Iron status of infants and their mothers prior to starting complementary feeding

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Abstract

Background: Women and children are vulnerable to developing iron deficiency (ID) due to their high iron requirements. In infancy, ID may cause irreversible deficits in cognitive functioning as well as adversely impacting immune functioning, growth, and oxygen transport. The iron store accumulated in utero is estimated to be depleted by 4-6 months of age, at which time infants are at risk of ID if insufficient iron is supplied from complementary foods. While there has been much research on the iron status of infants over six months old, little is known about the iron status of younger infants, particularly in New Zealand. Maternal postpartum anaemia is associated with a reduced quality of life and can adversely affect the bond between mother and child. The postpartum period is considered a time of low ID risk and is an opportune time to replenish iron stores lost during pregnancy. However, international studies have shown that some women are not able to adequately replace these losses and remain ID throughout the first postpartum year. Despite this evidence, only one New Zealand study has investigated maternal iron status at six months postpartum. Additionally, while maternal and infant iron status is likely correlated during pregnancy and the newborn period, there is a paucity of evidence regarding the existence of a relationship at mid-infancy.

Objectives: To determine the iron status of infants and mothers, to investigate the relationship between maternal and infant iron status and to determine the differences in infant iron status according to mode of milk feeding, prior to commencing complementary feeding.

Methods: This study reports the baseline iron status of 133 mother-infant pairs from a randomised controlled trial. Term infants, 3-6 months of age who had not yet started complementary feeding, and their mothers, were included in the analysis. Haemoglobin (Hb) and serum ferritin (SF) were measured to determine iron status alongside the inflammatory marker, C-reactive protein. Infant anthropometric measures were taken, and demographic and dietary information was obtained via questionnaires. Pearson's and Spearman's rho correlations determined the relationship between maternal and infant iron status. One-way ANOVA and Kruskal-Wallis tests determined the differences in infant iron status according to mode of milk feeding.

Results: Most infants (93.2%; SF ≥10 µg/L, Hb ≥110 g/L) and mothers (80.5%; SF ≥15 µg/L, Hb ≥120 g/L) were iron-replete. No infants had ID (SF <10 µg/L, Hb ≥110 g/L) or iron deficiency anaemia (SF <10 µg/L, Hb <110 g/L) but 6.8% had anaemia without ID (SF ≥10 µg/L, Hb <110 g/L). One mother had ID (0.8%; SF <15 µg/L, Hb ≥120 g/L), 9.8% had mild ID (SF <30 µg/L, Hb ≥120 g/L), 7.5% had SF ≥150 µg/L indicating iron overload and 1.5% had anaemia without ID (SF ≥15 µg/L, Hb <120 g/L). There was a weak positive relationship between maternal and infant serum ferritin (r=0.19, P(two-tailed)=0.03), however, no relationship between maternal and infant haemoglobin (P=0.91) was found. Infants fed breast milk only, infant formula only or mixed fed did not have significantly different serum ferritin (P=0.92) or haemoglobin (P=0.50) concentrations.

Conclusion: Prior to starting complementary feeding, most mothers and their infants were iron-sufficient, and their iron stores were weakly correlated at 3-6 months postpartum. Additionally, infant iron status did not differ by the type of milk infants were fed. Future research should focus on understanding the complex relationship between maternal and infant iron status. Furthermore, the iron status of infants and mothers from diverse socioeconomic and ethnic backgrounds, as well as those experiencing food insecurity, should be investigated, as these groups have a greater risk of poor iron status.

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List of Abbreviations

Abbreviation or Symbol	Definition
AI	Adequate intake
BM	Breast milk
BF	Breastfeeding
CF	Complementary feeding
CHr	Reticulocyte haemoglobin
CI	Confidence interval
CRP	C-reactive protein
EBF	Exclusive breastfeeding
ESPGHAN CoN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition - Committee on Nutrition
IF	Infant formula
IOM	Institute of Medicine
GUiNZ	Growing Up in New Zealand Study
Hb	Haemoglobin
MCV	Mean corpuscular volume
NZ	New Zealand
OR	Odds ratio
RDW	Red cell distribution width
RCT	Randomised controlled trial
RDI	Recommended dietary intake
SF	Serum ferritin
sTfR	Serum transferrin receptor
TS	Transferrin saturation
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organisation
ZPP	Zinc protoporphyrin
>	Greater than
≥	Greater than or equal to
≤	Less than or equal to
<	Less than

~	Approximately
±	Plus-minus
%	Percentage
mg	Milligrams
mg/d	Milligrams per day
mg/L	Milligrams per litre
mg/kg	Milligrams per kilogram
µg	Micrograms
µg/L	Micrograms per litre

Chapter 1: Introduction

1.1 Background

The period from preconception, through to postpartum and infancy are crucial times to optimise the health and nutritional status of both women and children. Nutrition during pregnancy and the first 1000 days influences health outcomes later in life (Burke, Leon, & Suchdev, 2014). Notably, iron deficiency (ID) may irreversibly alter the development of infant brain structures and thus cause lasting deficits in cognitive function (Coad & Pedley, 2014).

Iron deficiency and iron deficiency anaemia (IDA) disproportionately affect women and children. Worldwide, approximately 800 million women and children have IDA (WHO, 2017). While more people suffer from ID in developing countries, it remains a public health issue in many developed countries, including New Zealand (NZ) (WHO, 2021a). The World Health Organisation (WHO) estimates the prevalence of anaemia for NZ pregnant women and children to be 16% and 15%, respectively (WHO, 2021b), half of which is estimated to be due to ID (WHO, 2017).

Iron deficiency anaemia occurs due to the severe depletion of iron stores which reduces haemoglobin synthesis (Qasem, Fenton, & Friel, 2015). This results in symptoms such as fatigue, reduced cognitive function, pallor and poor immunity (Coad & Pedley, 2014; Percy, Mansour, & Fraser, 2017). These symptoms can also occur in ID without anaemia.

Women are vulnerable to developing ID with >20% of women experiencing ID during their reproductive lives (Percy et al., 2017). Iron deficiency is common in women of reproductive age as they need a high dietary iron intake to replace the iron lost in menstruation (Heath, Skeaff, Williams, & Gibson, 2001). Additionally, women tend to have diets low in meat, fish, and/or poultry, which are good sources of well absorbed haem iron (Beck, Conlon, et al., 2012) . Other dietary components may also enhance (i.e., vitamin C) or inhibit (i.e., calcium and phytate) the absorption of iron, particularly non-haem iron from plant foods, if they are consumed at the same time (Lynch et al., 2018). During pregnancy, iron requirements are even higher than in the non-pregnant state due to an increase in maternal blood volume and to ensure fetal and placental

iron needs are meet (WHO, 2017). Approximately 20% of pregnant women in developed countries develop IDA and women who enter pregnancy with suboptimal iron stores are at an increased risk of this (WHO, 2015). Iron deficiency during pregnancy adversely effects both mother and child by increasing the risk of maternal and perinatal mortality and morbidity, having a low birth weight or premature baby, reduced accretion of fetal iron stores and postpartum ID (Dewey & Chaparro, 2007; Percy et al., 2017; WHO, 2017). Postpartum anaemia is associated with a reduced quality of life and poor child development (Beard et al., 2005; Milman, 2011; Perez et al., 2005).

Optimal iron status is essential for the growth and development of infants. During the first year of life, infants triple their birth weight and blood volume (Cormack, 2013; Grant, Wall, Brewster, et al., 2007); this rapid growth and development means that iron requirements are higher during infancy than at any other stage of life, leaving infants vulnerable to developing ID (Moy, 2006). Iron deficiency, with or without anaemia, can adversely affect psychomotor development and cognitive function (Coad & Pedley, 2014). These effects appear to persist into childhood and adolescence, even after iron repletion (Georgieff, 2011). Hence, it is critical that ID is avoided during infancy.

Prevention of infant ID starts during fetal development. During pregnancy, iron stores accumulate in the fetus, with 80% of this accretion happening in the third trimester (Baker, Greer, & The Committee on Nutriton, 2010). In healthy, term infants (>2500g, 37-42 weeks gestation) this iron store is estimated to last approximately four to six months (Berglund & Domellöf, 2021). This endogenous iron combined with the small amount of iron supplied by breast milk is sufficient to meet infant's requirements. However, once iron stores are depleted, infants must dramatically increase their dietary iron intake to match their high iron needs for growth and development. Hence, complementary feeding of iron-rich foods is recommended to begin at around six months of age (Lopez, Cacoub, Macdougall, & Peyrin-Biroulet, 2016).

While the benefits of breastfeeding are undeniable, breastfed infants are more likely to have a lower iron status (Chen et al., 2020) and have an increased risk of ID (Hirata, Kusakawa, Ohde, Yamanaka, & Yoda, 2017) when compared to formula fed infants. This is of particular importance if the iron endowment from birth was poor, as this is a

major determinant of the degree of protection infants have against ID (Beard, deRegnier, Shaw, Rao, & Georgieff, 2007). Grant et al. (2007) found that the risk of developing ID increased 2.5-fold if breastfeeding was continued beyond seven months.

