

Iron status, inflammation and anthropometric nutritional status of four-to-thirteen month-old black infants from a rural South African population

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B.Sc Dietetics

Mini-dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in **Dietetics** at the Potchefstroom campus of the North-West University

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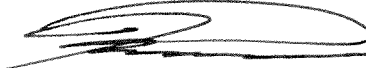
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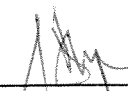
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**“You will keep in perfect peace him whose mind is steadfast,
because he trusts in You. Trust in the LORD forever, for the LORD,
the LORD, is the Rock eternal.”**

Isaiah 26:3 to 4 (NIV)



I would not have been able to complete this mini-dissertation without the peace and strength that comes from trusting in Jesus Christ as Saviour and Living God.

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My beloved husband,

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ABSTRACT

Background

The first 1000 days of life (from conception to two years of age) is a critical period of nutritional vulnerability, affecting lifelong health. Iron deficiency (ID) and iron deficiency anaemia (IDA) are considered major public health problems that adversely affect development and growth, impair immunity, and increase morbidity and mortality in infants. ID and IDA in sub-Saharan Africa can be attributed to poor dietary, socioeconomic and disease conditions. One of the major obstacles in determining the prevalence of ID, using serum ferritin (SF) as marker of iron status, is that it not only reflects the amount of iron that is stored in the body, but also functions as an acute phase reactant that is raised in the presence of infection or inflammation.

Aim

We conducted a re-analysis of the International Research on Infant Supplementation (IRIS) study's baseline data to determine a more accurate estimation of the ID prevalence in apparently healthy four to thirteen-month-old infants from rural KwaZulu-Natal while accounting for the effect of chronic and acute inflammation on SF.

Study design and methods

A cross-sectional analysis was performed on the baseline data (192 infants) of the IRIS study that was conducted in 2000. Infants' haemoglobin (Hb), SF, C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) concentrations were interpreted to determine the prevalence of ID. Literature of the past four years served as a guide to compare the ID prevalence obtained from four methods that account for the influence of inflammation on SF concentrations, to a reference method that does not take inflammation into consideration, and to what was reported in the original IRIS study. Weight and recumbent length measurements were converted to z-scores to interpret subjects' anthropometric nutritional status.

Results

A high prevalence of inflammation (52.6%) was present, with 11.5% of the subjects being in the incubation, 17.2% in the early convalescent, and 24% in the late convalescent phase of inflammation. SF was significantly associated with both CRP ($\beta = 0.200$; $P = 0.005$) and AGP ($\beta = 0.223$; $P = 0.002$) when adjusting for gender and age. The IRIS study reported an ID prevalence of 18.3%, whereas the results of this study ranged from 17.2 to 52.1%. We derived an IDA prevalence that ranged from 12 to 24.5% according to the different methods. The prevalence of stunting [length-for-age Z-score $<-2SD$] was 12.5%; while 25.1% of infants were overweight/obese [weight-for-length z-score $>2SD$].

Conclusion

A double burden of malnutrition was evident from the high prevalence of both overweight and ID, together with inflammation. The disconcertingly large variance in ID prevalence observed between the different methods that were employed highlights that iron supplementation interventions to treat anaemia must be based upon accurate estimates of IDA prevalence, otherwise they pose an increased risk of adverse effects to susceptible, iron-replete, but anaemic infants. Given the detrimental consequences of ID, it is imperative that governments, health care providers and parents must act to prevent or treat ID and IDA among vulnerable infants.

Key Words

Iron deficiency; Inflammation; Serum ferritin; Infants; Iron supplementation

OPSOMMING

Agtergrond

Die eerste 1000 dae van lewe (vanaf bevrugting tot op die ouderdom van twee jaar) is 'n kritieke tydperk wanneer babas besonder kwesbaar is in terme van voeding. Die voeding wat hulle tydens hierdie periode ontvang kan lewenslang hul gesondheid beïnvloed. Beide ystertekort en ystertekortanemie is groot openbare gesondheidsprobleme wat die ontwikkeling en groei van kinders nadelig kan beïnvloed, immuniteit aantas, en morbiditeit sowel as sterftes kan verhoog. Die hoë voorkoms van ystertekort en ystertekortanemie in sub-Sahara Afrika kan meestal toegeskryf word aan swak diëte, lae sosio-ekonomiese omstandighede, en siektetoestande. Serum ferritin (SF) reflekteer nie alleen die hoeveelheid yster wat in die liggaam gestoor word nie, maar tree ook op as akute fase reaktant wat styg in die teenwoordigheid van infeksie of inflammasie. Gevolglik is dit moeilik om die ware voorkoms van ystertekort te bepaal wanneer hierdie merker van ysterstatus op sy eie gebruik word.

Doel

Die doel van hierdie studie was om 'n meer akkurate beraming van die voorkoms van ystertekort, in 192 skynbaar gesonde, 4-13 maande-oue babas, te bepaal deur die effek van beide chroniese en akute inflammasie op SF konsentrasies in ag te neem.

Studie en metodes

Ons het 'n her-analise van die basislyndata van die Suid-Afrikaanse deel van die “International Research on Infant Supplementation” (IRIS) studie gedoen. Die oorspronklike intervensie studie is in 'n landelike gebied in KwaZulu-Natal in die jaar 2000 uitgevoer. Die babas se SF, C-reaktiewe proteïen (CRP), en alfa-1 glikoproteïen (AGP) konsentrasies is geïnterpreteer om die voorkoms van ystertekort te bepaal. Hemoglobien (Hb) konsentrasies is gebruik om die voorkoms van anemie, en die bydrae van ystertekortanemie tot anemie te bepaal.

Literatuur van die afgelope 4 jaar het ons gelei om vier metodes, wat die invloed van inflammasie op SF konsentrasies in ag neem, te vergelyk met 'n verwysingsmetode wat nie inflammasie in ag neem nie. Gewig en lengte metings is omgeskakel na z-tellings om die babas se antropometriese voedingstatus te interpreteer.

Resultate

'n Hoë voorkoms van inflammasie (52.6%) was teenwoordig, waarvan 11.5% van die studiedeelnemers in die inkubasie-, 17.2% in die vroeë herstelfase, en 24.0% in die laat herstelfase van inflammasie was. SF konsentrasies was beduidend met beide CRP ($\beta = 0.200$, $P = 0.005$) en AGP ($\beta = 0.223$, $P = 0.002$) geassosieer wanneer daar vir die effek van geslag en ouderdom aangepas is. Die IRIS studie het 'n ystertekort prevalensie van 18.3% gerapporteer, terwyl ons resultate gewissel het vanaf 17.2 tot 52.1%. Ons beraamde voorkoms van ystertekortanemie het dienoooreenstemmend tussen 12.0 en 24.5% gewissel, afhangende van die metode wat toegepas is. Die voorkoms van groei-inkorting [lengte-vir-ouderdom Z-telling <-2 standaard afwykings] was 12.5%, terwyl 25.1% van die babas oorgewig of vetsugtig [gewig-vir-lengte Z-telling >2 standaard afwykings] was.

Gevolgtrekking

Die hoë voorkoms van beide oorgewig en ystertekort, tesame met inflammasie dui op 'n dubbele las van wanvoeding in hierdie studiedeelnemers. Die ontstellende groot variasie in die voorkoms van ystertekort tussen die verskillende metodes beklemtoon dat ystersupplementering, met die doel om anemieë te behandel, gebaseer moet wees op akkurate bepalinge van die ystertekort prevalensie in 'n populasie. Ystersupplementering kan andersins negatiewe gevolge inhou vir vatbare babas wat nie 'n ystertekort het nie, maar wel anemies is. Gegewe die nadelige gevolge van ystertekort onder hierdie kwesbare ouderdomsgroep van babas, is dit noodsaaklik dat regerings, gesondheidsorgpersoneel, en ouers moet optree om ystertekort en ystertekortanemie te verhoed of te behandel.

Sleutelwoorde

Ystertekort; Inflammasie; Serum Ferritin; Babas; Ystersupplementasie

ABBREVIATIONS

ACD	Anaemia of chronic disease
AGP	α -1-glycoprotein
AI	Adequate intake
AIDS	Acquired immune deficiency syndrome
APP	Acute phase proteins
ASF	Animal-sourced food
ANOVA	Analysis of variance
BMI	Body mass index
CEN	Centre of Excellence for Nutrition
CF	Correction factor
CRP	C-reactive protein
DRI	Dietary reference intakes
EAR	Estimated average requirement
FAO	Food and Agriculture Organization
HAZ	Height-for-age z-score
Hb	Haemoglobin
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDE	Iron-deficient erythropoiesis
KZN	KwaZulu-Natal
LBW	Low birth weight
MRC	Medical Research Council
SAVACG	South African Vitamin A Consultative Group
SF	Serum ferritin
sTfR	Serum transferrin receptor
UNICEF	United Nations Children's Fund
UNU	United Nations University
WAZ	Weight-for-age z-score
WHO	World Health Organization
ZnPP	Zinc protoporphyrin

DESCRIPTION OF TERMS AND CONDITIONS

Several important terms that are used in this dissertation will be delineated to promote clarity.

Acute phase proteins	A class of proteins whose synthesis change — some increase and are called positive APPs, while others decrease and are called negative APPs — in response to inflammation, to protect the host against microorganisms via non-specific defence mechanisms (Dirckx, 2001).
Adequate intake	The average daily nutrient intake level — based on observed intake or experimentally determined estimations — by a group of apparently healthy people that is presumably adequate (DRI, 2003).
Anaemia	A condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiological needs (WHO, 2011), and diagnosed by a low concentration of haemoglobin in the blood (Biesalski & Erhardt, 2007). Anaemia is characterised by pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy and weariness.
Anthropometry	The science dealing with measuring the size, weight and proportions of the human body for comparison or classification purposes (Cogill, 2003; Dorland, 2007).
Body mass index	An index that uses the participants' weight and height to measure body fat stores (weight in kilograms divided by the square of height in metres) (Cogill, 2003).
Centrifugation	The separation of minute portions of matter in suspension in a fluid by spinning the fluid (Dirckx, 2001).
Complementary diet	Any solid or liquid food with nutritional value other than breast milk, offered to breastfed infants (Giugliani & Victora, 2000).
Convalescence	A period between the end of a disease and the patient's restoration to complete health (Dirckx, 2001).
Dietary diversity	The number of different foods or food groups consumed over a given time period (Ruel, 2003).

Energy density	The amount of energy per unit of volume or weight of the food (Giugliani & Victora, 2000).
Ferritin	The main protein in which iron is stored in the body cells (WHO, 2011a).
Food fortification	Addition of a single or more than one essential nutrients to a food, whether or not it is normally present in the food, to prevent or correct a deficiency of one or more nutrients observed in a population or specific population groups (FAO/WHO 1994).
Free radicals	An atom or atom group that may be highly active as intermediate in various reactions in living tissue, because it carries an unpaired electron and no charge (Dirckx, 2001).
Haem iron	Iron occurring in the oxygen-carrying, non-protein component of haemoglobin. Dietary sources of haem iron are meat, fish and poultry (Mosby, 2009).
Hepcidin	A small peptide hormone that mediates host defence and inflammation and regulates systemic iron metabolism. Hepcidin is measurable in human urine, plasma and serum (Tussing-Humphreys <i>et al.</i> , 2012).
Incubation	The period from the time an infectious agent gains entry, at this stage without sign or symptom, until the appearance of the first signs or symptoms (Dirckx, 2001).
Introduction of solids	The period during which an infant's diet is expanded, and the infant becomes less dependent on milk as the only source of nutrition (Anderson, 1997).
Iron	An essential metallic element that acts as a key component of oxygen-carrying proteins as well as enzymes, to play a vital role in cellular metabolism, cell growth and differentiation (Tussing-Humphreys <i>et al.</i> , 2012).
Iron bioavailability	The proportion of ingested iron that is absorbed by the body and available for use (Giugliani & Victora, 2000) in a form that is physiologically active (Miller, 1998).
Iron deficiency	A significant contributor to anaemia that is characterised by a reduction in total body iron to such an extent that iron stores become exhausted and some degree of tissue iron deficiency become present (Cook, 2004)
Iron deficiency anaemia	The combination of iron-deficiency and anaemia (Hay <i>et al.</i> , 2004) that indicates the final stage of iron deficiency, when iron stores are exhausted, circulating iron is very

low, red cell production is inhibited and anaemia develops. Indicated by age-appropriate haemoglobin and serum ferritin values.

Lactoferrin	A potent antimicrobial, immuno-modulating iron-binding glycoprotein that belongs to the transferrin family and is present in high concentrations in breast milk (Naot <i>et al.</i> , 2005).
Length-for-age	An index of past, or chronic, nutritional status, which assesses the prevalence of stunting (Cogill, 2003).
Malnutrition	A nutritional disorder or condition resulting from an unbalanced diet or inadequate nutrition (Cogill, 2003).
Non-haem iron	The other form of dietary iron that is less bio-available than haem iron, but present in all plant food sources and 60% of animal food sources (Mosby, 2009).
Standard deviation	A statistical measure of scattering away from the mean: the square root of the variance (Cogill, 2003).
Stunting	A measure of chronic malnutrition where skeletal growth slows down that usually results from extended periods of inadequate food intake and infection, especially during the years of greatest growth for children (Cogill, 2003) Reflected by a height-for-age < -2 standard deviations of the WHO Child Growth Standards median.
Underweight	A measure of both chronic and acute malnutrition that reflects on body mass relative to chronological age. Reflected by weight-for-age < -2 standard deviations of the WHO Child Growth Standards median (Cogill, 2003).
Wasting	A condition reflected by weight-for-height < -2 standard deviations of the WHO Child Growth Standards median that results from the loss of both muscle mass and fat mass. This form of malnutrition usually indicates severe food deprivation, malabsorption or nutrient losses, and current infection (Cogill, 2003).
Weight-for-age	An index of short- and long-term malnutrition referred to as under-nutrition; a valuable index for use with very young children (Cogill, 2003).
Z-score	The difference between the value for an individual, and the median value of the reference population, for the same age or height, divided by the standard deviation of the reference population (Cogill, 2003).

CHAPTER 1: INTRODUCTION



1.1. Background

Iron is a nutrient of critical importance during the period from conception to two years of age, which is known as the first 1000 days of life (Hay *et al.*, 2004). During early infancy, breast milk on its own provides all the required nutrients (including iron), in sufficient amounts to ensure optimal growth and development (WHO, 1998). Around six months of age, however, the iron in breast milk becomes insufficient, and the rapid growth that characterises this period may deplete an infant's iron stores (Thorisdottir *et al.*, 2011).

Most of the literature on the prevention of iron deficiency anaemia (IDA) in infants older than six months, centres on the importance of optimal complementary feeding practices (Kazal, 2002), since more than 90% of a breastfed infants' iron requirements must be met from complementary foods (Booth & Aukett, 1997). Poor iron status in infants is often caused by monotonous complementary diets that contain very little iron-rich food sources, or include food that interfere with iron absorption (Wharf *et al.*, 1997). These poor quality diets often result in the depletion of body iron stores that finally leads to IDA (Wharf *et al.*, 1997).

