with vitamin D.\textsuperscript{161-163} This strategy, however, first needs to be analyzed extensively to determine a safe and effective dosage of fortification for both children and adults. Furthermore, this strategy requires support of the local and European government. Moreover, cost-effectiveness analyses have to proof whether this strategy is suitable for the West-European setting.
In summary, compliance to the current national European guidelines for a healthy diet for toddlers, including the use of a vitamin D supplement, should be improved to prevent ID and VDD and their negative health consequences. Firstly, there should be more awareness about the future health consequences of an insufficient micronutrient intake and subsequent status in young growing and developing children hoping this will stimulate compliance to nutritional recommendations. This awareness should be created both in parents of young children and in health care professionals involved in the care for young children. Furthermore, we should realize that achieving a healthy diet for toddlers remains a huge challenge. Fortified products such as micronutrient-fortified YCF may help but we have to realize that this type of milk is about three times more expensive than regular CM and not every parent may be able to afford this. Government support through general fortification of regular CM should therefore be explored.

5. CONCLUSION

In Western-Europe, ID and VDD are common among moderately preterm infants and healthy young children but also in children with a chronic disease such as IBD and DM type 1. During the assessment of iron status it is important to differentiate between absolute ID (depleted iron stores due to increased demands, insufficient dietary intake, and malabsorption or chronic blood loss) and functional ID (iron-restricted erythropoiesis due to chronic infections/inflammation leading to increased hepcidin production that limits iron transfer from enterocytes and macrophages into the systemic circulation) because of therapeutic consequences. We propose a sequence for assessing iron status (Figure 1) that includes the measurement of SF, CRP, Hb, RDW and/or ZnPP/H. RDW and ZnPP/H are iron status biomarkers that reflect an impaired erythropoiesis either due to severely depleted iron stores or iron-restricted erythropoiesis. Therefore, in case of adequate iron stores, elevated RDW and/or ZnPP/H levels indicate the presence of functional ID. Furthermore, the identification of risk factors for ID and VDD in children can aid in the prevention and timely treatment of both deficiencies that are associated with several negative health consequences. Individualized instead of generalized iron supplementation should be sought to prevent infections in iron-replete children. Moreover, children and their parents should receive good but above all practical nutritional recommendations to stimulate an adequate iron and vitamin D intake. For example, for young children, the use of vitamin D supplements should be emphasized and, furthermore, the use of fortified products such as micronutrient-fortified YCF, but possibly also other...
food products, can be helpful. Preventive strategies and treatment options in children with a chronic condition are more complex because of the current lack of well-established risk factors for ID and diagnostic challenges in the presence of infection/inflammation.

6. SUMMARY OF FUTURE PERSPECTIVES

In this chapter, we stated several ideas for future studies which we will now summarize. Starting with our proposed sequence for assessing iron status in children (Figure 1); this sequence includes the measurement of RDW and/or ZnPP/H as iron status biomarkers reflecting an impaired erythropoiesis either due to severe absolute ID or a functional ID. However, no study has analyzed an association between elevated hepcidin concentrations and elevated RDW and/or ZnPP/H levels indicating functional ID. This association has to be investigated first and confirmed before our sequence should be implemented for assessing iron status in children. A new study, including the concurrent measurement of both RDW, ZnPP/H and hepcidin, should also aim to investigate which biomarker (RDW or ZnPP/H) with corresponding cut-off levels has the best diagnostic capacity for functional ID.

We mentioned several strategies to prevent iron and/or vitamin D deficiency in Dutch moderately preterm infants and in healthy young children living in Western-Europe. Firstly, an individual approach is preferred for these moderately preterm infants since not every infant develops absolute ID and because iron supplementation in iron-replete infants is harmful. The safety and efficacy of an algorithm for individualized iron supplementation in moderately preterm infants based on our investigated early risk factors first has to be investigated, preferably in a randomized controlled trial, while taken into account the current practice of delayed cord clamping. Secondly, it is likely that also for healthy young children living in Western-Europe an individual approach to prevent ID and VDD should be taken. Toddlers not consuming vitamin D supplements will benefit the most from micronutrient-fortified YCF as a strategy to improve their vitamin D status. The fortification of commonly used food products such as milk for European toddlers will increase compliance, compared to the low compliance to vitamin D supplements and, furthermore, it will also increase the dietary intake of other important micronutrients such as iron. These preventive strategies should always be aware of the risk of toxicity. Furthermore, one should keep in mind that most reasons for preventive strategies, like the use of micronutrient-fortified food
products, are studies that reported on biochemical changes in iron and vitamin D status and not functional outcomes like neuro-cognitive development (with ID) and infection risk (with VDD). Future studies should therefore focus on functional outcomes and long-term advantages. This research should also include a cost-effectiveness analysis, especially when exploring the effect of general fortification of regular CM.
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