While there has been much research on the iron status of infants over six months old, little is known about the iron status of younger infants, particularly in NZ. Furthermore, the most recent study investigating young NZ children's iron status is now 14 years old (Grant et al., 2007). The average age of children in this study was 15 months, therefore it is unlikely to accurately represent the iron status of young infants.

1.2 Purpose of the Study

The purpose of the study is to determine the iron status of infants prior to commencing complementary feeding, at a time when iron stores may be depleted. It will also look at maternal iron status during the postpartum period, the relationship between maternal and infant iron status and determine if infant iron status differs according to the type of milk infants are fed.

The postpartum period is an opportune time to replenish iron stores after pregnancy. Postpartum amenorrhoea and the minimal iron lost to breast milk results in a very low dietary iron requirement for mothers; therefore, the postpartum period is thought of as a time of low risk for ID. However, studies have shown that women can still be ID during the first postpartum year (Bodnar, Cogswell, & Scanlon, 2002; Finkelstein, O'Brien, Abrams, & Zavaleta, 2013; Jin et al., 2021). Only one study has assessed maternal iron status at six months postpartum in NZ (Jin et al., 2021). Hence, more studies are needed to confirm the postpartum iron status of NZ mothers.

The relationship between maternal iron status in pregnancy or at delivery and that of her newborn child has been well investigated. However, the relationship between maternal and infant iron status prior to complementary feeding is not as well understood. Therefore, this thesis will add further insight into the relationship between maternal and infant iron status at this time.

1.3 Aim

To determine the iron status of infants and mothers prior to commencing complementary feeding.

1.3.1 Objectives

1. To determine infants' iron status prior to commencement of complementary feeding.
2. To determine mothers' iron status prior to her infant commencing complementary feeding.
3. To investigate the relationship between the iron status of mothers and their infants.
4. To determine if infant iron status differs according to the mode of milk feeding.

1.3.2 Hypotheses

1. Infants will be iron replete prior to starting complementary feeding as term infants are estimated to have sufficient iron stores until approximately 4-6 months of age.
2. Mothers will be iron replete prior to her infant starting complementary feeding as postpartum amenorrhoea is likely to result in a replenishment of iron stores that were depleted during pregnancy.
3. There will be a relationship between maternal and infant iron status.
4. Infants fed breast milk only will have a lower iron status than those fed infant formula (exclusively and in combination with breast milk).

1.4 Thesis Structure

This thesis is divided into four chapters. **Chapter One** is an introduction to the background and purpose of the study. It includes the aim, objectives, hypotheses and researcher's contributions. **Chapter Two** is a literature review of the most up to date and relevant research in the field of infant and maternal iron status. **Chapter Three** is

the research manuscript which includes the abstract, introduction, methods, results and discussion of findings. The final chapter, **Chapter Four** is the concluding chapter that states how the aim and objectives have been met and acknowledges the impact this research may have on infant and maternal nutrition and future research recommendations. The **appendices** include the recruitment poster, participant information sheet, and questionnaires.

1.5 Researcher Contributions

Table 1.1 Summary of researcher's contributions to study

Author	Contribution to Thesis
Lesley Savage MSc Nutrition and Dietetic candidate	Primary author of the thesis and involved in all study components including recruitment and data collection for the primary study 'Vegetables as first foods for babies', co-designing the iron supplement questionnaire, literature review, statistical analysis and interpretation of results.
Associate Professor Cath Conlon Primary Academic Supervisor	Academic supervisor, developed the study design and part of the team who reviewed the study questionnaires. Advised about data analysis, assisted in dissemination. Revised and approved the thesis chapters and manuscript.
Professor Pamela von Hurst Co-Supervisor	Co-supervisor, developed the study design and part of the team who reviewed the study questionnaires. Advised about data analysis, assisted in dissemination. Revised and approved the thesis chapters and manuscript.
Jeanette Rapson NZRD, PhD candidate	Applied for ethics, designed the research and collected data for the primary study 'Vegetables as first foods for babies'.
Owen Mugridge Lab Manager	Phlebotomist, assisted with data collection.

Dr Hajar Mazahery School of Health Sciences	Assisted with statistical analysis and part of the team who reviewed the iron supplement questionnaire.

Chapter 2: Literature Review

2.0 Introduction

This chapter reviews the current literature on the topic of iron with a focus on the iron status of infants and women, iron metabolism, the clinical significance and factors that increase the risk of iron deficiency. PubMed, Google Scholar, Web of Science and Massey Discover were searched using different combinations of the search terms below (Figure 2.1), derived from the study objectives. Reference lists from relevant articles were also searched.

Date searched: January 2021 – September 2021

Search criteria:
"Iron status" OR iron OR anaemia OR "iron deficiency anaemia" OR "iron deficiency" OR "iron overload" OR relationship
Baby OR babies OR infant
Female OR woman OR postpartum OR postnatal OR "women of reproductive age" OR maternal
Lactation OR breastfeeding OR mother OR "infant formula" OR milk OR "human milk"
Weaning OR "complementary feeding" OR "first foods" OR solids
"Socioeconomic status" OR "low-income"
"New Zealand" OR "developed countries"

Filters: Past 5 years, Past 10 years, Nutrition and Dietetics

Electronic databases: Massey Discover, Pubmed, Google Scholar, Web of Science, WHO

Figure 2.1 Search strategy

2.1 Iron and its Functions

Iron is a mineral and an essential component of haemoglobin in red blood cells and of myoglobin in muscles (Lopez et al., 2016). It is needed for many functions within the body including metabolism, oxygen transport, DNA synthesis, immune function, growth and cognition (Helman, Anderson, & Frazer, 2019; Institute of Medicine, 2001; Mirza, Abdul-Kadir, Breymann, Fraser, & Taher, 2018; Qasem et al., 2015; WHO, 2020b).

2.2 Dietary Sources of Iron

Iron is found primarily in haem and non-haem forms. Haem iron is derived from myoglobin and haemoglobin in meat, fish and poultry (Anderson & Frazer, 2017; Institute of Medicine, 2001), whereas non-haem iron is found in animal and plant sources such as cereals, pulses, vegetables and meat (Coad & Pedley, 2014).

In the first six months of life, breast milk and/or infant formula is the main source of nutrition for infants and hence is their main source of exogenous iron. Infant formula contains non-haem iron (ferrous sulfate) (Collard, 2009; Qasem & Friel, 2015), and while the form of iron in breast milk is not well described, haem iron is usually absent (Collard, 2009; Helman et al., 2019).

2.3 Iron Bioavailability

Haem iron is tightly held inside a protoporphyrin ring making it inaccessible to factors that could inhibit its absorption, hence haem iron is highly bioavailable (Anderson & Frazer, 2017; Institute of Medicine, 2001). Absorption of haem iron is estimated to be between 15-35% (Lopez et al., 2016; Ministry of Health, 2008).

Non-haem iron is less bioavailable as its absorption can be influenced by dietary and intestinal factors (Anderson & Frazer, 2017). Absorption of non-haem iron is estimated to be between 5-15% (Heath & Fairweather-Tait, 2003). Non-haem iron absorption can be enhanced by dietary acids such as ascorbic and citric acid, the low pH of the stomach, and a component in meat, fish and poultry (Lynch et al., 2018). These factors, along with duodenal cytochrome B (DcytB), reduce non-haem iron from ferric

(Fe^{3+}) to the more soluble, ferrous (Fe^{2+}) form, which is needed for absorption into enterocytes (Anderson & Frazer, 2017; Cheng & Juul, 2011). Absorption is inhibited by dietary components such as phytic acid, calcium and polyphenols (Lynch et al., 2018).

Iron bioavailability in infant formula is low (~10%) compared to breast milk (~50%) (Lönnerdal, 2017a; Ministry of Health, 2008). The high concentration of lactoferrin in breast milk may contribute to its high iron bioavailability (Helman et al., 2019). Lactoferrin is a glycoprotein that binds iron and may increase its absorption by allowing lactoferrin-bound iron to be absorbed through the lactoferrin receptor-mediated pathway in the gut, and therefore providing an additional route of iron absorption (Griffin, 2020). The low iron bioavailability in infant formula may be partially due to the inhibitory effect of cow's milk proteins (Qasem & Friel, 2015). To compensate, infant formula is fortified with a high concentration of iron (7-12 mg/L), which overall results in more iron being absorbed from formula than breast milk (Table 2.1) (Institute of Medicine, 2001; Lönnerdal, 2017a; Ministry of Health, 2008).

Table 2.1 Iron absorption from breast milk and infant formula

	Iron concentration (mg/L)	Absorption (%)	Estimated iron absorption (mg/L)
Breast milk	0.3	50	0.15
Iron fortified infant formula	7	10	0.7
	12	10	1.2

Source: Adapted from Lönnerdal (2017a).