IDA remains the most common micronutrient deficiency for infants between the ages of six and 24 months and beyond (Stoltzfus & Dreyfuss, 1998), and adds to the burden of disease prevalent in developing countries (Oti-Boateng *et al.*, 1998). IDA contributes to poor overall health, delayed development and stunting in infants (Thorsdottir *et al.*, 2003).

Literature documenting the iron status, inflammation and anthropometric nutritional status of South African infants is scarce, and because very little of the above-mentioned data of the South African leg of the International Research on Infant Supplementation (IRIS) study had been published before, it was decided to reconsider the data, this time with a different aim.

Although there are many biochemical measures available to determine the prevalence of iron deficiency (ID) in a population, the World Health Organization (WHO, 2011) recommends the use of serum ferritin (SF) concentrations in resource-poor settings. Unfortunately, however, this measurement has a limited ability to distinguish between high SF concentration due to sufficient iron stores and increased SF concentration due to inflammation (Cook, 2005; Whitney & Rolfes, 2013). SF not only reflects the concentration of iron that is stored in the liver (Thurnham *et al.*, 2010), but also acts as an acute phase reactant that rises in the presence of inflammation that is associated with disease (Finch, 1994; Skinner *et al.*, 2010; Righetti *et al.*, 2013).

To detect the presence of inflammation and adjust for its elevating effect on SF concentrations, the WHO working group (WHO, 2011a) recommended that SF measurements should be accompanied by the analysis of one or more acute phase proteins (APPs). The prevalence of inflammation was determined in the IRIS study by measuring well-known APPs, namely C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) (Smuts *et al.*, 2005). The appropriate way of dealing with the effect of inflammation on SF concentration at the time of publication of the first results was to exclude all subjects with acute inflammation (defined as CRP > 12 mg/L) from the statistical analysis (Smuts *et al.*, 2005).

1.2. Motivation for study and study design

This mini-dissertation offers a re-assessment of the IRIS study's ID prevalence results, based upon recent advances in research, providing a number of methods to account for the effect of both acute and chronic inflammation on SF, as a marker of iron status. Four different methods — based on APP measurements to account for the effect of inflammation on SF concentrations — were used to determine the prevalence of ID. The results were compared to those of a reference method that did not take inflammation into account.

We used the baseline data of this double-blind, placebo-controlled intervention study that examined the prevalence of multi-micronutrient deficiencies and the efficacy of multi-micronutrient supplementation in black South African infants from the rural population of the Valley of a Thousand Hills in KwaZulu-Natal. The sample, consisting of 192 infants, was randomly selected to take part in the original study's six-month intervention period that lasted from April to September 2000 until the infants were around 12 months old (Smuts *et al.*, 2005).

The re-exploration and publication of the IRIS data provides a window of opportunity to document a more accurate estimate of the prevalence of ID and IDA in these South African infants that lived in a rural area with wide-spread inflammation, not attributable to malaria.

All infants included in the study were apparently healthy and had to comply with the exclusion criteria mentioned in the *Subjects and ethics* section of Chapter 3. This section also includes sufficient proof that the relevant ethical clearance and informed consent were obtained for the IRIS study. The baseline data were made available for further exploration by Prof. Mieke Faber (representing the MRC) and Prof. Marius Smuts (the principal investigator of the IRIS study and the student's co-study leader).

This information could provide a more precise assessment of the efficaciousness of the supplements used in the IRIS study if the intervention results were to be re-analysed. Furthermore, it may assist in the compilation of appropriate nutrition strategies that avoid the risks of unnecessary iron supplementation to infants who are not iron-deficient, or withholding iron supplementation when it is in fact indicated.

1.3. Basic hypothesis and study objectives

The hypothesis for this study is that the prevalence of ID will differ for the four methods that adjust SF concentrations for inflammation, and that there will be an increase in the measured prevalence of ID, when compared to the reference method. It is also hypothesised that certain socio-demographic and body composition characteristics will explain some of the variance observed in the distribution of APPs, as markers of inflammation.

The study objectives are therefore:

- to describe the prevalence of ID when applying four different methods to account for the effect of inflammation on SF concentrations;
- to compare the prevalence of ID obtained from the four methods, to a reference method that does not take inflammation into consideration; and lastly
- to determine socio-demographic and body composition characteristics associated with APPs, as markers of inflammation in these infants.

1.4. Structure of this mini-dissertation

This dissertation is written in article format, according to the postgraduate manual guidelines of the North-West University. The overall structure of the study takes the form of four chapters, including this introductory chapter.

Chapter 2 begins by discussing the available published literature, complementing the title of the dissertation, to investigate the iron status, inflammation and anthropometric nutritional status of young infants. The third chapter presents an article entitled, "Differential ferritin interpretation methods that adjust for inflammation yield discrepant iron-deficiency prevalence". This chapter is written following the authors' guidelines of *Nutrition*.

The *Participants and Methods* section of Chapter 3 includes a description of the *anthropometric measurements and analysis*, as well as the *blood collection and analysis*. *Although this information has previously been published (Smuts et al., 2005), it is still included in this mini-dissertation in order to meet the requirements of a method section. The word limit stipulated by Nutrition has therefore not been adhered to, but all parts that have been elaborated upon will be shortened, or just referred to, when submitting the article for publication.*

The fourth and final chapter presents an elaboration on the main findings of the research documented in Chapter 3, focusing on the three key objectives that have been identified earlier. It draws upon the entire dissertation, tying up the various theoretical strands in order to come to a final conclusion that explains the implications of the findings, and to identify further research areas.

Decimal numbers are used to ensure that the headings follow a logical sequence, except for Chapter 3, where headings are given without numbering, according to the instructions for authors of *Nutrition*. One combined reference list has been compiled for chapters 1, 2 and 4 and is presented after Chapter 4, followed by the addenda. Chapter 3 has a reference list according to the Vancouver reference style, as directed by the instructions for authors of *Nutrition*.

1.5. Contributions of the research team

Table 1.1 Level of involvement of the student in the exploration of the baseline data of the IRIS study, and authors' contributions to the article to be submitted

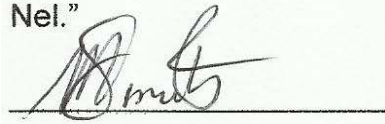
Team member	Institution	Role
Prof. Marius Smuts	* CEN, NWU Potchefstroom Campus	Principal Investigator of the original IRIS study (2000) and co-study leader of this mini-dissertation.
Prof. Mieke Faber	MRC, South Africa	Collaborator in the original IRIS study. Provided access to the IRIS data-set
Prof. Salome Kruger	* CEN, NWU Potchefstroom Campus	Main study leader who fulfilled an advisory role on all the content of this mini-dissertation.

Team member	Institution	Role
Dr Jeannine Baumgartner	* CEN, NWU Potchefstroom Campus	Co-study leader providing guidance and assistance with the statistical analysis and feedback on the writing of this mini-dissertation.
Dr Suria Ellis	Statistical Consultation Services, North-West University, Potchefstroom Campus	Provided guidance with the statistical analysis.
Mrs Elsmari Nel	* CEN, NWU Potchefstroom Campus	Developed and formulated the research questions after extensive data mining. Conducted the statistical analysis and was responsible for the final writing of all the content of the mini-dissertation.

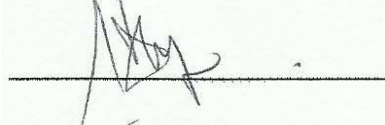
* CEN, Centre of Excellence for Nutrition, NWU, North-West University

The following statement and signatures confirm the co-authors' role in the article, and their permission to include the article (Chapter 3) into this dissertation: "I declare that I have approved the above-mentioned article, and that my role in the study, as indicated above, is representative of my actual contribution. I hereby give my consent that the article may be published as part of the M Sc dissertation of Mrs E.

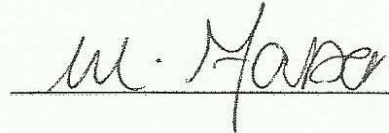
Nel."



Prof. C.M. Smuts



Prof. H.S. Kruger



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Dr J. Baumgartner

CHAPTER 2: LITERATURE REVIEW



2.1. Introduction

The first 1000 days of life (from conception to 24 months of age) is widely recognised as the most critical period of nutritional vulnerability that has a significant impact on lifelong health (Black, 2012; Pinhas-Hamiel *et al.*, 2003). A poor nutritional status often starts *in utero*, becomes evident during the first year of life, and continues beyond two years (Martorell & Zongrone, 2012). Breastfeeding and complementary feeding practices remain relevant in global public health (Vossenaar & Solomons, 2012), because of their importance in meeting the high nutritional requirements (especially for iron) that infants have. Infants need iron to support their rapid growth and to ensure optimal physical and mental development (Hay *et al.*, 2004; Thorisdottir *et al.*, 2011).

Infants who grow up in rural communities in developing countries are more vulnerable to malnutrition and more susceptible to infection, because of their disadvantaged circumstances (Faber, 2004; Faber *et al.*, 1997). They are therefore also predisposed to the adverse consequences of micronutrient deficiencies (including iron deficiency [ID]), such as a short stature, delayed cognition, less schooling, diminished work capacity and consequently reduced incomes (Martorell & Zongrone, 2012).

The inflammation that results from infection induces major changes in the handling of iron in the body. The acute phase proteins (APPs) redistribute iron from serum to the liver stores, to reduce the systemic availability of iron (Andersson, 2010) and thus protect the host against pathogens (WHO, 2006). Less iron is also released from the ferritin stores, or absorbed from the gastro-intestinal tract (Tussing-Humphreys *et al.*, 2012). The elevating effect of inflammation on serum ferritin (SF), as a marker of iron status, will be discussed in more detail in the next section.

Previous studies on the relationship between iron and growth mainly involved iron-deficient and stunted children (Bougle *et al.*, 2000; Gunnarsson *et al.*, 2005), while on the other end of the spectrum, many authors (Baumgartner *et al.*, 2012; Dallman *et al.*, 1980; Nead *et al.* 2004; Scheer & Guthrie, 1981) have found that children and

adolescents at risk of overweight, or already overweight and obese, were also more likely to be iron-deficient.

The aim of this chapter is therefore to give an overview of the literature available on possible relationships between indicators of iron status, inflammation and the anthropometric nutritional status of infants.

2.2. Iron deficiency in infants: A public health problem in developing countries, including South Africa

When infants are diagnosed with ID, it means that the amount of iron that is available in their bodies is inadequate to perform the normal physiological functions in tissues such as blood, the brain and muscles (Andersson, 2010). Iron deficiency anaemia (IDA) is the most advanced stage of ID that occurs when the body has been deprived of iron for a long time, or when large amounts of iron have been lost (Andersson, 2010). Simply looking at biochemistry, IDA is a combination of ID and anaemia, because the storage iron pool, namely SF, is exhausted, and the iron supply for red blood cell production (erythropoiesis) is insufficient to maintain normal haemoglobin (Hb) concentrations (Zimmermann, 2008).

IDA is the most common micronutrient deficiency for which infants aged six to 24 months represent one of the groups at highest risk (Stoltzfus & Dreyfuss, 2007). It is estimated that between 30% and 45% of children between the ages of six and 24 months in sub-Saharan Africa have IDA (Lutter, 2008). South African national representative data from the 1990s indicated that one child in 10 was iron-deficient and one in 20 had IDA (SAVACG, 1995). Faber and Benade (2007) found a much higher value for IDA prevalence (35%), when they investigated the nutritional status of infants from a rural population in South Africa.

It is important to note at this point that although ID is an important cause of anaemia, anaemia has a multifactorial aetiology (WHO/UNICEF/UNU, 2001). It can be caused by nutrient deficiencies (mostly iron, folate or vitamin B12), malaria, intestinal parasites, HIV, or certain congenital haemoglobinopathies (DeMaeyer *et al.*, 1989;

Gillespie & Johnston, 1998). In the past it was believed that the proportion of anaemia attributable to ID was approximately 50% (DeMaeyer & Adiels-Tegman, 1985); it may, however, be lower in developing countries where the prevalence of infections and other nutritional deficiencies are higher (Rohner, 2008).

In malaria-endemic areas, malaria represents the primary cause of anaemia in more than half of the cases of severe anaemia (Gillespie & Johnston, 1998). Malaria anaemia may be caused by acute or chronic destruction of red blood cells, defective red blood cell production, or a secondary folate deficiency (Gillespie & Johnston, 1998). Intestinal helminths affect 25% of the world's population at any one time, and are another important contributor to anaemia in the form of hookworms and schistosomes (Gillespie & Johnston, 1998). Hookworms feed on blood through the intestinal mucosa and thus cause chronic faecal blood loss that leads to anaemia (Roche & Layrisse, 1966). Anaemia is, however, also a very common complication of HIV infection that relates primarily to the reduced production of erythrocytes. HIV-associated anaemia may be a direct effect of HIV, or due to secondary infections and neoplasms, bone marrow suppression by medication, or micronutrient deficiencies (Bain, 1999). Iron deficiency does not, however, appear to be more common in HIV-infected than in uninfected children (Totin *et al.*, 2002). The last contributor to anaemia that will be discussed briefly in this review is congenital haemoglobinopathies, which can be conveniently grouped into thalassaemias or sickling disorders. Both of these may contribute to the prevalence of anaemia by altering the rate of globin-chain synthesis or modifying the structure of the globin chains, and thereby interfering with the release of iron from iron stores for erythropoiesis (Rohner, 2008).

IDA poses an enormous public health problem for the developing world because of its contribution to the burden of disease (Oti-Boateng *et al.*, 1998). The exact relationship between IDA and cognitive and psychomotor development is not well understood, but even ID without anaemia may impair the development, future learning ability and work productivity of infants (Baker *et al.*, 2010; Thorsdottir *et al.*, 2003; WHO/UNICEF/UNU, 2001). Iron plays a critical role as enzyme cofactor in

energy metabolism and deficiencies negatively affect the energy metabolism of the neurons, myelination, and memory function, explaining the negative consequences mentioned above (Beard, 2001).

Although most pathogens require iron to grow, iron is also required by the host to build up an effective immune response (Beard, 2001). ID lowers the human body's resistance to infections by reducing the leukocytes' capacity to kill ingested microorganisms, reducing the ability of the lymphocytes to replicate when stimulated, and lowering the concentration of cells responsible for cell-mediated immunity (Srikantia *et al.*, 1976). The adverse effect of ID on the immune system further impairs infants' health and growth (Nojilana *et al.*, 2007; Oti-Boateng *et al.*, 1998).

In conclusion, the spectrum of iron nutrition status can be viewed as a continuum that ranges from ID with anaemia to ID without anaemia, to sufficient iron status with iron stores that differ in size and lastly iron overload. The literature also documents some extent of overlap between ID and IDA that varies considerably among population, age, and gender groups (WHO/UNICEF/UNU, 2001).

2.3. Iron requirements during infancy

Iron is an essential nutrient that is required by most human tissues for growth, especially the brain, muscles for gain in lean body mass, and red blood cells to expand blood volume (Chaparro, 2008). Iron status in infancy is largely determined by four factors: the iron an infant is born with (which is related to his/her mother's iron status), the infant's post-natal needs for iron, the food sources of bio-available iron, and iron losses (Chaparro, 2008; Lozoff *et al.*, 2006). Eighty percent of the iron that an infant is born with is accreted during the third trimester of pregnancy. Premature babies therefore miss this important period of accretion and are therefore more prone to ID (Baker *et al.*, 2010).

Healthy, full-term, new-born infants would have formed sufficient iron stores prenatally so that they require very little exogenous iron for the first four to six months of life (Dallman *et al.*, 1980). Their iron needs of 0.27 mg/day can easily be supplied by breast milk (Baker *et al.*, 2010). The Adequate Intake (AI) for iron from

five months to one year old is 11 mg/day (Whitney & Rolfes, 2013), which is suddenly much higher than for the first four to six months. This jump is partly caused by the use of different methods when calculating these values, but even so, compared to adults, infants have a relatively higher iron requirement (Baker *et al.*, 2010).

A positive iron status can only be achieved when there is adequate, bio-available dietary iron intake to balance the infant's requirements for erythropoiesis, growth and development (Oti-Boateng *et al.*, 1998) and therefore one of the primary prevention strategies of IDA in infants depends upon healthy feeding practices (Kazal, 2002).

2.4. Iron homeostasis in infants

Iron is both an essential element as well as a potential toxicant to cells and therefore has to be regulated very tightly (Beard, 2001) to ensure proper growth, development and overall health throughout human life. Iron homeostasis is controlled mainly at the level of the intestine, through absorption, since iron cannot be actively excreted from the body (Andrews, 1999), besides through faeces when the intestinal cells are shed, and a minimal amount in urine (Chaparro, 2008).

Although iron absorption in infants appears to go through developmental changes throughout infancy (Chaparro, 2008), it is thought to be similar to iron absorption in adults that is regulated by several homeostatic mechanisms, namely the “stores regulator”, the “dietary regulator”, and the “erythropoietic regulator” (Dallman *et al.*, 1980). Lönnerdal and Kelleher (2007) explain that iron homeostasis matures from erythropoietic to dietary and then stores regulation in infants, and to stores, dietary, and finally erythropoietic regulation in adults, but these regulators may not be functioning appropriately until late infancy.

The erythropoietic regulator influences iron absorption based on the body's need for red blood cell production, which is important for infants because rapid growth early in life (Oti-Boateng *et al.*, 1998; Wharf *et al.*, 1997) requires a rapid expansion of blood volume (Fomon *et al.*, 2000). The dietary regulator, on the other hand, is influenced by recent dietary iron intake, independent of the size of iron stores, or the rate of red

blood cell production. Lastly, the stores regulator responds to total body iron, where the amount of iron being absorbed is inversely related to iron stores. The erythropoietic regulator may therefore have a greater capacity to increase iron absorption in infants when compared to the stores regulator (Andrews, 1999; Domellöf *et al.*, 2002; Finch, 1994).

Iron absorption varies between individuals and the human body has a remarkable capacity to conserve and re-use iron once it has been absorbed (Dallman *et al.*, 1980). Dietary iron mostly occurs in the ferric state (Fe^{+3}), and first has to be reduced to the ferrous form (Fe^{+2}) before it can be absorbed by the enterocyte in the intestine (Dallman *et al.*, 1980). A number of proteins are needed to coordinate the transfer of iron from the enterocyte into the systemic circulation. These proteins are located in the upper part of the duodenum where most iron is absorbed (Dallman *et al.*, 1980).

When the body needs iron, hephaestin oxidates Fe^{+2} back to Fe^{+3} to allow ferric iron to be incorporated into apo-transferrin in the circulation. The discovery of hepcidin, a small peptide synthesised by the liver, has enhanced researchers' understanding of iron metabolism (Lönnerdal & Kelleher, 2007). In adults, hepcidin status modulates the transport of iron from the enterocytes to facilitate an increase in iron absorption during ID (by expressing less hepcidin), and a decrease in iron absorption during iron repletion, inflammation, or infection (by increasing hepcidin expression) (Tussing-Humphreys *et al.*, 2012). This regulatory process is, however, not yet understood in infants. Iron repletion, via supplemental or dietary sources, may be limited by the high expression of hepcidin that is found in the presence of inflammation (Tussing-Humphreys *et al.*, 2012). This poses a problem when ID occurs because of poor dietary iron intake or helminth infestation, and coincides with anaemia of chronic disease (ACD), due to HIV/AIDS for example.

2.5. Iron status indicators and stages of iron deficiency

Ferritin and hemosiderin are the main storage proteins of iron in humans, while the bone marrow is the largest user of iron (Dallman *et al.*, 1980; Rohner, 2008). The iron-containing compounds that serve metabolic or enzymatic functions are

haemoglobin in the red blood cells and myoglobin in the muscle cells. These proteins carry oxygen to all the body cells (Whitney & Rolfes, 2013).

ID develops in stages, known as storage ID, functional ID, iron-deficient erythropoiesis (IDE), and IDA (Andersson, 2010). During the first stage iron stores become too low to meet the body's iron needs, but there is no dysfunction yet. Serum ferritin is the most valuable measurement in assessing storage ID (Whitney & Rolfes, 2013), since it is universally available and well-standardised, and the concentration of SF is directly proportional to the body iron stores in healthy individuals (Cook, 2005; Whitney & Rolfes, 2013).

The second stage of ID is characterised by severely depleted iron stores and now the human body adapts by increasing transferrin levels, to sequester needed iron. This is known as functional ID, and although serum transferrin receptor (sTfR) is a less sensitive parameter than SF, its measurement is valuable during this stage of ID to determine how severe the deficiency is (Biesalski & Erhardt, 2007). The higher sTfR and the lower SF, the more advanced is the deficiency (Whitney & Rolfes, 2013). SF is a measure of body iron stores, while sTfR is a measure of tissue ID (Zimmermann, 2008). The most important benefit of sTfR measurement is that the concentration is not as affected by inflammation as SF, and it can therefore more successfully distinguish between IDA and ACD (Ferguson et al., 1992).

During the next stage of ID, namely IDE, the amount of iron available for red blood cell production in the bone marrow becomes insufficient (Andersson, 2010). Various measurements additional to the ones already mentioned can be used to identify this stage, including low transferrin saturation, elevated zinc protoporphyrin (ZnPP), or low mean corpuscular volume. Iron-deficient erythropoiesis can occur despite normal or increased storage of iron (associated with inflammation) (Zimmermann, 2008).

The final stage of ID, known as IDA, is characterised by small (microcytic) red blood cells (erythrocytes). During this stage both haemoglobin and haematocrit concentrations become low because iron is not being delivered to the bone marrow

to produce haemoglobin (Andersson, 2010). Haematocrit is the measure of red blood cells in a given volume of blood, packed by centrifugation. Haemoglobin and haematocrit tests are often used because they are easy, quick and inexpensive, but their usefulness in detecting ID in the early stages is limited, and their concentrations may be affected by other nutrient deficiencies or medical conditions (Cook, 2005; Whitney & Rolfes, 2013).

The biomarker, sTfR, reflects the demand for Hb synthesis, and an inverse relationship exists between sTfR and Hb (George *et al.*, 2012). Haemoglobinopathies — mentioned earlier under paragraph 2.2 as a possible contributor to the prevalence of anaemia — are characterised by low Hb concentrations and high sTfR concentrations, similar to IDA, but in this case iron stores (SF) may appear adequate, or even high (Knowles *et al.*, 2012; Zimmermann, 2008).

Baker *et al.* (2010) recommend that infants should be screened for IDA at approximately 12 months of age, by means of an Hb concentration test together with an ID risk factor assessment. An Hb concentration < 110 g/L would diagnose anaemia in these subjects (WHO, 2011b), while ID risk factors would include a history of prematurity or low birth weight, exclusive breastfeeding beyond four months of age without supplementing iron, weaning onto cow's milk, not including iron-fortified cereals or naturally iron-rich foods as complementary food, feeding problems, or poor growth.

In conclusion, although bone marrow investigation to establish the absence of stainable iron is thought to be the “golden standard” (Zimmermann, 2008), it remains best practice to use a combination of indicators to determine the iron status of subjects, so that the different stages of ID can be identified. Bone marrow examinations are very expensive and invasive and require technical expertise; they cannot be performed for screening purposes (Zimmermann, 2008). Zimmermann (2008) further states that the best combination is usually Hb plus SF and, if CRP is elevated, sTfR and/or ZnPP.

2.6. Determining and interpreting the iron status of infants in the presence of inflammation

The World Health Organization (WHO, 2011a) recommends SF measurements to determine the prevalence of ID because it is the best biomarker of iron status in terms of cost and practicality (Biesalski & Erhardt, 2007). In previous years, the appropriate cut-off for SF to define ID in infancy was subject to debate, and the prevalence varied according to the cut-offs used (Lozoff *et al.*, 2006). The WHO working group (2011a) now recommends that SF concentrations $<12\mu\text{g/L}$ define ID in children less than five years of age.

SF concentrations, however, not only reflect the concentration of stored iron in the liver (Thurnham *et al.*, 2010), but also act as an acute-phase reactant that is elevated in inflammatory conditions or infection (Finch, 1994; Skinner *et al.*, 2010; Righetti *et al.*, 2013). The major diagnostic challenge, according to Zimmermann (2008), is to distinguish between IDA in otherwise healthy individuals and ACD, and for this SF is of limited usefulness (Cook, 2005; Whitney & Rolfes, 2013). Generally sTfR is not considered to be influenced by inflammation, but in a study conducted by George *et al.* (2012) among young Cambodian children, sTfR was also significantly elevated by chronic inflammation. The authors explain that when both ferritin and sTfR concentrations are raised, it may be related to the presence of certain Hb disorders (that were mentioned earlier) that limits effective erythropoiesis so that dietary iron absorption is increased, even when iron stores are adequate (George *et al.*, 2012).

In order to detect the presence of infection or inflammation, and to be able to adjust for its influence on SF concentrations, the WHO working group (2011a) recommended that SF measurements be accompanied by the measurement of one or more APPs. C-reactive protein (CRP) is the best laboratory marker for acute inflammation and gives an indication of the early influence of inflammation on SF concentrations, while alpha-1 glycoprotein (AGP) indicates chronic inflammation and thus predicts the influence of inflammation on SF concentrations at a later stage (Biesalski & Erhardt, 2007; Grant *et al.*, 2012). Whenever these APPs are increased,

it shows that iron metabolism is disturbed in reaction to inflammation (Biesalski & Erhardt, 2007).

The prevalence of ID — using SF concentrations alone — would be underestimated if not corrected for inflammation (Thurnham *et al.*, 2010), as proven by the results of Righetti *et al.* (2013) and Engle-stone *et al.* (2013) who demonstrated a significant increase in the measured prevalence of ID when correcting SF concentrations for inflammation. CRP and AGP identify different, but overlapping groups of people based on their status of inflammation (Grant *et al.*, 2012), and by using both APPs, underestimations can be better accounted for (Ayoya *et al.*, 2010; Thurnham *et al.*, 2010). If, however, only one APP is used, 50% of the underestimation of ID will remain because only half of the ferritin increase will be removed (Thurnham *et al.*, 2010).

A lot of uncertainty still remains about exactly how the APPs should be used to adjust SF concentrations for the effect of inflammation. The WHO workgroup (2011a) suggested two methods, one of which was to raise the SF cut-off concentration that defines deficiency to 30µg/L in populations with a high prevalence of inflammation. Thurnham *et al.* (2010), however, argued that this method is fraught with uncertainty because the increase in ferritin after infection follows a different pattern than that of either CRP or AGP. C-reactive protein rises within 10 hours of the onset of an acute infection, and reaches its peak concentration within 24 to 48 hours, whereas AGP responds more slowly and only reaches its peak after two to five days. CRP concentrations fall drastically as the intensity of the infection subsides, whereas AGP remains elevated for a longer period of five to six days (Ayoya *et al.*, 2010; Grant *et al.*, 2012). SF concentrations, on the other hand, rise significantly within a few hours of the onset of inflammation, and concentrations remain high even after CRP concentrations have subsided and while AGP concentrations are still elevated (Biesalski & Erhardt, 2007; Thurnham *et al.*, 2010). Kung'u *et al.* (2009) state that it is difficult to choose one specific cut-off concentration to provide a distinct international standard when the degree of inflammation varies considerably between individuals.

When Engle-Stone *et al.* (2013) used the higher cut-off concentration for SF in a population with inflammation, they found that the prevalence of ID was underestimated in those with inflammation and overestimated in those without inflammation. They consequently advised that this approach should only be used to estimate the prevalence of ID if data were collected long ago and inflammation was known to be present in the population, but was not measured. It should not be included in studies that are still in the planning stages (Engle-Stone *et al.*, 2013).

Phiri *et al.* (2009) even arrived at a much higher cut-off concentration for SF (273 µg/L instead of 30 µg/L) when they compared SF measurements with bone marrow iron findings in 381 Malawian children with severe anaemia. Their recommended cut-off is, however, much higher than any other cut-off appearing in the literature, and may need more support from other studies before changes in recommendations can be made.

The second method proposed by the WHO working group (2011a) was to exclude individuals with elevated concentrations of CRP and/or AGP when calculating the prevalence of ID based on SF concentrations. Thurnham *et al.* (2010) felt that this method could bias the results if iron-deficient persons were more prone to infection, and Engle-Stone *et al.* (2013) added that this method could substantially reduce the sample size in populations with a high prevalence of inflammation, causing an underestimation of the prevalence of ID (WHO, 2011a).

A third method was devised by Thurnham *et al.* (2010) from a meta-analysis consisting of 32 studies, which included infants, children, men and women. The SF concentrations of individuals were mathematically adjusted for the presence of inflammation based on two APP measurements. Individuals were categorised into four groups based on their CRP and/or AGP concentrations. Categories included an apparently healthy reference group (CRP ≤ 5 mg/L and AGP ≤ 1 g/L), an incubation group (CRP > 5 mg/L and AGP ≤ 1 g/L), an early convalescence group (CRP > 5 mg/L and AGP > 1 g/L), and lastly a late convalescence group (CRP ≤ 5 mg/L and AGP > 1 g/L).

The authors then determined correction factors for each of the three inflammation sub-groups, depending on the elevating effect that inflammation had on SF concentrations. In the incubation group SF concentrations were elevated by 30% and this was converted to a correction factor of 0.77; the 90% elevation observed in the early convalescence group was converted to a correction factor of 0.53; and the 36% elevation observed in the late convalescence group was converted to a correction factor of 0.75. Individual SF concentrations were adjusted by using the relevant, group-specific correction factors as multiplier, and repeating the calculation to determine the prevalence of ID after correction (Thurnham *et al.*, 2010).