2.4 Iron Requirements

Healthy term infants are born with a total iron store of 75 mg/kg (Widdowson & Spray, 1951) which should be sufficient to cover their iron needs until four to six months of age (Friel, Qasem, & Cai, 2018). Hence, infants require minimal dietary iron (0.2 mg/day) before this time (Institute of Medicine, 2001). From 7-12 months of age, iron requirements increase dramatically to 11mg/day (RDI) (National Health and Medical Research Council Australian Government Department of Health and Ageing New Zealand Ministry of Health, 2006), based on the assumption that endogenous iron stores have been exhausted (Burke et al., 2014; Moy, 2006). At this stage, iron

requirements per kilogram of body weight (0.9-1.3 mg/kg/day) are higher than at any other stage of life (Domellöf et al., 2014). Hence, infants are highly susceptible to iron deficiency, especially if dietary iron intake is inadequate (Table 2.2).

Iron requirements in the non-pregnant state reflect iron losses, predominantly through menstruation (Table 2.2). Figure 2.2 shows that iron requirements increase dramatically during pregnancy and are lowest during lactation. The iron requirement during lactation covers the small amount of iron excreted into breast milk and reflects the amenorrhoeic state (WHO, 2017). However, iron needs may still be high if ID was experienced during pregnancy or if severe blood loss occurred during childbirth (Milman, 2011).

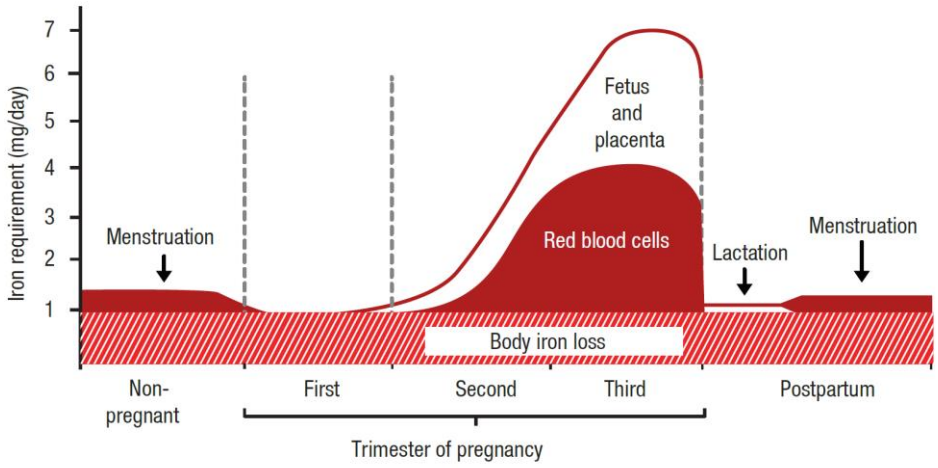


Figure 2.2 Estimated daily iron requirements for non-pregnant women, pregnant and postpartum women. Original figure from Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. Oxford, United Kingdom: Blackwell Scientific Publications, 1979 - accessed in Bothwell (2000).

Table 2.2 Iron requirements for infants and women

	Age	Iron Requirement (mg/d)
Infants	0-6 months	0.2
	7-12 months	11
Premenopausal Women	19-50 years	18
Pregnancy	19-50 years	27
Lactation	19-50 years	9

Source: National Health and Medical Research Council Australian Government Department of Health and Ageing New Zealand Ministry of Health (2006). All figures reported as RDI, except infants 0-6 months reported as AI.

2.5 Iron Metabolism

2.5.1 Absorption and Transport

Iron is primarily absorbed from the duodenum and proximal jejunum (Anderson & Frazer, 2017). Non-haem iron (Ferric - Fe^{3+}) is first reduced to ferrous (Fe^{2+}) iron, by acid or the ferroxidase enzymes, such as DcytB, on the apical surface of enterocytes and absorbed via the transmembrane protein, divalent metal transporter 1 (DMT1) (Collard, 2009; Institute of Medicine, 2001). The mechanism of haem iron absorption is still unclear, however, two mechanisms have been proposed. Haem iron may be absorbed into enterocytes via endocytosis (Anderson & Frazer, 2017) or transported by a transport protein such as haem carrier protein 1 (Friel et al., 2018). Once inside the enterocyte, haem iron is released by haem oxygenases (Anderson & Frazer, 2017). Haem and non-haem iron enter an intracellular iron pool, where it can be used for cellular metabolism, stored as ferritin or transported to the basolateral membrane of enterocytes and into plasma via ferroportin (FPN1) (Cheng & Juul, 2011; Collard, 2009). Once in the bloodstream, iron is oxidised back to Fe^{3+} by membrane-bound ferroxidase enzymes, such as hephaestin, so that it can bind to transferrin. Iron is then transported around the body to tissues, such as the bone marrow, to aid in erythropoiesis. Body tissues requiring iron have transferrin receptors on their cell membranes which bind transferrin and allow iron to enter the cell by endocytosis (Cheng & Juul, 2011; Institute of Medicine, 2001).

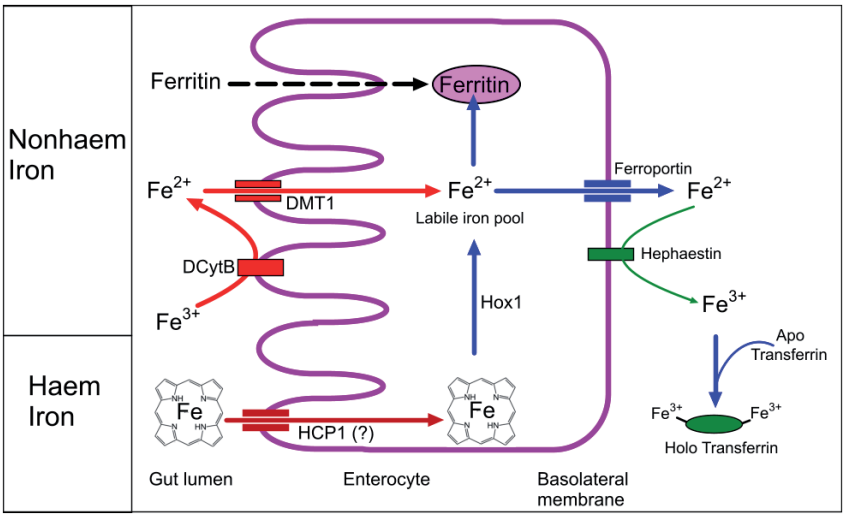


Figure 2.3 Diagram of iron absorption from the intestines.
DMT1 - Divalent metal transporter 1; DCytB - Duodenal cytochrome B; Hox1 - haem oxygenase-1; HCP1 - haem carrier protein 1. Source: Coad & Pedley, 2014.

2.5.2 Recycling and Storage

Most (90%) of the daily iron requirement is met by recycling iron (Coad & Conlon, 2011; Coad & Pedley, 2014). Macrophages export iron from senescent erythrocytes via ferroportin, iron is then bound by transferrin and can be used or stored (Collard, 2009; Qasem & Friel, 2015). The main storage sites for iron are the liver, enterocytes, spleen and bone marrow, where iron is stored as ferritin or hemosiderin (Institute of Medicine, 2001). Iron stored in enterocytes are lost as these cells are shed during intestinal mucosal cell turnover and excreted in faeces (Domellöf, Lönnerdal, Abrams, & Hernell, 2002).

2.5.3 Iron Homeostasis

Hepcidin, a peptide hormone, is the primary regulator of iron homeostasis (Berglund, Chmielewska, Domellöf, & Andersson, 2021). It controls iron absorption, recycling and availability to body tissues (Lynch et al., 2018). Hepcidin expression is controlled by iron status, erythropoiesis and inflammation. When iron stores are high, hepcidin (predominantly produced by hepatocytes) is upregulated. Hepcidin binds to FPN1, inducing its internationalisation and degradation, and decreasing iron release from enterocytes, hepatocytes and macrophages into the bloodstream (Lopez et al., 2016; Qasem et al., 2015). Hepcidin upregulation also results in decreased synthesis of DMT1 to reduce intestinal iron absorption (Lynch et al., 2018). Conversely, hepcidin production decreases when iron stores are low to allow greater iron absorption and

availability to body tissues (Lopez et al., 2016). Therefore, iron absorption in iron-sufficient/overloaded individuals will be minimal while iron deficient individuals will have maximal absorptive capacity. This is the body's main mechanism for controlling iron status within a normal range. Increased erythropoiesis results in reduced hepcidin production to ensure iron is available for this process. This effect overrides the control by iron status. Inflammation increases hepcidin production thereby reducing iron bioavailability (Lynch et al., 2018). Transferrin receptors control cellular uptake of iron by up- or down-regulating their expression on the cell surface. When intracellular iron concentration is low, transferrin receptor expression is increased to allow more iron to enter the cell. In states of iron sufficiency or overload, transferrin receptor expression is reduced (Cheng & Juul, 2011).

During the second and third trimesters of pregnancy, hepcidin production is decreased to allow for greater iron absorption, increased iron recycling and the release of maternal iron stores (Cheng & Juul, 2011; Cross, Prentice, & Cerami, 2020; Sangkhae et al., 2020). The placenta regulates iron transfer to the fetus and therefore determines the size of fetal iron endowment. A recent study by Sangkhae et al. (2020) investigated maternal iron status during pregnancy and its effect on regulatory mechanisms of iron transfer to the fetus by using mice models and in vitro human placentas. This study indicated that during mild to moderate maternal ID, placental transferrin receptor expression increases to ensure sufficient fetal iron transfer but FPN1 remains unchanged. Interestingly, they found that in maternal IDA, FPN1 expression decreased, reducing iron transfer from the placenta to the fetus. Therefore, favouring placental iron homeostasis to ensure its continued functioning, over fetal iron accruement. Hence, maternal IDA may result in a reduced fetal iron endowment through reduced iron transfer to the fetus. However, more studies are needed to confirm these findings.