SF concentrations often vary greatly between different age and sex groups, but the results of Thurnham *et al.* (2010) concluded that neither age nor gender significantly influenced the increase in SF concentrations associated with inflammation. They found that the increase observed in SF concentrations, at each stage of the infection cycle, were proportionate to the initial SF concentrations of each group, and therefore they recommended that these correction factors be applied in other studies (Thurnham *et al.*, 2010).

Righetti *et al.* (2013) found considerable differences between the estimated ID prevalence when using SF versus sTfR concentrations as measures of iron status. It is, however, important to note that the application of correction factors will enable researchers to implement the recommendation of the WHO to use SF (WHO, 2011a), while adjusting for the elevating effect of inflammation on SF concentration, to arrive at a more accurate measurement of ID (Thurnham *et al.*, 2010; Grant *et al.*, 2012).

2.7. Dietary measures to meet the iron requirements of infants

2.7.1. *Breast milk, formula milk and cow's milk*

Breastfeeding is the ideal feeding practice (Kazal, 2002) and breast milk is recognised as the only food that can singly provide all the nutrients required for optimal growth in early infancy (WHO, 1998). Domellöf *et al.* (2004) found no correlation between the iron content of a mother's breast milk and any of the iron-status variables that can be measured in her, or even her dietary iron intake.

Instead, they found that the differences in breast milk iron concentrations that they observed were caused by differences in milk volume, rather than differences in maternal iron status (Domellöf *et al.*, 2004). The higher the volume of breast milk being produced by the mother, the lower the concentration of iron was. Breast milk is produced in response to the baby's demand, and in infants not consuming adequate amounts of complementary food, breast milk intake will be higher. In support the researchers found that a high complementary food energy intake was also associated with higher breast milk iron concentrations (Domellöf *et al.*, 2004). Their results therefore underline the importance of adequate complementary feeds together with breast milk intake during the second part of the first year.

In a Zambian study population where more than 50% of the infants had IDA at six months of age, exclusive breastfeeding at four months was found to be protective of iron status when compared to early complementary foods (Van Rheezen *et al.*, 2008). At four months of age exclusively breastfed infants will have the same iron status as infants fed iron-fortified formula milk, even though the formula milk contains about ten times more iron than breast milk (Dube *et al.*, 2010). The lower calcium and phosphate concentrations, together with the presence of lactoferrin in breast milk, ensure that the low iron concentration (0.3mg/L) (Institute of Medicine, 1991) is uniquely well absorbed (12–56%) and utilised (Kazal, 2002; Dewey *et al.*, 2007). Iron is absorbed less efficiently from formula milk, but because of the higher concentration of iron found in iron-fortified formulas, infants are usually able to

maintain sufficient iron stores without additional iron supplementation (Booth & Aukett, 1997; Committee on nutrition, 1999; Kazal, 2002).

Breast milk iron may, however, become insufficient in infants around six months of age when iron stores become depleted because growth velocity is high. For this reason many authors recommend an iron supplement of 1 mg/kg per day for exclusively breast-fed infants after the fourth month of life, and some countries even routinely give supplementary iron drops until appropriate iron-containing complementary foods have been introduced (Baker *et al.*, 2010; Calvo *et al.*, 1992; Engelmann *et al.*, 1998). Baker *et al.* (2010) recommended that even partially breastfed infants, who receive more than half of their daily feedings as breast milk, and who are not yet eating complementary foods containing iron, must be supplemented with 1 mg/kg per day of iron.

One should, however, be cautious with the routine supplementation of iron during infancy, because iron is a pro-oxidant that stimulates the production of free radicals. Free radicals can affect the genes that regulate growth factors and thus reduce linear growth, head circumference and weight gain (Dewey *et al.*, 2002; Kelleher, 2006). When lactoferrin (a major protein in human milk that protects breastfed infants from infection) is saturated with iron, the protective effect against infection is reduced and excess iron increases the risk of infection among breastfed infants (Dewey *et al.*, 2002). This is not a risk among formula-fed infants, because lactoferrin is not present in formula milk. Iron supplementation will be discussed in more detail in section 2.8.

Domellöf *et al.* (2002a) conducted a study to investigate the influence of iron supplementation on the absorption of iron from breast milk in healthy, full-term infants at six and nine months of age. Iron absorption from breast milk at six months was the same for iron-supplemented and non-supplemented (placebo) infants, while at nine months it was significantly higher in non-supplemented infants. This finding was expected because the non-supplemented infants had significantly lower SF concentrations, indicative of smaller iron stores. The authors also found that iron absorption was inversely related to dietary iron intake at nine months, but not at six months, which suggests that dietary iron intake regulates iron absorption at nine

months, but that this regulation may still be immature at six months (Domellöf *et al.*, 2002a).

Hicks *et al.* (2006) conducted a similar intervention study on Peruvian infants at five to six and nine to 10 months of age, to determine whether healthy infants at risk of ID would regulate their iron absorption based on their iron stores. Again, results indicated that iron absorption in infants was related to iron stores as assessed by SF. These authors, however, found an up-regulation in iron absorption from breast milk in both age categories, which was different from what Domellöf *et al.* (2002a) found.

The results found by Domellöf *et al.* (2002a) and Hicks *et al.* (2006) do not imply that breastfed infants need no additional source of iron, besides that obtained from breast milk, during the second half of infancy. They do, however, show a valuable compensatory mechanism in partially breastfed infants consuming low-iron diets, where their iron absorption from non-haem dietary sources will be up-regulated (Chaparro, 2008).

Exclusive breastfeeding for longer than six months has consistently been associated with an increased risk of developing ID (Thorsdottir *et al.*, 2003). Even so, breast milk iron can provide sufficient iron, even beyond the six-month limit, if the infant was born full-term at a normal birth weight, the mother had an adequate iron status during pregnancy, and the infant underwent delayed cord clamping (Dewey & Chaparro, 2007). Breastfeeding per se, during the second half of the first year, may improve iron status by influencing growth, because breastfed infants have appropriate, lower energy intake and therefore do not gain excessive weight and grow at a more appropriate, slower rate that protects iron stores (Thorsdottir *et al.*, 2003).

While iron-fortified formula can help to ensure an adequate iron status in infants (Faber, 2007), low-iron formulas (containing less than 6.7 mg of iron per L) place infants at risk of IDA (Kazal, 2002). The usefulness of formula milk in developing countries, such as South Africa, is very limited, because illiteracy, innumeracy and

language barriers often lead to formula feeding instructions not being followed and feeds being over-diluted or contaminated by bacteria (Faber, 1997; Faber, 2007).

The last type of milk that will be discussed in this literature review is cow's milk. Oti-Boateng *et al.* (1998) found that cow's milk had a dose-related inhibitory effect on iron absorption, and consumption was associated with the depletion of body iron stores. In addition, cow's milk intake was found to have a significantly negative impact on the duration of breastfeeding. According to Szymlek-Gay *et al.* (2009), iron-fortified formula milk instead of regular cow's milk increased mean SF concentration by 44% and Thorrisdottir *et al.* (2011) similarly concluded that the improvement in iron status they observed among 12-month-old Icelanders was attributed to the replacement of regular cow's milk by iron-fortified formula. Formula milk is therefore a better choice for infants than cow's milk, but breast milk remains the absolute best, since the benefits of breastfeeding go far beyond providing adequate iron.

2.7.2. The complementary diet

It is difficult to meet the micronutrient requirements of infants during the period of complementary feeding (Allen, 2008), especially for poor populations in the developing world who often follow monotonous diets. Many authors (Faber, 2004, Faber, 2008; Faber & Benade, 1998, Faber & Benade, 2001; Hotz & Gibson, 2001; Oti-Boateng *et al.*, 1998) have observed that the dietary inadequacies found in the complementary diet of these infants were largely attributed to the predominance of starch (particularly maize in South Africa), and lack of animal-sourced foods (ASF) in the diet. Faber and Benade (1998), as well as Lutter and Rivera (2003), identified poor quality rather than insufficient quantity as the main cause of poor micronutrient intake. The bulky diets consumed by infants were often deficient in iron, resulting in the depletion of body iron stores, which led to IDA and contributed to overall poor health and sub-optimal growth (Faber *et al.*, 1997; Onyango, 2003; Wharf *et al.*, 1997).

2.7.2.1. *Dietary diversity*

By six months of age, all infants should receive a variety of energy- and nutrient-dense foods to supply the full range and quantities of nutrients to fill the gap and ensure optimal health, growth and development when breast or formula milk becomes insufficient (Hoddinott & Yohannes, 2002; Onyango, 2003). The amount of nutrients that breastfed infants need from complementary foods depends on the quantity of nutrients provided by breast milk. This ranges from 90–100% for iron, to 0% for vitamin C (Booth & Aukett, 1997; Institute of Medicine, 1991).

Dietary diversity was included as a specific recommendation in the guidelines for complementary feeding of the breastfed child, to avoid the poor organoleptic qualities and associated micronutrient deficiencies of monotonous diets (Arimond & Ruel, 2004; Onyango, 2003). Monotonous diets are associated with poor appetites that reduce energy intake from complementary foods and consequently lead to poor growth (Golden, 1991). Dietary diversity is, however, even more important for non-breastfed infants living in poor socio-economic circumstances with limited access to formula milk, because they rely solely on complementary food to meet all of their energy and nutrient needs (Arimond & Ruel, 2004).

2.7.2.2. *Iron bioavailability*

Poor iron status in infants is very often caused by consuming a complementary diet with a low iron content, or selecting food that interferes with iron absorption (Wharf *et al.*, 1997). Iron bio-availability is the term used to refer to the percentage of ingested iron that is eventually absorbed by the body and available for use (Giugliani & Victora, 2000). Dietary iron is categorised as either haem or non-haem, based on the pathway by which it is absorbed. The bioavailability of haem iron is relatively high and is not much affected by the composition of the diet, while the bio-availability of non-haem iron is largely determined by the solubility of the iron in the upper gastrointestinal tract (Miller, 1998).

The iron that is best absorbed is found in breast milk, because of its low content of inhibitors of non-haem iron (Fomon *et al.*, 2000). Animal-sourced foods, such as red

meat and organ meat, contain more iron than plant-based foods, and the iron they contain is also more bio-available. This is because ASF contain both haem iron and non-haem iron, while plant-based foods only contain non-haem iron (Giugliani & Victora, 2000; Whitney & Rolfes, 2013). Egg-yolk is rich in iron but it is poorly absorbed, and similarly, beans, lentils, soy beans and leafy vegetables such as Swiss chard, kale, broccoli, mustard and chicory contain reasonable amounts of poorly absorbable iron (Giugliani & Victora, 2000).

The absorption of non-haem iron is inhibited by many factors present in food, all which may influence an infant's iron stores negatively. These include tannin in tea, as well as calcium and phosphate that are present in high concentration in unmodified cows' milk (Dallman & Yip, 1989; Booth & Aukett, 1997; Wharf *et al.*, 1997). Iron bioavailability from cereal products is usually low because of the presence of phytic acid, another major inhibitor of non-haem iron absorption (Davidsson, 1996; Miller, 1998; Gibson *et al.*, 2010). Phytic acid forms an insoluble complex with iron in the gastrointestinal tract that cannot be absorbed by humans owing to a lack of the enzyme phytase. More than 90% of the phytates found in maize is located in the germ, but can be removed by processing (Wesley & Ranum, 2004; Miller, 1998). Other strategies, such as cooking, germination, fermentation and soaking, can be implemented to reduce the phytic acid content of cereal-based complementary foods, without reducing the iron content and availability. Soaking of maize flour is both practical and acceptable and can be readily undertaken at home (Gibson *et al.*, 1998; Miller, 1998).

On the other hand, the absorption of non-haem iron is enhanced by the presence of meat, fish and poultry (Booth & Aukett, 1997; Dallman & Yip, 1989) because of the existence of naturally occurring mineral chelates in animal protein (Gibson *et al.*, 1998). Ascorbic acid (vitamin C) also enhances non-haem iron absorption, and by adding ascorbic acid in high enough quantities the negative effect of phytic acid can be partly overcome (Davidsson, 1996; Miller, 1998), if the food is not cooked excessively so that part of the vitamin C is destroyed (Giugliani & Victora, 2000). Gibson *et al.* (1998) encourage the addition of vitamin-C containing foods (orange,

guava, lemon, mango, papaya, melon, passion fruit, peach, tomato, green pepper, green leaves, cabbage, broccoli, and cauliflower) to plant-based complementary diets, even though breast milk contains sufficient amounts of ascorbic acid to meet the requirements of breastfed infants (Giugliani & Victora, 2000).

Cooking in iron pots can also increase the iron content of food, depending on the pH of the food and the cooking time. Acidic food cooked for long periods gain most iron, though different iron pots release different amounts of iron. The bio-availability of the iron from the pot will be the same as the bio-availability of the food being cooked (Miller, 1998). Iron absorption is, however, not only influenced by the type of iron (haem or non-haem) and presence of inhibitors and enhancers in the diet, but also by the amount of iron (known as the iron density) being ingested (Domellöf *et al.*, 2002).

2.7.2.3. Iron density and sources of iron-dense foods

A WHO report (1998) specifically identified iron as a problem nutrient because of the large discrepancy between the amounts of iron found in typically consumed complementary foods, compared to the amount of iron required by infants during the complementary food phase. Infants need to absorb about 0.8 mg of iron per day from the diet (of which 0.6 mg is needed for growth and 0.2 mg to replace losses), and therefore the recommended density (mg/100 kcal) of iron in complementary foods is 4 mg/100 kcal from six to eight months, 2.4 mg/100 kcal from nine to 11 months and 0.8 mg/100 kcal from 12 to 24 months (Giugliani & Victora, 2000).

As already discussed ASF, particularly red meat, poultry and fish, are rich sources of readily available iron that in addition have an enhancing effect on non-haem iron absorption (Hotz & Gibson, 2001; Szymlek-Gay *et al.*, 2009). Krebs and Hambridge (2007) suggested that the earlier introduction of ASF — at, or shortly after six months of age — could substantially contribute to meeting infants' iron requirements. According to a review of complementary feeding practices (Krebs & Hambridge, 2007), the recommendations for food choices and the sequence of introduction have

generally not been evidence-based, and in earlier centuries meat was commonly pre-chewed and offered to infants as a weaning food.

Krebs (2000) compared pureed beef versus iron-fortified cereal as first complementary food, and found that for the age-group five to seven months, the beef group consumed significantly more protein, while iron intake was significantly higher in the cereal group at seven months. In later years the same author suggested that both a daily meat (30-45 g) and a multi-micronutrient fortified cereal (of equal calories) were relatively effective at maintaining, or building iron stores in infants aged six to 18 months. Krebs *et al.* (2012) further speculated that meat from foraging and scavenging animals may be more readily available and affordable to individual households in rural communities than micronutrient-fortified products.