The control of iron homeostasis in infants is not fully understood (Berglund & Domellöf, 2021). Some regulatory mechanisms that are present in adults may still be developing during infancy (Berglund & Domellöf, 2021). A study by Domellöf et al. (2001) found that when infants <6 months old were given iron supplements, all infants had similar increases in haemoglobin despite differences in iron status. However, after six months of age, the ID infants had greater increases in haemoglobin than the iron-replete infants, suggesting that iron status does not regulate absorption until late infancy.

Conversely, recent studies have shown hepcidin regulation to be active in infants <6 months old. Neonates have a high concentration of hepcidin, thought to be induced by the inflammatory effect of childbirth. This causes hypoferremia and may protect the neonate from infection while their immune system is immature (Cross et al., 2020; Núñez, Sakamoto, & Soares, 2018). Berglund et al. (2021) showed that hepcidin is responsive to iron stores at four months of age, increasing with high iron stores and decreasing with low stores. Uyoga et al. (2020) also found hepcidin to be responsive to iron supplementation in infants 5-14 months old. Furthermore, hepcidin concentration is thought to decrease throughout infancy in order to release stored iron, absorb dietary iron and prevent ID (Qasem et al., 2015).

2.6 Excess Iron and Toxicity

Too much iron can be detrimental. Free iron is a pro-oxidant that can form harmful reactive oxygen species via the Haber-Wiess and Fenton reactions (Lönnerdal, 2017a). Hence, excessive iron can damage lipids, DNA, and proteins (Cheng & Juul, 2011; Domellöf, Thorsdottir, & Thorstensen, 2013; Helman et al., 2019), resulting in tissue fibrosis, dysfunction (Anderson & Frazer, 2017) and adverse impacts on infant brain development (Janbek, Sarki, Specht, & Heitmann, 2019). High iron intakes can also cause other nutrient deficiencies, such as copper, calcium and zinc, as they compete with iron for absorption by DMT1 (Coad & Pedley, 2014; Domellöf et al., 2013; Lönnerdal, 2017a). The human body is not able to actively excrete iron (Coad & Pedley, 2014; Helman et al., 2019), increasing the risk of iron overload if absorption is not tightly regulated. Other mechanisms to protect the body from iron's pro-oxidant potential include storing excess iron in ferritin, transporting iron as ferric iron (less reactive state), and sequestering iron in cells thereby reducing serum iron levels (Anderson & Frazer, 2017).

2.7 Definitions

2.7.1 Anaemia, Iron Deficiency and Iron Deficiency Anaemia

Anaemia occurs when the concentration of red blood cells or the haemoglobin concentration within them is lower than normal (WHO, 2017). This results in a reduced

capacity to transport oxygen around the body (WHO, 2011a) and the presentation of clinical symptoms such as fatigue (WHO, 2017). Anaemia has three main causes including blood loss, haemolysis and insufficient erythropoiesis (WHO, 2017).

Iron deficiency is a state of depleted iron stores (Lopez et al., 2016) leading to a reduced supply of iron to tissues. If iron stores continue to deplete it can lead to iron deficiency anaemia (IDA) – where haemoglobin production is compromised and oxygen delivery to tissues is reduced (Qasem et al., 2015). Iron deficiency anaemia results in microcytic (small) and hypochromic (pale) red blood cells due to a reduced concentration of haemoglobin (WHO, 2017).

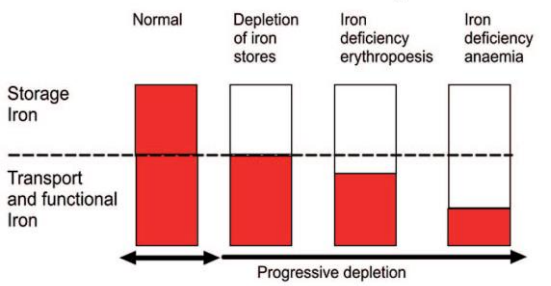
2.8.3 Iron Sufficiency and Iron Overload

Iron sufficiency is defined as having enough iron to sustain normal physiological functions (Baker et al., 2010). Iron overload is an accumulation of excess iron in body tissues and is characterised by high levels of serum ferritin (Baker et al., 2010). Iron overload is usually caused by genetic conditions such as hereditary haemochromatosis or thalassaemia that affects iron absorption or erythropoiesis - rather than excessive dietary intake (Heath & Fairweather-Tait, 2003). The early symptoms of iron overload are non-specific and include fatigue and depression hence, symptoms of iron excess may be confused with ID (Heath & Fairweather-Tait, 2003). Iron overload can result in an increased risk of heart disease, liver cirrhosis, cancer and diabetes (Heath & Fairweather-Tait, 2003; Wood, Skoien, & Powell, 2009).

2.8 Iron Deficiency Stages

Iron status is a continuum from IDA to iron overload. Within ID there are three stages, mild ID, marginal iron-deficiency and IDA (Coad & Pedley, 2014). Mild iron deficiency (or iron depletion) occurs when iron stores decrease due to a negative iron balance and is characterised by a decrease in serum ferritin. Marginal iron deficiency (or iron deficient erythropoiesis) results in depleted iron stores and a decreased production of iron-dependent proteins, however haemoglobin synthesis continues (Coad & Pedley, 2014). Iron deficiency anaemia is characterised by severely depleted iron stores,

decreased iron-dependent proteins and low haemoglobin levels (Coad & Pedley, 2014).



Iron status	Normal	Mild deficiency	Marginal deficiency	Severe deficiency
Haemoglobin	Normal	Normal	Normal	Low
Other Fe functions	Normal	Normal/low	Low	Low
Stores (SF)	Normal	Normal/low	Low	Low

Figure 2.4 Iron deficiency stages. Source: Coad & Pedley, 2014.

2.9 Iron Biomarkers and Cut-off Values

Early identification of ID is important to prevent the progression to IDA (Coad & Pedley, 2014). The best measure of ID in adults is to test bone marrow for its iron content (Lopez et al., 2016; Moy, 2006). However, this is invasive and impractical in most clinical settings (Lopez et al., 2016). It is also less useful in infancy, as bone marrow stores are minimal until adolescence (Grant, Wall, Brewster, et al., 2007). Fortunately, there are several biomarkers that can be measured concurrently to assess the adequacy of iron storage, transport and tissue utilisation (Beard et al., 2007; Grant, Prestidge, & Noel, 2016).

Research investigating the iron status of individuals uses different biomarkers and cut-off values in their assessment as there is no universal definition of ID (Domellöf, Hernell, Dewey, Lönnerdal, & Cohen, 2002). This makes comparing studies difficult and contributes to the variation seen in ID and IDA prevalence. This section will discuss the following commonly used iron biomarkers and their cut-off values: serum ferritin, transferrin saturation, serum soluble transferrin receptor and haemoglobin. It will also discuss the use of hepcidin as an iron biomarker, the relevance of iron biomarkers in the postpartum period and the effect of inflammation. For more information about other iron biomarkers, please refer to the review by Lopez et al. (2016).

2.9.1 Serum Ferritin

Ferritin is an iron storage protein (Camaschella & Pagani, 2018). Serum ferritin (SF) is a sensitive and commonly used (Baker et al., 2010) marker of iron stores, as the amount of ferritin in the blood is positively correlated to the body's total iron stores (Anderson & Frazer, 2017; Domellöf et al., 2013; Institute of Medicine, 2001). A low SF indicates the individual is ID and in combination with a low Hb it indicates the presence of IDA; conversely, a high SF indicates iron overload. However, SF does not show the severity of iron depletion (WHO, 2011b).

The current WHO (2011b) guidelines defines ID as SF <12 µg/L for children under five years of age, however a range of SF cut-off values (SF <10-15 µg/L) are used in research (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013). Other biomarkers such as mean corpuscular volume, zinc protoporphyrin, serum iron, transferrin saturation or total iron binding capacity, may be used in combination with SF to diagnose ID (Domellöf, Hernell, et al., 2002). There is no specified upper limit for term infants to indicate iron overload (WHO, 2011b).

2.9.2 Transferrin Saturation

Transferrin saturation (TS) is a measure of the amount of iron bound to transferrin. In ID, serum iron is reduced and total iron binding capacity is increased causing TS to decrease (Lopez et al., 2016). Erythropoiesis is reduced when TS falls below 16%, indicating ID (Anderson & Frazer, 2017). However, not all studies report transferrin saturation when investigating infant iron status.