Engelmann *et al.* (1998) added 25 g of meat to a home-prepared vegetable puree meal for infants aged 10 months. This intervention resulted in a 2.7-fold increase in the absorption of non-haem iron and enabled infants to meet 30% of their daily iron requirements. They also compared the effect of a low (10 g/d) versus high (27 g/d) intake of meat on non-haem iron absorption in eight-month-old, partially breastfed infants. Protein and iron intakes were higher in the “high” meat group and haemoglobin concentrations were minimally lower, compared to the significant declines observed in the haemoglobin concentrations of the “low” meat group (Engelmann *et al.* 1998).

Szymlek-Gay *et al.* (2009) found that during the second year of life each additional gram of red meat consumed was associated with a 0.6% higher SF concentration. A moderate increase in the consumption of red meat may therefore prevent a decline in, or even increase, body iron stores. Allen *et al.*'s (2012) report on the Nutrition Collaborative Research Support Program, which was conducted in Egypt, Kenya and Mexico in the 1980s, found that children who consumed a higher proportion of energy as ASF were taller, heavier, and displayed better cognitive and school performance than those with lower intakes. The authors remarked that the main factor determining dietary quality in most low-income populations was the proportion

of daily energy intake that was consumed as ASF. By introducing meat products as a component of the complementary diet, the micronutrient status of infants, as well as their growth and development, can be improved (Engelmann *et al.*, 1998b).

Providing meat as an early complementary food is often viewed as challenging, and Faber (2007) cautioned that the cost of ASF may prevent daily consumption in areas of low socio-economic status. However, in view of the potential benefits, the addition of small, realistic amounts of ASF to plant-based diets should be encouraged (Perlas & Gibson, 2005; Daviddson, 2003). This is in line with the non-formal publication of the WHO's (2002) recommendation of one rounded teaspoon of cooked liver to be provided daily as a complementary food. Chicken liver is readily available and affordable, and was found by Perlas and Gibson (2005) to be consumed by some young children in the Philippines. Implementation of this guideline may lead to excessive vitamin A intake (Allen, 2008), but this does not form part of the scope of this literature reviews' discussion.

The findings of Vossenaar and Solomons (2012), however, suggest that one cannot assume that family foods will be able to fill the gap left by milk to meet the nutritional requirements of infants. They argue that it is only possible to meet the full recommended intake for iron from family foods if breast milk is consumed in quantities below current international recommendations. To avoid the displacement of breast milk by complementary foods in older infants, they suggest that paediatric practitioners and public policy decision-makers should seriously contemplate the need for some contribution to home fortification or pre-fortified complementary food sources that can be integrated into family foods to ensure that all the nutrient requirements are met throughout the first critical 24 months of life.

Iron-fortified foods specifically targeting young children, such as commercial infant cereals, are valuable to improve growth and decrease the rates of anaemia (Lutter & Riveriera, 2003; Faber *et al.*, 2005). The use of these products was found not to be uncommon in rural South African populations (Faber, 2004, Faber, 2007; Faber & Benadé, 2001), but the duration of consumption was on average only three months, and the quantities were on average only a quarter of the recommended portion size.

It is therefore unlikely that infants received the intended nutritional benefits from these fortified products. The high cost and unacceptable palatability were reasons cited for the low consumption (Faber & Benadé, 2001).

Although regulations for the mandatory fortification of maize meal and wheat flour came into effect in South Africa in 2003, researchers in the field of complementary foods are of the opinion that these would not have a significant enough effect to address the micronutrient deficiencies observed in infants, because of the small amounts that they consume (Faber & Benadé, 2001). A portion of 200 g raw maize meal or wheat flour is able to provide a 10-year-old child with significant amounts of vitamin A, thiamine, niacin, pyridoxine, folate, riboflavin, iron and zinc (Kruger *et al.*, 2008). It is, however, unrealistic to rely on fortification programmes, which were developed for broad populations, to meet the relatively high nutrient needs of infants, because the density of fortification appropriate for adult consumption is unlikely to be realistic for infants or young children (Krebs & Hambridge, 2007).

It becomes clear that infants' iron requirements during the complementary feeding phase can only be met by improving the diversity of complementary foods, increasing knowledge of the caregivers on dietary factors that influence iron bio-availability, large amounts of ASF, the consumption of iron-fortified foods, or oral supplementation (Giugliani & Victora, 2000; Faber, 2007). Food-based approaches should always be made the first priority when aiming to meet the micronutrient needs of infants (Allen, 2008). These approaches are cost-effective strategies to improve health and reduce morbidity and mortality in infants (Krebs & Hambridge, 2007).

2.8 Iron supplementation

Iron supplementation can reverse the anaemia of IDA and restore iron stores sufficiently, but the poorer cognitive and psychomotor functioning associated with early IDA may persist life-long (Kazal, 2002; Thorsdottir *et al.*, 2003). In developing countries, however, ID is often worsened by conditions such as malaria, helminth infestation and HIV, resulting in a cycle of anaemia and infection. On the other hand, many authors have found IDA to be protective in individuals exposed to the above-mentioned diseases (Sazawal *et al.*, 2006; Jonker *et al.*, 2012).

Bacteria and parasites rely on free iron for multiplication and growth. The acute phase response therefore protects infected subjects by reducing levels of free circulating iron (causing low serum iron concentrations), and increasing concentrations of circulating binding proteins (causing high SF concentrations) to make iron less available to these pathogens (Kung'u *et al.*, 2009).

In a review of Gordeuk *et al.* (2001) one study found that high iron stores were associated with shorter survival times in patients with HIV infection. Excess iron may have inhibited zinc absorption and consequently led to a compromised immune response (Sazawal *et al.*, 2006). None of the clinical studies included in their review, however, found a relationship between iron stores and the progression of HIV infection (Gordeuk *et al.*, 2001).

Sazawal *et al.* (2006) highlighted the potential risk of routine supplementation with iron and folic acid in populations with high rates of malaria and other infections after they had to stop an iron and folic acid supplementation trial midway because of the associated significantly higher rates of adverse events. The authors queried global recommendations to routinely supplement iron for the prevention of ID, and suggested that only children with anaemia associated with ID benefited from supplementation in terms of hospital admissions and mortality. In response to their results the WHO (2006) published guidelines stipulating that “universal iron supplementation should not be implemented without the screening of individuals for iron deficiency”.

Crowley *et al.* (2012:561) explain that there are two approaches that may be followed in terms of iron supplementation: either universal supplementation or targeted supplementation after an initial screening. In comparing the options one needs to weigh the risks of receiving a surplus of iron, when not required, versus not receiving iron when iron-deficient. In healthy infants with a normal iron status, fortification or supplementation strategies may result in the consumption of excessive amounts of iron (Hicks *et al.*, 2006). As mentioned earlier in section 2.4, the absorption of iron is regulated, but younger infants may not be able to compensate for an oversupply of iron above their requirements (Lönnerdal & Kelleher, 2007).

Zimmermann *et al.* (2010) found that the form of iron often used for supplementation and fortification purposes is poorly absorbed and results in more than 90% of the iron being passed through to the colon. The authors explain that there is no system in the gut lumen to bind free iron and consequently the growth of enteric pathogens (e.g. *Salmonella*, *Shigella*, or pathogenic *Escherichia coli*) that require iron are supported. The result is an unfavourable ratio of faecal enterobacteria to bifidobacteria and lactobacilli. Bifidobacteria and lactobacilli are beneficial barrier bacteria that play an important role in the prevention of colonisation by enteric pathogens, but do not require iron for growth (Archibald, 1983). This imbalance may increase the risk of diarrhoea or increase gut inflammation in children (Zimmermann *et al.*, 2010).

The South African National Department of Health's (Department of Health, 2010) guidelines for the management of HIV-infected children propose that a trial dose of 2 mg/kg of elemental iron be given three times daily with meals— for a trial period of three weeks— if a baseline Hb indicates anaemia. If, after three weeks, Hb concentrations have increased by ≥ 2 g/dL, IDA can be assumed and treatment should continue for three more weeks.

To avoid the increased risk of infection associated with iron supplementation that was mentioned earlier, treatment of ID should occur under simultaneous and sustained control of prevalent infections (Sazawal *et al.*, 2006). Also, more emphasis should be placed on the prevention of infections, which can markedly contribute to the prevention of ID through decreased loss of iron through blood loss (in the instance of hookworm infection), and increased absorption of dietary iron (Jonker *et al.*, 2012).

2.9 The anthropometric nutritional status of infants

Anthropometry is a relatively easy way to determine the nutritional status of individuals, and by implication the availability of proper food, because the quantity and quality of food available are often the main determinants of growth rate (Hotz & Gibson, 2001). Dietary diversity is generally associated with a child's anthropometrical nutritional status, but it is sometimes difficult to interpret

associations between dietary diversity and anthropometrical nutritional status because both are strongly linked to household socio-economic factors. Arimond and Ruel (2004) explained that families with greater incomes and resources tend to have more diverse diets, but they are also more likely to have better access to health care and better environmental conditions. Hatløy *et al* (2002) consistently found positive associations between dietary diversity and each of the nutritional status indicators examined, namely height-for-age, weight-for-age, weight-for-height, mid-upper-arm circumference and triceps skinfolds.

The WHO child growth standards are recommended to assess the growth of infants, being based on a sample of healthy breast-fed infants, and designed to describe how all children should grow rather than how certain children grew at a specified time and place (De Onis *et al.*, 2006). These standards demonstrate that healthy children around the world, who are raised in healthy environments where the recommended feeding practices are followed, grow amazingly similarly (WHO Multicentre Growth Reference Study Group, 2006). Any deviation from these standards should therefore be assumed to reflect that something has gone wrong that requires correction. Although the WHO standards recognise the adequacy and superiority of breast milk to support healthy growth and development, the standards are recommended to assess the anthropometric indicators of all children, whether they are breastfed or not (De Onis *et al.*, 2006).

Using the above-mentioned standards, underweight is reflected by a low weight-for-age (below -2 standard deviations [SD] from the median), and is a measure of both chronic and acute malnutrition, yet it cannot distinguish between the two (Cogill, 2003). Stunting is reflected by a low length-for-age (below -2SD from the median) and is a sign of chronic nutritional deficiencies, rather than shortfalls in energy intake *per se*, where the deficit often starts at a very early age (between two and four months) when complementary feeding is wrongly introduced (Faber *et al.*, 2001; Hotz & Gibson, 2001; Oelofse *et al.* 2003). Stunting does not change rapidly and may be irreversible in children older than two years. It is also associated with a number of long-term complications, including failure to reach their linear growth

potential, frequent infections and micronutrient deficiencies of particularly iron and zinc (Cogill, 2003).

Wasting is reflected by a low weight-for-height (below -2SD from the median), and is a measure of acute malnutrition that can change rapidly with changes in the availability of food or disease prevalence. Wasting is often caused by insufficient food intake, poor feeding practices, disease and infection, or mostly, a combination of these factors (Cogill, 2003). Overweight, on the other hand, is reflected by a high weight-for-length or body mass index (BMI)-for-age (above +2SD from the median) and reflects on the subject's body mass in relation to his/her chronological age (De Onis *et al.*, 2006).

A number of observational studies have found a relationship between stunting and obesity in childhood (Faber *et al.*, 2001; Kimani-Murage *et al.*, 2010; Mamabolo *et al.*, 2005). This implies that stunting may be associated with both under- and over-nutrition, which may increase these children's risk to develop chronic diseases of lifestyle later in life (Kimani-Murage *et al.*, 2010). Timaeus (2012), however, differs in opinion about such a relationship, and proposes that because the value for height is used as the numerator for stunting, as well as the denominator for obesity, random errors made by fieldworkers who measured heights could have produced negative bias in the estimates of this relationship. He applied four methods to adjust for random errors in the measurement of height and found no relationship between stunting and obesity in childhood. He emphasised that the validity of the results of those studies that found a relationship between stunting and overweight depend on the accuracy with which height was measured. He further highlighted that associations between stunting and being underweight may be masked by these biased associations, which can be avoided by rather using waist circumference or related measures to measure obesity instead of weight-for-length or BMI-for-age (Timaeus, 2012).

2.10 The relationship between growth and iron status

ID has been implicated as a cause of stunting, developmental delay, reduced cognition and impaired immunity. Bougle *et al.* (2000) suggested that even a

marginal iron status may be a limiting factor of growth, which could be improved by optimizing iron status. The results of Engle-Stone *et al.* (2013) support this finding by showing that the height-for-age Z-score and weight-for-height Z-score both independently predict one or more of the iron status indicators among children.

An infant's birth weight determines the size of his/her iron stores; higher birth weight babies have larger iron stores. Birth weight is also negatively associated with postnatal growth, where the rate of growth will determine how quickly iron stores will be depleted (Wharf *et al.*, 1997). Domellöf *et al.* (2001) compared the iron status of Honduran and Swedish infants at four months of age and found a significant difference, even though infants from both countries were exclusively breastfed. Honduran infants had a significantly lower birth weight and larger weight gain from birth to four months when compared to the Swedish infants and this catch-up growth had a negative impact on SF concentrations and placed the Honduran infants at higher risk of ID. A high birth weight therefore offers protection against ID through slower growth (Dewey *et al.* 2007). Low-birth-weight (LBW) (<2500g) infants, on the other hand, are at greatest risk of developing ID (Schulman, 1954) and sadly the prevalence of LBW infants is high in many developing countries (Davidsson, 2003).

ID often develops during periods of rapid growth because the demand for iron exceeds the supply so that iron stores are depleted gradually (Bougle *et al.*, 2000; Gunnarsson *et al.*, 2005; Dewey *et al.* 2007). Yang *et al.* (2009) investigated the prevalence and predictors of ID in fully breastfed infants at six months of age, and found that greater weight gain after birth was associated with significantly lower SF concentrations, but normal haemoglobin concentrations. The rapid growth of the first five months of life mobilised iron from iron stores (SF) to synthesise Hb, but Hb values only diminished when iron stores had been depleted for some time.

2.11 The relationship between overweight or obesity, and iron status

Obesity is associated with ID, but not with anaemia (Yanoff *et al.*, 2007). ID is significantly (approximately twice) more prevalent among obese children and obese adolescents compared to their non-obese counterparts (Baumgartner *et al.*, 2012;

Dallman *et al.*, 1980; Nead *et al.* 2004; Scheer & Guthrie, 1981). Many different factors have been proposed to explain this association, including genetic differences, physical inactivity, periods of rapid growth, increased body surface area, or low dietary iron intake (Dallman *et al.*, 1980; Miraglia del Giudice *et al.*, 2009; Nead *et al.* 2004; Pinhas-Hamiel *et al.*, 2003).