2.9.3 Serum Soluble Transferrin Receptors

Serum soluble transferrin receptors (sTfR) result from the breakdown of transferrin receptors (Lopez et al., 2016). When iron stores in tissues are low (high demand for iron), transferrin receptors are upregulated to allow greater iron uptake hence, sTfR is a marker of early functional ID and will be high in individuals with ID or IDA (Baker et al., 2010). The WHO (2017) recommends the assessment of sTfR is most beneficial

in populations with a high prevalence of infection and inflammation, as it is not affected by inflammation. This biomarker is not commonly used to assess infant iron status in developed countries.

2.9.4 Haemoglobin

Haemoglobin is used to diagnose anaemia (Baker et al., 2010; WHO, 2011a) however, it lacks specificity as it does not indicate the cause of the anaemia. Anaemia can be caused by several factors such as genetic haemoglobin disorders, chronic and infectious diseases and nutrient deficiencies such as vitamin B_{12} and folate. However, ID accounts for approximately half of all anaemias in women and 42% in children worldwide (WHO, 2017). Therefore, the WHO recommends assessing haemoglobin and SF together to diagnose IDA (WHO, 2011a, 2017). When measured alone, haemoglobin is an insensitive marker of ID as it does not decrease until iron stores are severely depleted (Baker et al., 2010).

The WHO does not have a haemoglobin cut-off value for children under six months of age (WHO, 2011a). Therefore, studies examining anaemia in this age group use a variation of cut-off values, further complicating the comparison of IDA between studies. Some studies use the WHO (2011a) Hb value of <110 g/L recommended for 6-59 months (Friel et al., 2003), whilst others (Chen et al., 2020; Dube, Schwartz, Mueller, Kalhoff, & Kersting, 2010; Yang et al., 2009) use <105 g/L as recommended by Domellöf, Hernell, et al. (2002).

2.9.5 Hepcidin

Hepcidin has been proposed as a biomarker to determine iron status as its expression increases with high iron stores, decreases with low stores and correlates strongly to SF (Berglund et al., 2021; Camaschella & Pagani, 2018). However, there are currently no standardised laboratory methods or cut-off values for infants (Berglund & Domellöf, 2021).

2.9.6 Measuring Iron Status in the Postpartum Period

The physiological changes that occur during pregnancy, such as the increased blood volume, return to pre-pregnancy levels within 5-6 weeks post childbirth (Milman, 2011). Therefore, after this period iron status can be measured in the same way as non-pregnant women.

2.9.7 Inflammation

An inflammatory marker, such as C-reactive protein (CRP), should always be measured concurrently with iron biomarkers as inflammation can confound the interpretation of iron status (Grant et al., 2016). The effect of inflammation on iron biomarkers is shown in Table 2.3. Infection and inflammation cause a transient decreased in serum iron, transferrin saturation and haemoglobin levels (Grant et al., 2016; Moy, 2006). Conversely, serum ferritin may appear elevated in the presence of inflammation as it is a positive acute phase reactant (Baker et al., 2010; Hay, Sandstad, Whitelaw, & Borch-Iohnsen, 2004; WHO, 2011b).

In the presence of inflammation, the WHO (2020b) recommend excluding individuals with elevated CRP values however, no cut-off limit is suggested. Participants with CRP >5-10 mg/L are typically excluded from analysis.

Table 2.3 Effect of inflammation on iron status biomarkers

Biomarker	Effect of inflammation
Serum iron	↓
Transferrin saturation	↓
Haemoglobin	↓
Serum ferritin	↑
Hepcidin	↑
Mean corpuscular volume	↔
Red cell distribution width	↔
Serum transferrin receptor	↔

Source: Adapted from WHO (2011b); Grant et al. (2016); Moy (2006); Baker et al. (2010).

2.10 Iron Status

Iron status and the prevalence of ID varies greatly between developed and developing countries, with developing countries often having significantly higher rates of deficiency (Domellöf et al., 2001). Therefore, this literature review focuses on the iron status of infants and their mothers in developed countries, to ensure it is comparable to the New Zealand (NZ) population.

2.10.1 Infant Iron Status

Iron status and the risk of deficiency largely depends on the size of iron stores at birth. Healthy term infants are estimated to be born with a sufficient iron store to cover their needs for the first four to six months of life (Berglund & Domellöf, 2021). However, several factors can reduce the iron endowment at birth including prematurity and prenatal conditions such as intrauterine growth restriction, preeclampsia, diabetes mellitus, IDA and smoking (Baker et al., 2010; Beard et al., 2007). These conditions reduce placental function and therefore reduce iron transfer to the fetus, or increase fetal iron requirements (Beard et al., 2007).

Trends in Infant Iron Status

Iron stores, as measured by ferritin, are high at birth and continue to rise over the first two months of life (Domellöf, Lönnerdal, et al., 2002) as iron is released from broken down haemoglobin (Domellöf et al., 2014). Iron stores then decrease as they are used for growth and development until about mid-infancy when the iron endowment is exhausted and SF concentrations plateau (Domellöf, Hernell, et al., 2002; Larsson et al., 2019; Siimes, Addiego, & Dallman, 1974; Ziegler, Nelson, & Jeter, 2014).

The birth iron endowment is the major determinant of the amount of protection infants have against ID (Beard et al., 2007). However, the size of iron endowment varies considerably between infants (Ziegler et al., 2014). A study by Morton et al. (2014) determined the iron status of newborns in a subset of the longitudinal study ‘Growing up in New Zealand’ (GUiNZ). They measured cord serum at birth and found 7% of newborns were ID (SF <35 µg/L), 2% were anaemic (Hb <130 g/L), however fortunately, no newborns had IDA. Georgieff, Wewerka, Nelson & deRegnier (2002) found that infants born with low ferritin stores (SF <70 µg/L (<5th percentile)) continued

to have lower iron stores at nine months of age when compared to infants born with normal iron stores. They also found that birth ferritin concentrations were significantly correlated to ferritin concentrations at nine months (r=0.50, P=0.03). Similarly, Ziegler et al. (2014) found that all breastfed infants who developed ID by six months had significantly lower iron stores at one month of age than iron replete infants (P<0.001).

Global Prevalence of Iron Deficiency

Prevalence rates of ID and IDA in studies depends on the definition and cut-off values used. Heterogenous definitions and cut-off values makes comparing studies difficult.

There is limited research on the iron status of healthy, term infants <6 months of age, as it is assumed they are protected from ID by birth iron stores; nevertheless, ID does occur in this population. Worldwide estimates of ID and IDA are between 0-15% and 0-4%, respectively, for six month old infants (Burke et al., 2014). In Europe, <2% of infants have IDA before six months of age and 2-3% have IDA at 6-9 months (Domellöf et al., 2014). After six months of age the frequency of ID and IDA increase (Burke et al., 2017), with the highest prevalence occurring during late infancy and the toddler period (Lozoff et al., 2006).

A German study comparing the iron status of breastfed and formula fed infants at four months of age found 6% of the breastfed and 4% of the formula fed infants to be ID (Dube et al., 2010). This prevalence increased when measured again at seven and 10 months of age, with ID only occurring in breastfed infants (7m: ID 19%, IDA 4%; 10m: ID 21%, IDA 2%) (Dube et al., 2010). A Taiwanese study by Chen et al. (2020) investigated the iron status of infants in the first year of life and found 3.7% and 2.7% of infants (mean age 5 months) to have ID and IDA, of which all were breastfed (Chen et al., 2020). A randomised controlled trial (RCT) looking at iron supplementation in breastfed Canadian infants found that 8% of infants receiving a placebo were ID at 3.5 months of age, compared to only 3% of infants receiving an iron supplement (Friel et al., 2003). Similar to the study by Dube et al. (2010), at six months of age, infants not receiving supplemental iron had higher rates of ID (placebo 33%; iron supplement 7%) and IDA (placebo 14%; iron supplement 0%) (Friel et al., 2003). These studies show that ID is more prevalent in breastfed than in formula fed infants, likely due to the high level of iron fortification in infant formula (Table 2.4).

New Zealand Prevalence

The most recent study on the iron status of Auckland children (6-23 months), found the prevalence of ID and IDA to be 14% and 6% respectively, after controlling for ethnic differences (Grant, Wall, Brunt, et al., 2007). Two NZ studies investigating ID in predominately European children, found ID and IDA to range between 6-22% and 4-7% respectively, in children 6-24 months old (Heath, Reeves Tuttle, Simons, Cleghorn, & Parnell, 2002; Soh, Ferguson, McKenzie, Homs, & Gibson, 2004). In the BLISS study, they found 3-5% of children had depleted iron stores (PF <15 µg/L) and 5-7% had IDA at 12 months of age (Daniels et al., 2018). In contrast to the BLISS study, Heath et al. (2002) and Lovell et al. (2018) found high rates of ID in 12 month old children at 21% and 22% respectively. These studies show that suboptimal iron status may be common in young NZ children.

Older research in NZ infants primarily determined the prevalence of anaemia therefore, it is unknown if iron deficiency was the underlying cause. Studies were often conducted in hospital or acute care settings in which anaemia may be present due to disease or inflammation, and therefore iron biomarkers may be inaccurate (Grant, Wall, Brunt, et al., 2007; Wham, 1996).

Table 2.4 provides a summary of studies from developed countries that investigated the prevalence of ID and IDA in infants. It highlights the heterogenicity between definitions and shows that ID typically increases with age. This is not an exhaustive list of studies.

Table 2.4 Summary of studies in developed countries investigating the prevalence of ID and IDA in infants

First author, year, country	Number of participants	Age (months)	Prevalence		Iron status definition	
			ID (%)	IDA (%)	ID	IDA
Tuthill, 2002, Wales†	149	3	BM: 0.0 IF: 1.1	NR	SF <10 µg/L	NR
Friel, 2003, Canada†	77	3.5	8.0[Ø]	0.0[Ø]	SF <12 µg/L	ID + Hb <110 g/L or HCT <0.33
Dube, 2010, Germany‡	76	4	BF: 6.0 IF: 4.0	BF: 0.0 IF: 0.0	SF <12 µg/L	ID + Hb <105 g/L
Libuda, 2016, Germany‡	73	4	BF: 8.0 IF: 0.0	BF: 2.0 IF: 0.0	SF <12 µg/L	ID + Hb <105 g/L
Chen, 2020, Taiwan‡	328	1 to 6 (x̄ 5)	3.7	2.7	SF <15 µg/L	ID + Hb <105 g/L
Friel, 2003, Canada†	77	6	33.0[Ø]	14.0[Ø]	SF <12 µg/L	ID + Hb <110 g/L or HCT <0.33
Hay, 2004, Norway‡	278	6	4.0	2.0	SF <12 µg/L	ID + Hb <110 g/L
Lind, 2003, Sweden†	267	6	9.0[ł]	2.0[ł]	SF <12 µg/L	ID + Hb <110 g/L & MCV <73 fL
Domellöf, 2001, Sweden†	101¥	6	2.0[§]	NR	2 of 3 from MCV ≤70 fl, ZPP ≥80mmol/mol, SF <12 µg/l	ID + Hb <110 g/L
Dube, 2010, Germany‡	76	7	BF:19.0 IF: 0.0	BF: 4.0 IF: 0.0	SF <12 µg/L	ID + Hb <105 g/L
Georgieff, 2002, USA‡	22	6 to 12 (x̄ 9)	0.0	0.0	SF <10 µg/L	ID + Hb <115 g/L
Heath, 2002, New Zealand‡	74	9	19.0	7.0	MCV <77 L & ZPP >80 µg/dL	ID + Hb <110 g/fL
Dube, 2010, Germany‡	76	10	BF: 21.0 IF: 0.0	BF: 2.0 IF: 0.0	SF <12 µg/L	ID + Hb <105 g/L

Chen, 2020, Taiwan‡	181	7 to 12 (x̄ 11**)	20.4	6.6	SF <15 µg/L	ID + Hb <105 g/L
Oti-boateng, 1998, Australia‡	88	6 to 12 (x̄ NR)	C: 20.0 A: 5.0	C: 3.0 A: 11.0	SF <15 µg/L &/or TF >3 g/L, TS <12% & SI <8 µmol/L	ID + Hb <110 g/L
Soh, 2004, NZ‡	231	6 to 12 (x̄ NR)	2.8	5.6	2 of 3 from MCV ≤73 fL, ZPP ≥70mmol/mol, SF <10 µg/l	ID + Hb <110 g/L
Health, 2002, New Zealand‡	142	12	22.0	7.0	MCV <77L & ZPP >80 µg/dL	ID + Hb <110 g/fL
Friel, 2003, Canada†	77	12	32.0[Ø]	5.0[Ø]	SF <12 µg/L	ID + Hb <110 g/L or HCT <0.33
Hay, 2004, Norway‡	249	12	10.0	5.0	SF <12 µg/L	ID + Hb <110 g/L
Lind, 2003, Sweden†	267	12	18.0[ł]	1.0[ł]	SF <12 µg/L	ID + Hb <110 g/L & MCV <73 fL
Makrides, 1998, Australia†	62	12	11.3[ł]	0.0[ł]	SF <10 µg/L	ID + Hb <105 g/L
Lovell, 2018, NZ & Australia†	160	12	21.0[§]	0.0[§]	2 of 3 from MCV ≤70 fL, ZPP ≥80mmol/mol, SF <12 µg/l, SI <5 µmol/L or TS <0.10%	ID + Hb <100 g/L
Daniels, 2017, NZ†	59	12	5.0[Ø]	5.0[Ø]	PF <15 µg/L	ID + Hb <110 g/L
Grant, 2007, NZ‡	324	6 to 23 (x̄ 15)	14.0	6.0	2 or more abnormal values from SF <10 µg/L, iron saturation <10% and MCV <73 fL	ID + Hb <100 g/L

*mean age ID: 5 months, IDA: 4.8 months IDA; **mean age ID: 11.2 months, IDA: 11.3 months; †RCT; łtotal prevalence from all participants; [Ø]control/placebo group data; [§]Baseline data; ‡Observational study; [¥]Swedish infants only; x̄ = sample mean.
A – Asian; BF – breastfed; C – Caucasian; Hb – haemoglobin; HCT – haematocrit; IF - infant formula fed; MCV - mean cell volume; NR - not reported; RCT – randomised controlled trial; SF - serum ferritin; SI - serum iron; TF – Transferrin; TS - transferrin saturation; ZPP - zinc protoporphyrin.

2.10.2 Maternal Iron Status

The iron status of women of reproductive age is important to consider as it provides a baseline estimate of iron stores when women enter pregnancy. Women with low iron stores at the onset of pregnancy have a greater risk of developing ID during their pregnancy (Bothwell, 2000; Butwick & McDonnell, 2021). Unfortunately, suboptimal iron status is common with approximately 40-55% of European premenopausal women having depleted iron stores (SF <30 µg/L) (Milman, Taylor, Merkel, & Brannon, 2017). Similarly, up to 12% and 6% of NZ women have ID and IDA, respectively, according to the 2008/9 NZ Adult Nutrition Survey (University of Otago and Ministry of Health, 2011).

Iron deficiency is the most common nutrient deficiency in pregnancy (Burke et al., 2014), with the WHO estimating that IDA affects 20% of pregnant women in developed countries (WHO, 2015). A NZ study looking at rates of ID diagnosed by midwives found ID and IDA prevalence to be 8% and 0.5% in the first trimester, increasing to 37% and 6% in the third trimester (Calje & Skinner, 2017). The high rates of ID during pregnancy are due to the high iron requirements for the expansion of maternal blood volume and to ensure fetal and placental iron needs are meet (WHO, 2017). It is estimated that a women must enter pregnancy with at least 500 mg of iron reserves, (equivalent to SF 70-80 µg/L) however, only 15-20% of women achieve this (Milman et al., 2017). Therefore, most women will need to take iron supplements to maintain their iron status during pregnancy.

Postpartum Women

The postpartum period is a time of low iron requirements (Figure 2.2) and hence, in contrast to pregnancy, is a time of low ID risk. During childbirth, women lose iron to the fetus and placenta, and through blood loss; because of this, anaemia in the immediate postpartum period (~48 hours) is common and estimated to be between 15-50% in developed countries (Calje & Skinner, 2017; Milman, 2011). Nevertheless, the postpartum period represents an opportunistic time to replenish iron stores as iron losses are low due to postpartum amenorrhea and minimal iron lost to breast milk (~0.3 mg/day). Iron stores are also increased from iron recycling as maternal red cell mass returns to pre-pregnancy levels (Bothwell, 2000; Lopez et al., 2016; Milman, 2011). A Norwegian study found that rates of ID progressively declined throughout the

first postpartum year (30% at 6 weeks to 10% at 11 months) (Bjørke-Monsen, Torsvik, Ueland, Sætran, & Sandberg, 2012). Likewise, a Peruvian study found the prevalence of maternal IDA to decrease from 37% to 21% between two and six months postpartum (Finkelstein et al., 2013).

A Japanese study found 10.5% of women were anaemic at 6-7 months postpartum. While a US study found that 12.7% and 4.2% of low-income women had ID and IDA between childbrith and six months postpartum (Bodnar et al., 2002). A recent NZ study investigating iron status in relation to thyroid function found that most (90%) women were iron replete six months postpartum (Jin et al., 2021) . At this time point, no women had IDA, three (4.2%) had ID, and four had anaemia without ID; iron overload was not investigated. Studies investigating iron status in the postpartum period, especially late postpartum, are lacking. However, there is a general trend of women becoming anaemic post-delivery but managing to restore their iron status by 2-6 months postpartum. Little is known about the prevalence of iron overload in the postpartum period.

2.10.3 Relationship Between Maternal and Infant Iron status at Mid-infancy

Most research investigates the relationship between maternal iron status in pregnancy or at delivery to that of her child at birth (discussed in relation to fetal iron accretion in section 2.12.1). However, this section will discuss the research investigating the relationship between maternal and child iron status at mid-infancy.