Iron balance is regulated mainly in the gastrointestinal tract through absorption, and because obese children gain weight easily, Pinhas-Hamiel *et al.* (2003) assumed that these individuals would experience no problem with iron absorption. Studies in ob/ob mice confirmed that obese mice absorb approximately twice as much iron as wild-type lean mice (Failla *et al.*, 1988); however, despite the increased absorption, concentrations of iron throughout the body were significantly lower in obese mice (Kennedy *et al.*, 1986). Menzie *et al.* (2008) showed that neither differences in iron intake, nor dietary factors known to affect iron absorption, explained the lower serum iron concentrations found in obese individuals.

Kennedy *et al.* (1986) long ago proposed that chronic obesity may alter the nutritional requirements for iron, while today researchers reason that hepcidin may explain the link between overweight and ID through an inflammation-induced increase in its expression (Yanoff *et al.*, 2007). Hepcidin is a key regulator of iron homeostasis and is produced primarily in the liver but also expressed in adipose tissue (Nemeth *et al.*, 2006; Richardson *et al.*, 2009). It effectively lowers serum iron and the bioavailability of iron by decreasing intestinal iron absorption and promoting iron binding in macrophages. Nicolas *et al.* (2002) found that mice with an inactive hepcidin gene developed iron overload, whereas transgenic mice who over-expressed hepcidin developed severe IDA.

Hepcidin expression is positively associated with increased body iron (Bekri *et al.*, 2006), and conversely the production of hepcidin is inhibited rapidly during ID, anaemia or IDA when erythropoiesis needs to be increased (Ayoya *et al.*, 2010). Iron is then absorbed from the intestine, or released from macrophages, to support the red blood cells' demands. Serum hepcidin is also positively associated with leptin concentrations, which are highly correlated with BMI (Zekanowska *et al.*, 2011).

Leptin up-regulates hepcidin expression via the same signalling pathway than interleukin-6 (Chung *et al.*, 2007), which is also higher in obese than non-obese children (Baumgartner *et al.*, 2012; Nemeth *et al.*, 2004; Richardson *et al.*, 2009). It is thus clear that both obesity and inflammation increase hepcidin expression (Nemeth *et al.*, 2004; Zimmermann *et al.*, 2008).

The adipose tissue in obese individuals acts as an endocrine organ that contributes to the inflammatory process by secreting a variety of pro-inflammatory cytokines. Richardson *et al.*, (2009) found that obese children have higher values of high sensitivity C-reactive protein than non-obese children, indicating that even very early obesity is associated with inflammation.

Cook *et al.* (2000) and Visser *et al.* (2001) all concluded that adiposity was the most important determinant of CRP levels in children aged three to 16 years old. It was also noted that children from non-Caucasian backgrounds had higher CRP concentrations than Caucasian children (Cook *et al.*, 2000). The CRP concentrations observed by both these groups of researchers can be classified as low-grade inflammation, since it ranged from 0.22 – 10 mg/L (Cook *et al.*, 2000; Visser *et al.*, 2001).

Skinner *et al.* (2010) were, however, unable to identify a relationship between low-grade inflammation ($1 > \text{CRP (mg/L)} \leq 10$) and obesity in infants and children younger than three years of age, although the very obese one-to-two-year-olds included in this study did show a trend toward elevated inflammatory markers. Their results suggest that inflammatory markers do not increase immediately with obese status. The researchers did, however, mention that the number of one-to-two-year-old children in their sample was lower than the number of children in the other age groups, and that it was therefore possible that a more balanced age distribution would have confirmed a relationship even for the one-to-two-year-olds (Skinner *et al.*, 2010).

Persistent low-grade inflammation in obese infants may not only increase their risk of metabolic and cardiovascular events later in life (Tam *et al.*, 2010), but may also predispose them to an ID similar to what has been observed in children

(Baumgartner *et al.*, 2012). If such a relationship exists, it may highlight a new need to screen for poor iron status among infants with elevated BMI (Zekanowska *et al.*, 2011), since the interactions of a double burden of malnutrition may be particularly detrimental to their development (Baumgartner *et al.*, 2012).

2.12 The relationship between gender and iron status

Another factor that appears to have an influence on iron status that will be discussed in this literature review is gender (Hay *et al.*, 2004). From the literature it was noted that girls consistently had a better iron status when compared to boys (Zetterström, 2004), which may be related to the faster growth velocity among boys, and hence the larger need for iron.

Lozoff *et al.* (2006), however, found that being male predicted having a poorer iron status, even after controlling for birth weight and growth. Thorsdottir *et al.* (2003) also found that the difference in growth between genders disappears when incremental growth is calculated as a ratio of birth size, and hence concluded that the difference in iron status between the genders cannot be explained by differences in growth rate alone. Domellöf *et al.* (2002b) and Chaparro (2008) proposed that the gender difference in iron status during infancy may be due to genetic and hormonal factors causing iron to be metabolised differently, or otherwise differences in body composition.

2.13 Conclusion

On the basis of the literature reviewed, it can be expected that rapid growth following a lower birth weight, consuming staple-based complementary diets with very poor variety and limited sources of dietary iron, consuming cow's milk in the place of breast or formula milk, consuming diluted formula or sub-optimal amounts of commercial infant cereal, being at risk of overweight, being overweight or obese, or being male, would be the major risk factors for ID among infants in the second half of their first year of life.

Even though there is still much to be learned and discovered on the iron status, inflammation and anthropometric nutritional status of infants, there is already a large body of knowledge available on the discoveries made by researchers over the past 50 years. This literature review was only able to touch briefly on many of the complexities found in the determinants of ID and IDA in infants, but with this knowledge it can be concluded that it is possible to reduce the prevalence of ID and IDA if interventions follow a life-cycle approach.

Stoltzfus (2012) recommends a strategy that deserves attention. She recommends that prevention of ID should start in utero, by ensuring an optimal iron status in pregnant women, and then continue at birth by supporting the delayed clamping of the umbilical cord to ensure that infants are born with adequate iron stores. Exclusive breastfeeding should then be practised for the first six months of age, and continued until infants reach the age of two years or beyond, while appropriate complementary foods should be introduced when the infant reaches the age of 180 days (six months)(Dewey, 2001). This will reduce the need for supplemental iron during infancy.

When needed, food-based strategies such as the increase of affordable sources of animal protein food intakes and ways to improve the bioavailability of non-haem iron should be favoured above supplementation to improve iron intake in this sub-group of the population. These approaches are preferred because they do not require screening for ID, nor pose a risk for accidental iron overdose and they appear to be safe for infants who are iron-sufficient (Szymlek-Gay *et al.*, 2009). Stoltzfus (2012:581) agrees that it is better to give iron in, or with, food at the lowest possible adequate dose (the Recommended Dietary Allowance), because in this way the iron will be absorbed more slowly and the amount of free iron in the body will be limited.

CHAPTER 3: ARTICLE



Differential ferritin interpretation methods that adjust for inflammation yield discrepant iron-deficiency prevalence

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Abstract

Objective: We re-assessed the iron-deficiency (ID) prevalence (18.3%) reported by the South African leg of the International Research on Infant Supplementation study to account for the effect of the high prevalence of acute (28.6%) and chronic (41.8%) inflammation, using data of 192 apparently healthy 4-13-month-old infants.

Research Methods and Procedures: Serum ferritin (SF) was the marker of iron status, while Alpha-1 glycoprotein and C-reactive protein determined acute and chronic inflammation status, respectively. We compared the ID prevalence obtained from four methods that adjust for inflammation: 1) excluding infants with inflammation, 2) using a higher cut-off (SF < 30 µg/L), 3) using different cut-offs for infants with inflammation (SF < 30 µg/L) versus those without inflammation (SF < 12 µg/L), and 4) adjusting SF concentrations with correction factors (CF), to a reference method (SF < 12 µg/L) not accounting for inflammation.

Results: ID prevalence ranged from 17.6% for the exclusion method to 52.1% using the higher SF cut-off, compared to the reference method (17.2%). Using two different cut-offs resulted in 31.8% and CFs in 21.9% subjects being classified as ID. The prevalence of iron-deficiency anaemia (IDA) ranged from 13.2% to 24.5%, with the lowest prevalence (12.0%) obtained from the reference method.

Conclusion(s): Our results highlight the challenge of assessing the prevalence of ID and IDA using SF in the presence of inflammation. We demonstrated that SF should be interpreted in relation to inflammation when planning iron intervention, otherwise the possibility of over- or under-supplementation poses a risk of adverse consequences in susceptible infants.

Introduction

Iron-deficiency anaemia (IDA) is the most common micronutrient deficiency in infants between the ages of 6 and 24 months [1]. These infants grow at a relatively fast rate that requires an expansion in blood volume [2], for which large amounts of iron are needed [3, 4]. Around 6 months of age breastfed infants' iron stores may become depleted, owing to the low concentration of iron in breast milk [5]. Furthermore, it is difficult to meet the iron requirements of infants from complementary foods in the second 6 months of life [6].

South African national representative data from the 1990s indicated that one child in 10 was iron-deficient (ID) and that one child in 20 had IDA [7], while more recently the National Food Consumption Survey reported that one child in 7 had low iron stores [8]. In the greater part of sub-Saharan Africa the estimated prevalence of IDA is, however, higher and ranges between 30% and 45% for children between the ages of 6 and 24 months [9]. The pooled International Research on Infant Supplementation (IRIS) study [10], which was conducted in four developing countries, reported the highest ID prevalence for Peru (38.6%) and Indonesia (34.5%), while in South African and Vietnam the prevalence of ID was 18.3% and 12.4%, respectively.

IDA has been implicated as a cause of stunting, developmental delay, reduced cognition and impaired immunity [11]. It is, however, very important to confirm IDA before initiating iron supplementation, since iron-replete infants do not have an excretion pathway for the oversupply of iron, besides through faeces, and minimal amounts in urine [12]. The excess iron will act as a pro-oxidant in their bodies,

affecting the genes that regulate their growth, which will result in reduced linear growth, head circumference and weight gain, similar to the effect of IDA [13, 14].

Although serum ferritin (SF) is the recommended measure to describe the prevalence of ID if only one indicator of iron status can be measured [15], it is well known that inflammation has an elevating effect on SF concentrations [16-18]. Therefore, SF measurements are usually coupled with the analysis of one or two acute-phase proteins (APPs) to detect whether inflammation is present, and to adjust for its elevating effect on SF, when present [15].

In areas without safe and clean water, and with poor sanitation, chronic inflammation may be caused by intestinal parasites, gastrointestinal, respiratory or other infections. Alternatively, being overweight or obese — even in children as young as 3 years of age — may contribute to inflammation through the secretion of a variety of pro-inflammatory cytokines [19-21]. Skinner [17] showed that even very obese 1-2-year-olds already had elevated inflammatory markers.

We revisited the baseline ID prevalence (based on SF) of the South African leg of the IRIS multi-centre study reported by Smuts *et al.* [22]. The IRIS researchers [22] reported an ID prevalence of 18.3% for infants ranging from 4–13 months from a rural region in the KwaZulu-Natal Province. The IRIS study only considered the influence of acute inflammation on SF as marker of iron status, at the time of the study. Recent literature, however, strongly demonstrates that the prevalence of chronic inflammation cannot be ignored, especially in an area such as KwaZulu-Natal, where the prevalence of HIV/AIDS is widespread [23] and may possibly contribute to the prevalence of chronic inflammation.

We therefore re-calculated the ID prevalence using four different methods that account for the effect of both acute and chronic inflammation on SF concentrations [15, 24]. This was compared to a reference method that does not take inflammation into account [15]. We postulated that the ID prevalence derived would vary depending on the methods chosen, and that it would be higher when accounting for both acute and chronic inflammation. If so, the differences in ID prevalence should be documented for researchers to be aware of the implications of each method in order to derive a more accurate estimation of the ID prevalence so that, in the end, appropriate interventions that avoid the risks of excessive or inadequate iron supplementation can be recommended.

We further explored whether there were differences in the distribution of APPs (markers of inflammation) between subgroups based on socio-demographic and body composition characteristics within this study population, since a notable proportion of the infants had chronic inflammation [Alpha-1 glycoprotein (AGP) \geq 1g/L, 41.8%] or acute inflammation (CRP $>$ 12 mg/L, 12.4%) [22].

Participants and Methods

Study design. Secondary, cross-sectional data analysis was performed on the baseline data of the South African trial of the IRIS study. The IRIS study was a double-blind, placebo-controlled intervention that examined the efficacy of multi-micronutrient supplementation on infants from a rural South African population.

Subjects and ethics. The study sample of 192 infants aged 4–13 months, was randomly selected to take part in the IRIS study, which lasted 6 months (April to

September, 2000), until the participants were around 18 months old. Exclusion criteria included premature birth or low birth weight (< 2.5 kg), congenital defects, chronic illness, severe wasting, fever (> 39 °C) or severe anaemia (Hb < 8g/L). The relevant ethical clearance from the Ethics Committee of the South African Medical Research Council, as well as permission from local community leaders and written informed consent from the caregivers of all participating infants, was obtained for the South African leg of the IRIS study [22].

Study area and population. The South African trial of the IRIS study was conducted in the Valley of a Thousand Hills, a rural area situated 40 km northwest of Durban, a coastal city of KwaZulu-Natal, South Africa. Around 200 000 predominantly Zulu-speaking people live scattered over the mountainous area, which is somewhat better off in terms of welfare than many other rural areas in South Africa [22].

The next two sections report on the methods that were used in the IRIS study.

Anthropometric measurements and analysis. In the original study infants' weight was measured to the nearest 50 g on a load cell operated digital scale (UC-300 Precision Health Scale), while they wore only light clothing. Recumbent body length was measured to the nearest 0.1 cm on a horizontally placed measuring board. To exclude individual variation, all anthropometric measurements were taken by one experienced nutrition monitor with more than 5 years' experience in a community-based growth-monitoring programme [22].

The IRIS researchers [22] used the United States National Center for Health Statistics median as reference to yield three measures of nutritional status. We re-

calculated the nutritional status of the infants by using the World Health Organization (WHO) 2006 [25] growth standards to express their length-for-age (HAZ) and weight-for-length z-scores (WHZ). Stunting or chronic under-nutrition was defined as a HAZ below -2 standard deviations (SD) of the reference median. Overweight was defined as a WHZ above +2 SD, and obesity as a z-score for the same indicator above +3 SD of the median of the reference population [26].

Blood collection and laboratory analysis. A paediatrician collected 3 mL of blood from each infant, in heparinised tubes, by means of antecubital venepuncture. Hemoglobin (Hb) concentrations were determined on-site in whole blood, by using the cyanomethemoglobin method and a portable photometer, as described previously in the IRIS report [22]. The rest of the blood was centrifuged immediately, and the plasma was transferred to Eppendorf tubes and frozen on the same day at -20 °C. After completion of the baseline survey the samples were stored at -80 °C until they were shipped on dry ice to Germany for the analysis of SF, CRP and AGP. All procedures were conducted as fast as possible to limit the samples' exposure to direct light. CRP and AGP were measured by means of a sandwich enzyme-linked immunosorbent assay (ELISA), and SF was also assessed with an ELISA test, as described previously for the IRIS study [22].