A large American study (*n*=21,264 mother-infant pairs) found a significant relationship between maternal and infant anaemia (*P*<0.001) (Leslie, Park, Briggs, El-Banna, & Greene, 2020). Infants whose mothers had mild anaemia during pregnancy were 33% more likely to develop anaemia between 6-11 months of age, compared to infants of non-anaemic mothers. Several other studies have found maternal IDA in pregnancy to be related to ID, IDA or anaemia in their six-month-old infants (Abioye et al., 2019; Kilbride, Baker, Parapia, & Khoury, 2000; Liu, Xiao, Zou, & Zhao, 2015). Conversely, Marques, Taddei, Lopez, and Braga (2014) did not find a relationship between maternal and infant ID and IDA at six months of age. One explanation for the difference could be the timing of maternal iron status measurements. Those measuring maternal iron status in pregnancy found correlations (Abioye et al., 2019; Leslie et al., 2020; Liu

et al., 2015), whereas Marques et al. (2014) measured this within the first postnatal month, a time when haemodynamic changes are occurring to return the body to a pre-pregnancy state (Milman, 2011). This may have confounded the diagnosis of ID and IDA (Moy, 2006; WHO, 2011b).

Finkelstein et al. (2013) found that infants were more likely to become ID at 5-6 months of age if their mother was ID at two months postpartum (R=0.33, P=0.008). They also found significant associations between maternal and infant haemoglobin at 2-3 (R=0.38, P=0.007) and 5-6 (R=0.31, P=0.02) months of age, indicating that low maternal haemoglobin at two months postpartum may result in low infant haemoglobin concentrations. However, the strength of the relationship decreased at 5-6 months suggesting that the association between maternal and infant haemoglobin decreases as the infant gets older. Equally, two studies investigating the relationship between maternal and infant haemoglobin concentrations during exclusive breastfeeding, failed to find significant correlations at 6-7 months postpartum (Amano & Murakami, 2019; Marques et al., 2014).

A recent systematic review and meta-analysis concluded that only maternal ferritin concentration in pregnancy was significantly, inversely related to infant sTfR concentrations (Quezada-Pinedo et al., 2021). No consistent associations were found with infant haemoglobin, ferritin or transferrin saturation. One reason for this may be due to sTfR not being affected by inflammation, whereas the other biomarkers are. This result suggests that there is a relationship between maternal and infant iron status.

2.11 Clinical Significance of Iron Deficiency

2.11.1 Signs and Symptoms

Figure 2.5 denotes the common signs and symptoms of ID in women and infants, many of which are the same. The signs and symptoms of ID in infancy may be subtle (Amano & Murakami, 2019) and therefore hard to detect by parents or caregivers. Chen et al. (2020) found that infants with IDA had no obvious signs of anaemia, other than pallor, which resulted in unrecognised deficiencies. The severity of symptoms

depends on how rapidly the deficiency develops, a gradual onset may allow the body to adjust so that less symptoms are felt (Lopez et al., 2016).

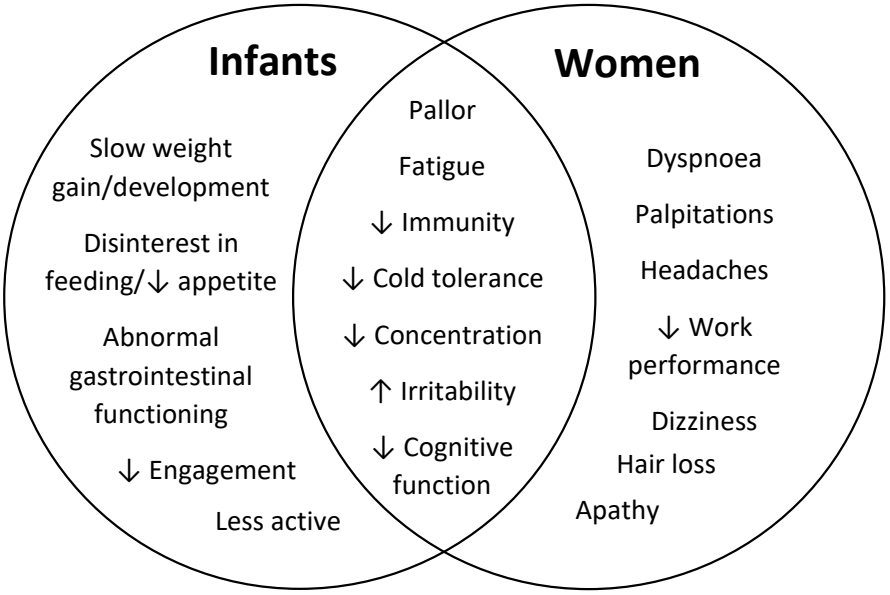


Figure 2.5 Signs and symptoms of iron deficiency in infants and women.
Sources: Canadian Paediatric Society Nutrition Committee (2007); Coad and Pedley (2014); Grant et al. (2016); Lopez et al. (2016); Percy et al. (2017).

2.11.2 Infancy

Neurodevelopment

Brain development begins in utero and continues for the first two years of life (Radlowski & Johnson, 2013). During this time iron is essential to develop the structure of the brain and for normal cognitive functioning. Iron is concentrated in oligodendrocytes, microglia and astrocytes (Coad & Pedley, 2014) and has several roles including myelination of nerves, dendritogenesis, neuronal energy metabolism and neurotransmitter production (Georgieff, 2011). Iron deficiency during infancy may impair myelination of the central nervous system and brain structures; resulting in irreversible neurological damage and adverse effects to cognitive, behavioural and motor functioning (Moy, 2006).

Iron deficiency during pregnancy is associated with adverse cognitive, behavioural and academic outcomes in offspring (Janbek et al., 2019). Tran and colleagues (2014) found that infants whose mother experienced IDA in late pregnancy had poorer motor function skills at six months. Prenatal anaemia, of which ID is the most common cause (Burke et al., 2014), is associated with an increased risk of autism spectrum disorder

(ASD), attention-deficit/hyperactivity disorder (ADHD) and intellectual disability in children (Santa-Marina et al., 2020; Wiegersma, Dalman, Lee, Karlsson, & Gardner, 2019). Wiegersma et al. (2019) found that this risk increased when anaemia occurred before 30 weeks gestation (ASD – Odds ratio (OR) 1.54; ADHD - OR 1.48 and intellectual disability - OR 2.85), potentially due to the anaemia coinciding with fetal brain development or due to restricted oxygen supply to the fetus. However, participant's iron status was not measured and therefore we cannot conclude that IDA specifically, is associated with a greater risk of ASD, ADHD and intellectual disability in children.

Infants with ID are more hesitant, wary and easily fatigued than those with good iron status (Lukowski et al., 2010). During behavioural testing infants have been shown to remain closer to their mother and are less playful and attentive when iron deficient (Lukowski et al., 2010). Iron deficiency anaemia appears to alter infant sleeping patterns (Peirano et al., 2010). A study by Peirano, Algarín, Garrido, Algarín, and Lozoff (2007) found that six month old infants with IDA were more restless at night and had more daytime naps than infants without IDA. Similarly, Kordas et al. (2008) found that infants (6-18 months) with IDA slept less and woke more in the night, based on parent reports. Poor sleep during infancy may adversely impact cognition, behaviour and emotional regulation which can be displayed as irritability, short attention span and hyperactivity. However, it is important to note that an infant's sleep may be disrupted for many reasons such as waking in the night to feed or sleeping longer when unwell (Finn Davis, Parker, & Montgomery, 2004). Sleep in infancy is likely involved in brain maturation, memory consolidation and learning therefore, altered sleeping patterns in infants with IDA may correlate to cognitive impairments later in childhood (Peirano et al., 2010).

Unfortunately, even when ID is treated, the adverse effects can be long-lasting. Low iron stores at birth are also associated with poor language ability and fine-motor skills at 5-years of age (Tamura et al., 2002). A study by Congdon et al. (2012) found that 10-year-old children who experienced IDA in infancy had slower neurological processing than those who did not, which may be a result of hypomyelination during infancy. Adolescent children (11-14 years) who had been treated for IDA in infancy found they had lower test results for mathematics, writing and motor functioning, than iron-sufficient infants (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000). The same

individuals were followed-up at 25 years old, those treated for IDA in infancy were less likely to complete secondary school and pursue higher education, had lower ratings of self-perceived health and experienced more negative emotions (Lozoff et al., 2013). This indicates a relationship between IDA in infancy and persistent lower cognitive functioning and behaviour problems that can prevent individuals from achieving their developmental potential.

Growth

Infancy demands high iron requirements to support growth, particularly for the expansion of blood volume and tissue mass (WHO, 2017). Iron deficiency is associated with stunting in children from low-resource settings (Bougle & Laroche, 2000). However, observational studies in children <2 years old from high-resource settings, report that growth was not significantly different between those with a normal or deficient iron status (Chen et al., 2020; Georgieff et al., 2002; McCarthy et al., 2018).

Two meta-analyses investigated the effect of iron supplementation on growth in children under five years, in mostly developing countries which have high incidences of ID and IDA. Ramakrishnan, Nguyen, and Martorell (2009) concluded there was no significant effect on growth, even when stratified by baseline haemoglobin. Whereas Pasricha, Hayes, Kalumba, and Biggs (2013) concluded that children who received iron supplements gained significantly less weight and length than controls. However, Pasricha et al. (2013) did not exclude the study by Majumdar, Paul, Talib, and Ranga (2003) which was an outlier with significantly smaller effect sizes for changes in weight and length, likely accounting for the different conclusions. Iron supplements should be given with caution in developed countries with a low risk of ID, as iron supplementation in iron replete infants may impair weight, length and head circumference growth (Capozzi, Russo, Bertocco, Ferrara, & Ferrara, 2011; Dewey et al., 2002).