Methods used to re-assess ID and IDA prevalence. An Hb cut-off of < 110 g/L identified anaemia [27], whereas IDA was defined as the co-existence of anaemia and ID, which varied depending on the method used to interpret SF concentrations. CRP indicated the effect of short-term infectious status, whereas AGP measured the effect of long-term inflammatory status.

The IRIS researchers excluded all cases with elevated CRP (> 12 mg/L) at baseline from the statistical analysis for SF [22]. We, however, defined elevated APP concentrations as CRP > 5 mg/L or AGP > 1 g/L [24]. We opted to apply the commonly used cut-off values for the APPs to facilitate comparison with other studies, and to add to the limited understanding of the relationship between markers of inflammation and SF as an indicator of iron status. We then interpreted SF concentrations by applying four methods that adjust for the effect of inflammation on SF and comparing the results to the ID prevalence obtained from a reference method that did not take inflammation into account.

Our re-assessment was based upon the WHO working group's [15] recommendations together with the recently published approach of Thurnham [24]. We first raised the SF cut-off concentration that defines ID to 30 $\mu\text{g/L}$ (Higher cut-off method), because our study sample suffered from widespread inflammation [15]. The second method (Exclusion method) excluded the SF values of all individuals with elevated concentrations of CRP and/or AGP from the determination of ID prevalence and only included the SF values of the remainder of the study participants [15]. Thirdly, we proposed a different interpretation of the WHO working group's recommendation to apply the higher cut-off of 30 $\mu\text{g/L}$ only to individuals with inflammation (elevated CRP and/or elevated AGP), while applying the normal cut-off of 12 $\mu\text{g/L}$ to individuals without inflammation (Two different cutoffs method). Our fourth method adjusted individuals' SF concentrations by means of correction factors (CFs) specific to each subject's inflammatory status (CF method) [24]. For this method, individuals were categorised into four groups based on their CRP and/or AGP concentrations: 1) an apparently healthy reference group (CRP ≤ 5 mg/L

and $AGP \leq 1$ g/L), 2) an incubation group ($CRP > 5$ mg/L and $AGP \leq 1$ g/L), 3) an early convalescence group ($CRP > 5$ mg/L and $AGP > 1$ g/L), and 4) a late convalescence group ($CRP \leq 5$ mg/L and $AGP > 1$ g/L). Individual SF concentrations were then adjusted by using the relevant, group-specific CF as a multiplier and repeating the calculation to determine the prevalence of ID after correction. For the incubation group a CF of 0.77 was used and for the early and late convalescence groups CFs of 0.53 and 0.75 were used, respectively. Different CFs were needed to account for inflammation during the various stages of inflammation, because the increase in SF after infection follows a different pattern from that of either CRP or AGP. SF concentrations rise significantly within a few hours of the onset of inflammation, and concentrations remain high even after CRP concentrations have subsided, and while AGP concentrations are still elevated [24].

We wanted to determine whether associations existed between low-grade inflammation and overweight or obesity in infants. In order to be able to compare our results to a similar study [17], we excluded all subjects with $CRP > 10$ mg/L from the analysis for the weight categories. The researchers explained that CRP concentrations above 10 mg/L are indicative of infection that is not related to the subjects' weight status [17].

Statistical analysis. All statistical analyses were conducted using the 21st version of SPSS. Significance was defined as $P < 0.05$. Descriptive statistics were done for all variables. Continuous variables were visually examined for adherence to the normal distribution using Q-Q plots and histograms. Abnormally distributed variables were log-transformed for statistical analyses, or non-parametric methods were used for analyses.

The Kruskal-Wallis and chi-square tests were used to explore whether there were differences in the distribution of APPs, as markers of inflammation, between subgroups based on certain socio-demographic (i.e. age and sex) and body composition characteristics of the infants. A Bonferroni post-hoc test was used to make multiple-comparisons. We performed multiple regression analyses to determine associations between the markers of inflammation (CRP and AGP), and SF concentrations (dependent variable), while controlling for age and gender as covariates. Two-way frequency tables described the prevalence of ID, IDA and anaemia not associated with ID, for the four methods that account for inflammation, as well as for the reference method. The McNemar test for dependent data was used to analyse the differences between the ID prevalence obtained by the four methods that adjusted for inflammation, and the reference method.

Results

Characteristics of the study participants are shown in **Table 1**. The mean HAZ z-score was below the WHO growth standards [25] population median, while the mean WHZ z-score was found to be above +1. The prevalence of elevated APPs indicating inflammation was 52.6%. We determined that 11.5% of the subjects with inflammation were in the incubation (CRP > 5 mg/L and AGP ≤ 1 g/L), 17.2% in the early convalescent (CRP > 5 mg/L and AGP > 1 g/L), and 24% in the late convalescent stages (CRP ≤ 5 mg/L and AGP > 1 g/L).

TABLE 1 Characteristics of study participants

Characteristic	N*	Distribution / Prevalence
Age (mo)	191	8.37 ± 2.02
Male:female ratio (%)	192	47.4: 52.6
Length-for-age (z-score)	184	-0.73 ± 1.12
Weight-for-length (z-score)	184	1.29 ± 1.00
CRP (mg/L)	192	1.84 (0.54;6.29)
AGP (g/L)	192	0.29 (0.75;1.19)
Inflammation (% with AGP > 1g/L or CRP > 5mg/L)	192	52.6
Haemoglobin (g/L)	192	11.19 ± 1.18
Anaemia (% , Hb < 110 g/L)	192	40.1
Unadjusted SF (µg/L)	192	29.68 (15.1; 52.76)

Values are mean ± SD for variables with a normal distribution, median (IQR) for variables not normally distributed and % for frequency. *Number of participants varies because of missing values.

Table 2 shows differences in the distribution of the APPs between subgroups of socio-demographic and body composition characteristics of the participating infants. Characteristics for which literature suggested a possible link with inflammation were included. We only found significant differences in both APP concentrations between age subgroups ($P = 0.016$ and 0.031 for CRP and AGP, respectively). The Bonferroni post-hoc test showed that the significant differences in CRP concentrations were between the age groups < 6 months and 10-13 months ($P = 0.015$); while for AGP, significant differences in concentrations were observed between infants younger than 6 months and those 6-9 months ($P = 0.046$), as well as between the youngest and the oldest age groups ($P = 0.041$). Trends of higher AGP concentrations were observed in infants who were stunted ($P = 0.059$), or who drank water of questionable safety ($P = 0.058$), while female infants tended to have lower CRP concentrations than the boys ($P = 0.063$).

TABLE 2 Differences in the APPs between subgroups of socio-demographic and body composition characteristics

Background characteristics of infants	Subgroup	N	Plasma CRP (mg/L)		P*	Plasma AGP (g/L)		P*
			Median	IQR		Median	IQR	
Infant's sex	Boy	91	3.49	0.43; 5.43	0.063	0.98	0.60; 1.24	0.109
	Girl	101	2.39	0.62; 6.77		0.89	0.72; 1.15	
Infant's age (mo)	< 6	26	1.14 ^b	0.36; 3.61	0.016	0.78 ^b	0.61; 0.98	0.031
	6-9	120	1.58 ^{a,b}	0.48; 5.20		0.94 ^a	0.77; 1.18	
	10-13	45	4.44 ^a	0.78; 9.44		0.94 ^a	0.79; 1.31	
Infant's weight (kg)	Normal	120 [#] /136	1.5	0.46; 4.33	0.120	0.95	0.76; 1.28	0.131
	Overweight & Obese	40 [#] /48	1.03	0.30; 3.98		0.88	0.67; 1.08	
Infant's length (cm)	Normal	161	1.59	0.46; 6.33	0.343	0.90	0.74; 1.19	0.059
	Stunted	23	2.74	0.83; 7.37		1.06	0.92; 1.37	

Background characteristics of infants	Subgroup	N	Plasma CRP (mg/L)		P*	Plasma AGP (g/L)		P*
			Median	IQR		Median	IQR	
BF duration (mo)	Never	8	1.81	0.29; 9.98	0.429	0.81	0.70; 0.93	0.100
	< 6	52	1.37	0.44; 4.85		0.89	0.67; 1.08	
	6-9	98	1.80	0.45; 6.62		0.97	0.80; 1.30	
	10-13	31	3.97	0.73; 7.33		0.92	0.79; 1.3	
Water source	More safe (public)	177	1.84	0.48; 6.34	0.825	0.91	0.74; 1.19	0.058
	Less safe (nature)	14	1.17	0.62; 4.06		1.09	0.88; 1.56	

P* indicates the significance of the difference between mean rankings of CRP or AGP concentrations for various characteristics, using the Kruskal-Wallis and chi-square tests. Median values with different letters in superscript differed significantly ($P < 0.005$). N[#] subjects with CRP > 10 mg/L were excluded from the analysis for the weight categories to enable comparison with the results found by Skinner *et al.* [17].

Age showed a significant association with both APPs (**Table 2**) in this study and was entered as a predictor in the multiple regression analyses with SF concentration as dependent variable. Sex was also entered as a predictor into both models (CRP and AGP), based upon literature showing that girls consistently have better iron status than boys [28,29]. Both CRP ($P = 0.005$) and AGP ($P = 0.002$), as well as age and sex, were significantly associated with unadjusted SF concentrations, (both, $P < 0.005$).

Figure 1 presents the differences in the prevalence of ID, IDA, and proportion of anaemia not associated with ID for the five different methods to interpret SF concentrations.

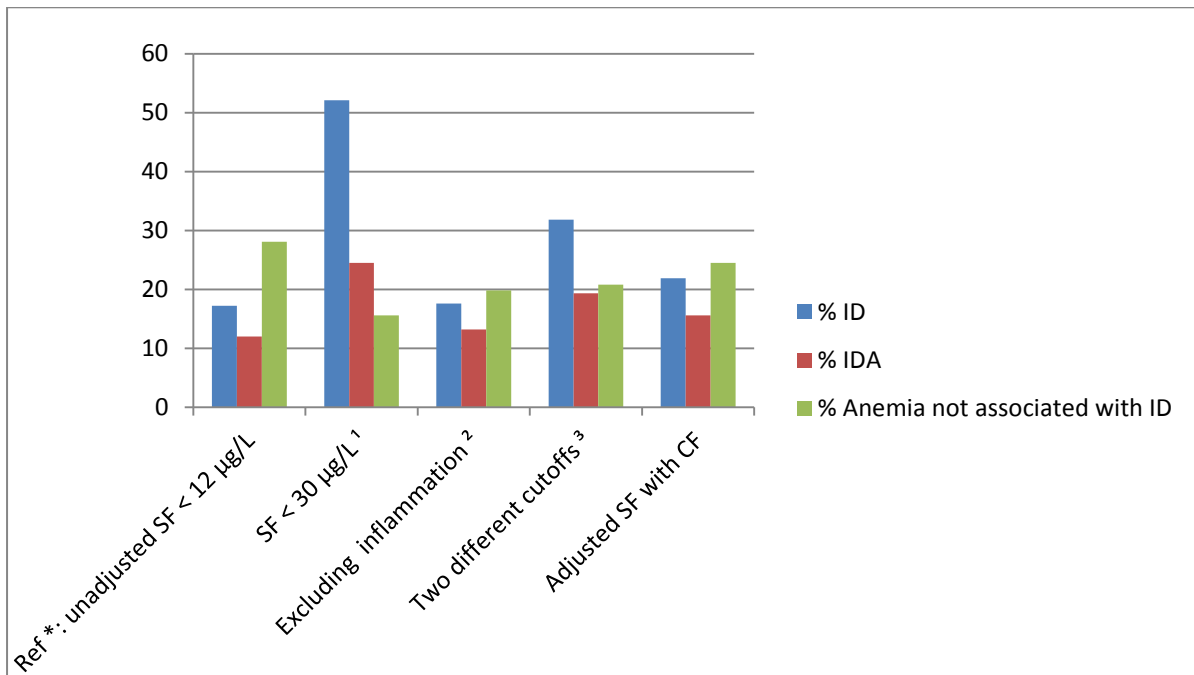


Figure 1. Differences in the prevalence of ID, IDA and anaemia not associated with ID for the five methods employed to interpret SF concentrations.

¹ Higher cut-off method; ² AGP \geq 1g/L and/or CRP > 5 mg/L; ³ Different cut-offs for SF for subjects with inflammation (30µg/L), vs. no-inflammation (12µg/L).

Compared to the reference method, the ID prevalence obtained from all the other methods was significantly higher ($P < 0.05$). The higher cut-off method classified the highest number of infants as having an ID, and the reference method the lowest number. The reference method classified 34.9% less infants as ID when compared to using the higher cut-off method, 14.6% less when compared to using two different cut-offs, and 4.7% less when compared to using the CF method. The ID prevalence from the exclusion method could not be compared to the other methods, because of differences in N. Accordingly, IDA prevalence varied from 12–24.5% for the four methods and the reference method. The contribution of IDA to anaemia varied from 29.9–61% for the different methods.

Discussion

We re-assessed the baseline ID prevalence of the South African trial of the IRIS multi-centre study, using recently proposed methods to interpret SF concentrations in the presence of both acute and chronic inflammation. The most important findings of this study were that the different methods we employed to account for the effect of inflammation on SF concentration yielded different estimates of the prevalence of ID and the proportion of anaemia associated with ID. The methods that adjusted for inflammation — higher cut-off (52.1%), exclusion method (17.6%), two different cut-offs (31.8%) and the CF method (21.9%) — found higher ID prevalence than the reference method (17.2%) that used an SF cut-off of 12 µg/L, which did not account for inflammation.

The high prevalence of inflammation, especially chronic inflammation, that we found in this sample of infants from a rural, malaria-free region in South Africa, led us to explore whether there were any differences in the distribution of the markers of inflammation (CRP and AGP) between subgroups of socio-demographic and body composition characteristics. The higher CRP and AGP concentrations in older infants may have been due to the fact that more than half of the infants studied were breastfed for six to nine months and received the immunity benefit of breast milk for this period. Over time, however, breastfeeding rates diminished and therefore the infants became more exposed to possible sources of infection, such as complementary food and other sources of fluid. Infants also become more mobile from around 9 months of age and start to explore their surroundings, which increases their exposure to infection.

Infants who were stunted or who drank water of questionable safety tended to have elevated AGP concentrations, while male infants tended to have higher CRP concentrations than females. Although overweight and obesity are potential confounders of low-grade inflammation in older children and adolescents [30], we were unable to identify a relationship between low-grade inflammation and obesity. Our results were therefore similar to those of Skinner [17], who reported that although inflammatory markers rose with increasing weight status from the age of 3 years, obesity did not affect the inflammatory status of younger children. We had no data on the HIV status of the study sample's infants or their mothers, but HIV/AIDS may have contributed to the high prevalence of chronic inflammation that we found. We base this notion on the high rate of HIV infection (36.2% among pregnant women attending antenatal clinics) that was already present at the time of the IRIS study in KwaZulu-Natal [23].

Our results show that both CRP and AGP concentrations were positively correlated with SF, with AGP being more strongly associated. Thus, it is important to measure both APPs when interpreting iron status based on SF concentrations [31], because the sensitivity of SF may be reduced if only one marker of inflammation is taken into account. We found that not accounting for inflammation when interpreting SF concentrations resulted in significantly lower ID prevalence when compared to any of the four methods that adjust for inflammation ($P < 0.005$). Our results therefore reflect the findings of Thurnham [24], who showed that the prevalence of ID is underestimated in a population with high rates of inflammation, if SF concentrations are not corrected for inflammation. Righetti *et al.* [18], who studied infants and children from the Ivory Coast, also demonstrated a significant increase in the prevalence of ID when SF concentrations were corrected for inflammation, regardless of the method of adjustment. Similar increases in ID prevalence were found by Engle-Stone [32] who studied Cameroonian households with children aged 12–59 months old.

We found a significantly higher ID prevalence when using the higher SF cut-off ($< 30 \mu\text{g/L}$) compared to the ID prevalence obtained from any of the other methods. This result is in agreement with the findings of Engle-Stone [32], who observed that the prevalence of ID was underestimated in infants with inflammation and overestimated in those without. Subjects with infection can be categorised into different phases of the inflammatory process, and the extent of elevation seen in SF concentrations varies between these phases [24].

It is therefore imprecise to use only one specific SF cut-off concentration as an international standard [33], especially since the increase that is observed in SF concentration after infection follows a different pattern from that of either CRP or AGP [24]. We agree with Engle-Stone [32] that the higher cut-off (< 30 µg/L) method should be used only to estimate the prevalence of ID if data have already been collected in a population known to have high rates of inflammation, and CRP and/or AGP were not measured. It is very important that researchers using this method, however, keep in mind that overestimations of the prevalence of ID are very likely. We furthermore postulate that this cut-off needs to be adapted, since it appears to be too high for this particular study sample.

The danger of overestimating and treating ID lies in the fact that bacteria and parasites rely on free iron for multiplication and growth. Zimmermann *et al.* [34] found that the poorly absorbable iron often used for fortification resulted in a more pathogenic gut microbiota profile in anaemic African children. They observed changes in the ratio of faecal enterobacteria to bifidobacteria and lactobacilli that relate to the iron becoming available for pathogenic bacteria in the colon to grow on [34], while the beneficial barrier bacteria do not require iron for growth [35]. To avoid the increased susceptibility to infection and poor growth associated with iron supplementation to iron-replete infants [36, 37], IDA should be confirmed before starting supplementation [38]. If iron supplementation is, however, routinely prescribed to all anaemic children it is important to treat coincident infestations or infections simultaneously [37].

When we used different SF cut-offs for infants with inflammation ($< 30 \mu\text{g/L}$) and without inflammation ($< 12 \mu\text{g/L}$), we obtained a lower ID prevalence than when employing a higher cut-off to all infants, but a higher prevalence than when excluding all infants with inflammation. This supports our assumption that the cut-off proposed by the WHO [15] for subjects with inflammation might be too high for our particular study population, leading to an overestimation of the ID prevalence. A limitation of the method using different cut-offs for subjects with inflammation and without inflammation is that it can only be applied when both APPs are measured. Excluding subjects with inflammation, on the other hand, could bias the results, as iron-deficient individuals might be more prone to infection [24] and this may lead to an underestimation of the ID prevalence [15]. Engle-Stone [32] added that excluding subjects with inflammation could substantially reduce the sample size in populations with a high prevalence of inflammation, so that they become unrepresentative of the study population. This was the case in our study, since we had to exclude more than 50% of our subjects when using the exclusion method.

By using correction factors to adjust SF concentrations, we derived an ID prevalence higher than what was previously reported for infants [22] in the same study or obtained from the reference or exclusion methods, but lower than what was found using the higher cut-off, or different cut-off methods. Using this method, researchers are able to assess the iron status of populations with high rates of inflammation while retaining the use of all data [39]. Furthermore, it enables researchers to follow recommendations of the WHO — to use SF as a marker of iron status — whilst incorporating the effects of APPs [24, 31] to provide, in our opinion, a more accurate

estimation of ID prevalence. The CFs published by Thurnham [24] can be applied to other subject populations because the author found that the elevating effect on SF concentrations were proportionate to baseline SF measurements at each stage of the infection cycle, irrespective of age or gender. The single limitation of the CF method is that it can be used only when both APPs are measured, which is not always possible in population studies subject to budget constraints.

Figure 1 shows that depending on the method applied to define ID, the prevalence of IDA in our study population ranged from 12.0% to 24.5%, while the prevalence of anaemia not associated with ID in our study population ranged from 15.6% to 28.1%. The causes of anaemia are, however, often multifactorial in settings with high rates of inflammation [40], such as this study sample, and may include nutrient deficiencies (folate or vitamin B12), malaria, helminth infestation, HIV, or certain congenital hemoglobinopathies [41, 42], for which we had no data. The results of Sazawal [37] suggest that only children with IDA benefited from iron supplementation in terms of hospital admissions and mortality. Infants with anaemia not associated with ID may even show adverse effects to iron supplementation, such as an increased susceptibility to infection or poor growth [37]. Therefore, misdiagnosis, or assuming that 50% of anaemia is due to ID [43] when specific iron status indicators are not available, may lead to inappropriate and/or ineffective treatment [32, 39].

Our study contributes to an increasingly rich literature [24, 31, 32, 39] that documents the effect of inflammation on SF, and reinforces the pivotal role of APP measurements to account for the effect of inflammation, when using SF as a marker of iron status. We recognise, however, that because we only had SF and Hb,

together with the APPs, to interpret the iron status of these infants, we were unable to conclude which of the four methods came closest to the true ID prevalence. We therefore propose that additional research, which investigates the effects of inflammation on different indicators of iron status and in various populations, is needed to be able to advise on the best measure of iron status when inflammation is highly prevalent. Furthermore, studies should aim to validate peripheral blood iron markers against bone marrow iron smears (the golden standard for assessing iron status), similar to what was conducted by Phiri [44] in Malawian children aged 6–59 months. It should, however, be noted that the collection of bone marrow iron smears are very invasive and therefore not suitable for screening purposes [44].

Finally, our results affirm that SF should rather not be used on its own as proxy indicator of ID in infant populations with widespread inflammation, because the ID prevalence will be underestimated. When APPs cannot be measured in a population known to have high rates of inflammation and SF is the only available measure of ID, we recommend that the higher cut-off method be used. The SF cut-off for this method should, however, be redefined to be more specific for different populations and particular age-categories, and for this more research is still needed. When SF is used as singular indicator of iron status, and both APP measurements are available, we strongly recommend using CF to adjust SF concentrations.

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CHAPTER 4:
GENERAL SUMMARY, CONCLUSIONS AND
RECOMMENDATIONS



4.1. Introduction

This study initially set out to explore whether any relationship existed between the iron status, markers of inflammation and the anthropometric indicators of nutritional status of apparently healthy, black infants aged four to 13 months. The WHO working group (2011a) recommend that if only one indicator of iron status can be measured, it should be serum ferritin (SF), complemented by haemoglobin (Hb). We re-analysed the baseline data of the South African leg of the International Research on Infant Supplementation (IRIS) study conducted in 2000, that used SF and Hb measurements to determine infants' iron status. The study subjects came from a rural, Zulu-speaking population in the Valley of a Thousand Hills, KwaZulu-Natal, in South Africa (Smuts *et al.*, 2005).

The very high rates of both chronic and acute inflammation that were observed in this study sample made us realise that SF, being an acute phase reactant, would have been raised in the data-set (WHO, 2011a). The richness of literature that was reported over the past four years was therefore employed as a guide on how to account for the effect of both of these types of inflammation on SF concentrations, so that a more accurate estimation of iron deficiency (ID) prevalence could be obtained.

The purpose of this chapter is therefore firstly, to synthesise the main findings of our investigation, as was discussed in chapter 3, and then to draw conclusions based on the results, with regard to the given hypotheses that:

- the prevalence of ID will differ for the four methods that account for the effect of inflammation on SF concentrations;
- the prevalence of ID will be higher after reassessment, when compared to the reference method; and
- certain socio-demographic and body composition characteristics may be significantly associated with C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) (markers of inflammation).

This chapter will be closed by reviewing the research contributions of this mini-dissertation, and including recommendations for future research and guidance to programme managers who work in the field of iron nutrition in similar rural areas and communities where inflammation is highly prevalent.

4.2. Main findings and conclusion

In the study sample of 192 apparently healthy infants with a mean age of eight months and an almost equal sex distribution, a high prevalence of both anaemia and inflammation were observed. Most infants were in the late convalescent stage of inflammation, as defined by the acute phase proteins (APPs), namely CRP for acute inflammation, and AGP for chronic inflammation. As expected, both CRP and AGP correlated significantly with SF concentrations. The regression model that included age and gender as predictors showed a stronger association between AGP and SF concentrations than between CRP and SF. Age and gender, however, were also significantly associated with SF concentrations.

It would have been valuable to know the exact causes of the chronic inflammation that was observed in this study sample. From the available data, however, it was only possible to assess the differences between APP concentrations by categories of some of the socio-demographic and body composition characteristics. Our results indicated that the prevalence of both CRP and AGP was significantly higher in infants older than six months than in younger infants. The current cross-sectional study, however, does not permit causal inferences to be made. The high rates of inflammation observed in this malaria-free region may have been related to other predictors of inflammation, including HIV/AIDS, helminth infestations, and infection of the gastrointestinal or lower respiratory tract.

The prevalence of ID varied depending on the method being applied. The highest ID prevalence was found when using the higher cut-off (SF < 30 µg/L) approach, while the lowest prevalence was found when using the reference method (SF < 12 µg/L). The ID prevalence obtained from all the methods that accounted for inflammation,

was significantly higher than the prevalence obtained from the reference method, emphasising the importance of measuring both APPs to account for both chronic and acute inflammation to avoid underestimating the ID prevalence. ID prevalence ranged from 17.6% for the exclusion method, to 52.1% using the higher SF cut-off, compared to the reference method (17.2%). Ranging in-between, the method using two different cut-offs and CFs, resulted in 31.8% and 21.9% infants being classified as ID, respectively.

In settings with high rates of inflammation the causes of anaemia are multifactorial, although the contribution of ID to anaemia is generally assumed to be 50% (DeMaeyer & Adiels-Tegman, 1985). The percentage of anaemia associated with ID, in this study varied in accordance to the ID prevalence obtained from the different methods. The higher cut-off yielded the highest prevalence of iron deficiency anaemia (IDA) of 24.5% (61% of all anaemia cases), and it was concluded that this cut-off may have been too high for this specific subject population. The reference method, on the other hand, yielded the lowest prevalence of IDA of 12% (30% of all anaemia cases) which we realised from an early stage would be inaccurate, since inflammation was not taken into account by this approach, and the study was conducted in an area known to have very high rates of inflammation. Using the correction factor (CF) method, 39% of the anaemia cases were attributable to ID, thus yielding a lower rate than what is generally expected, which in our opinion seems to be the more accurate approach to use.

In terms of iron supplementation, the risk of not receiving iron when needed needs to be weighed against the risk of receiving an excess of iron when not needed, since both these scenarios could lead to serious adverse consequences (Crowly *et al.*, 2012). Universal iron supplementation strategies may waste precious resources and pose an increased risk of infections to iron-replete subjects or subjects with anaemia that is not associated with ID, while ID subjects who are not anaemic would not be exposed to a decreased or increased risk of adverse effects when receiving iron supplements (Sazawal *et al.*, 2006).

4.3. Recommendations

- It is well known that one single indicator is not enough to describe the iron status of an individual reliably (Zimmermann *et al.*, 2008). We therefore recommend that more research using other indicators of iron status, and in different populations, be undertaken to reach consensus on the best method to assess the iron status of populations where inflammation and infection are highly prevalent.
- The results of this study suggest that the higher cut-off method may have overestimated the ID prevalence in the study participants, compared to other methods. More research is therefore needed to identify a more appropriate higher cut-off point for populations with a high prevalence of inflammation, in settings where APPs cannot be measured.
- The application of correction factors to SF concentrations may help to make a more accurate estimate of the prevalence of ID in populations where high rates of inflammation are present. This method accounts for the presence of both acute and chronic inflammation, while retaining the use of all data.
- Low-cost field screening is urgently needed so that iron interventions can be focussed on infants who are most likely to benefit from it without being exposed to any risk (Crowly *et al.*, 2012).

4.4. Conclusion

The findings of this study show that a careful assessment of the aetiology of anaemia, for each setting and population group, is of the utmost importance prior to launching national intervention programmes. In the results of this study IDA emerged as a bigger nutritional problem than previously reported for these infants; however, ID still did not contribute to 50% of all anaemia cases, as is generally accepted. We suspect that this trend would probably also be observed in other studies, conducted in areas with a high prevalence of infection or inflammation during the same time that this study was undertaken, if they should be re-analysed as well.

With recent advances in knowledge on how to deal better with the effect of inflammation on SF concentrations, we were able to derive a more precise estimation of the ID prevalence in this population. The estimation was higher than what was reported before (Smuts *et al.*, 2005), but literature demonstrates that there is more harm in overestimating the contribution of ID to anaemia than in underestimating it, especially in areas where inflammation is widespread. Although Smuts *et al.* (2005) concluded that the multiple micronutrient supplementations they tested were successful in improving the micronutrient status (and more specifically the iron status) of these South African infants; this study's secondary analysis demonstrates that the outcome may have been even more desirable, because more infants, being ID, would have responded positively to iron supplementation.

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ADDENDA



ADDENDUM 1: NUTRITION, AUTHORS GUIDELINES

ADDENDUM 2: STATISTICAL CONSULTATION SERVICES

ADDENDUM 1: NUTRITION, AUTHORS GUIDELINES

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Acknowledgments

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Article

Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;266:497-509.

Article in Book
 Johnson RK. Energy. In: Mahan LK, Escott-Stump S, editors. Krause's food, nutrition & diet therapy. 10th ed. Philadelphia: W.B. Saunders; 2000, p. 19-30.

Book
 Kline P. The handbook of psychological testing. London: Routledge; 1993.

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23 October 2013

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We hereby confirm that the Statistical Consultation Services of the North-West University had advised the student on the analyses of the data. However, any opinion, findings or recommendations expressed in this document are those of the author and the Statistical Consultation Services of NWU (Potchefstroom Campus) do not accept responsibility for the statistical correctness of the results reported. Kind regards

A handwritten signature in black ink, appearing to read 'S M Ellis'.

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Head: Statistical Consultation Services

I.D. NO. 851118 0042 08 5



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