2.11.3 Women

Iron deficiency, with or without anaemia, can have intergenerational consequences. Arguably, the most important consequence of ID is its impact on cognitive function. Attention, learning, memory and intelligence may be adversely impacted. Fortunately,

unlike when ID occurs in infancy, iron supplements can restore cognitive functioning in adults (Greig, Patterson, Collins, & Chalmers, 2013).

Iron deficiency during pregnancy adversely effects both mother and child. It increases the risk of maternal, fetal and neonatal mortality and morbidity, obstetric haemorrhage, low birth weight or prematurity, reduced accretion of fetal iron stores and postpartum ID (Dewey & Chaparro, 2007; Grant, Wall, Brewster, et al., 2007; Mirza et al., 2018; Percy et al., 2017; WHO, 2017). Postpartum anaemia is a significant global issue with approximately 20% of all maternal deaths being linked to this (WHO, 1999). Iron deficiency and IDA in the postpartum period is associated with reduced breast milk production which can decrease the duration of breastfeeding (Mirza et al., 2018). Postpartum anaemia is associated with a reduced quality of life including increased fatigue, infections, stress and depression (Beard et al., 2005; Milman, 2011; Wassef, Nguyen, & St-André, 2019). Consequently, these outcomes can negatively affect the bond between mother and child, and child development (Perez et al., 2005).

2.11.4 Immunity

Iron plays an important role in the immune system of adults and children. A delicate balance of iron is required as ID can decrease immune function, and excess iron can increase the risk of infections as iron is required by pathogens to survive and grow. During times of high microbial load, hepcidin expression is increased to sequester iron in cells so that it is unable to be used by pathogens (Núñez et al., 2018).

Iron supplements have been shown to increase the risk of infections, diarrhoea, vomiting and fever in infants (Pasricha et al., 2013; Soofi et al., 2013). Potentially due to greater iron availability in the gut for pathogenic growth or due to altered gut microflora (Nairz & Weiss, 2020); these effects may be more pronounced in areas with poor hygiene (Lönnerdal, 2017b). One study found iron supplements reduced the incidence of diarrhoea in anaemic infants (Dewey et al., 2002), likely due to the restoration of essential immune functions with greater iron stores (Lönnerdal, 2017b). Additionally, the low iron content of breast milk and strong affinity between lactoferrin and iron is likely an evolutionary mechanism to protect infants from infections (Lönnerdal, 2017b).

2.12 Risk Factors for Iron Deficiency

This section describes several factors that increase the risk of infant and maternal ID. The presence of multiple risk factors can have an additive effect, thereby further increasing the chance of deficiency (Brunt, Grant, Wall, & Reed, 2012).

2.12.1 Maternal Iron Status During Pregnancy

In utero, iron is preferentially transported to the fetus, via the placenta, at the expense of maternal iron stores to protect the fetus from ID (Cheng & Juul, 2011; Domellöf et al., 2014). Mild maternal ID is unlikely to affect fetal iron accretion. Several studies have found no differences in the iron status of infants born to mothers with normal iron stores, mild ID or mild anaemia (Ervasti, Sankilampi, Heinonen, & Punnonen, 2009; Paiva et al., 2007; Shao et al., 2012). However, severe ID may result in inadequate placental iron transfer (Cheng & Juul, 2011; Dewey & Chaparro, 2007).

Several studies have found that neonates born to mothers with IDA or severe ID have lower iron stores. Shukla, Srivastava, and Verma (2019) found that healthy term infants born to IDA mothers had lower haemoglobin and SF concentrations at birth and 14 weeks old, than those born to non-IDA mothers. Likewise, Bernhardt, Jhancy, Shivappa, Bernhard, and Pinto (2021) found cord haemoglobin, ferritin and iron to be significantly (P<0.05) lower in infants born to anaemic mothers. Significantly lower cord SF concentrations have been found in neonates born to ID compared to iron sufficient mothers (Jaime-Perez, Herrera-Garza, & Gomez-Almaguer, 2005; Kohli, Rajput, & Venkatesan, 2019; Shao et al., 2012). Interestingly, Lee et al. (2016), found that cord iron indices (ferritin, sTfR, total body iron and hepcidin) were significantly related to maternal iron status at mid-gestation but only sTfR remained significantly correlated at delivery (r=0.18, P=0.02); this may be partly explained by the labour-induced inflammation affecting iron biomarkers. These studies indicate that severe ID in pregnancy can limit the ability of the fetus to accumulate a sufficient iron endowment. One explanation for the diminished neonatal iron stores could be the recent finding that placental expression of FPN1 decreases during maternal IDA but

is unchanged in mild to moderate ID (Sangkhae et al., 2020). This would result in less iron being transferred from the placenta to the fetus during maternal IDA.

2.12.2 Milk Feeding

While the benefits of breastfeeding are undeniable, many studies have found breastfeeding beyond six months to be associated with a greater risk of infant ID (Burke et al., 2018; Chen et al., 2020; Clark et al., 2017; Grant, Wall, Brunt, et al., 2007; Hirata et al., 2017; Maguire et al., 2013). Hirata et al. (2017) found breastfed infants to have a significantly (*P*=0.007) greater risk of developing anaemia in late infancy (6-9 months), than mixed fed (breast milk and infant formula) or formula fed infants. This is of particular importance if complementary foods are low in iron, especially haem iron (Moy, 2006). Grant, Wall, Brunt, et al. (2007) found that the risk of developing ID increased 2.5-fold if breastfeeding was continued beyond seven months. While, Maguire et al. (2013) estimated that the risk of ID increases by 5% for every additional month of breastfeeding. However, ID risk will depend on the volume of breast milk consumed in relation to iron-rich solids. Hopkins et al. (2007) found that infants who had <6 breastfeeds each day had higher iron intakes than infants having ≥6 breastfeeds. Indicating that high breast milk intake can displace iron rich foods in the diet.

Breastfeeding has been associated with greater maternal iron status as most women who breastfeed are amenorrhoeic and hence have minimal iron losses (Aggett et al., 2002; Lopez et al., 2016). A large (*n*=32,947) US study by Bodnar, Scanlon, Freedman, Siega-Riz, and Cogswell (2001) found that women who breastfed for ≥7 weeks were 24% (OR 0.76; 95% CI 0.70-0.83) less likely to develop postpartum anaemia compared to women who never breastfed, and breastfeeding for ≥26 weeks reduced their risk of anaemia by 35% (OR 0.65; 95% CI 0.61-0.68). However, Amano and Murakami (2019) did not find a significant difference in the prevalence of maternal anaemia at 6-7 months postpartum between mothers who breastfed and those that did not.

2.12.3 Maternal Diet

Vegetarians have a higher risk of developing ID as their diet has low iron bioavailability (predominantly non-haem iron consumption) and high levels of inhibitors, such as phytates. Therefore, vegetarians have higher iron requirements and are recommended to increase their iron intake 1.8-fold (Burke et al., 2014; Institute of Medicine, 2001). A study by Beck et al. (2013) identified a 'meat and vegetable' dietary pattern to be associated with a low risk of ID development in premenopausal women. This dietary pattern contains haem and non-haem iron. The haem iron is well absorbed, and the non-haem iron absorption is increased due to the 'meat, fish, poultry' factor in meat and vitamin C in vegetables (Anderson & Frazer, 2017).

2.12.4 Birth Age and Weight

Premature (<37 weeks gestation) and low birthweight (<2500g) infants have a greater risk of developing ID, owing to an inadequate accumulation of iron stores in utero (Grant et al., 2016). Low birth weight infants have smaller livers and hence a smaller store of iron (Scholl, 2011). Infants born <37weeks or <2500g were found to be three times as likely to develop ID (Grant, Wall, Brunt, et al., 2007). Healthy, term infants born to mothers with normal iron status are assumed to have enough endogenous iron for the first four to six months of life (Lopez et al., 2016).

2.12.5 Iron Supplements

The American Academy of Paediatrics recommends EBF infants and infants who consume >50% of their intake from breast milk, use iron supplements (1 mg/kg/d) from four months old until they start iron-rich complementary foods (Baker et al., 2010). However, this recommendation is opposed by ESPGHAN CoN due to insufficient evidence that iron supplementation reduces IDA in populations with low ID prevalence (Domellöf et al., 2014). Rather than universal supplementation, iron status should be measured to reduce the potential harm of iron overload, especially in low-risk populations.

Maternal iron supplementation does not increase the iron status of breastfed infants (Baykan, Yalçın, & Yurdakok, 2006). Breast milk iron concentration remains constant

(Keikha, Shayan-Moghadam, Bahreynian, & Kelishadi, 2021; Mello-Neto et al., 2012), despite fluctuations in dietary intake, as iron is actively transported into breast milk (Domellöf et al., 2014; Domellöf, Hernell, Lönnerdal, Dewey, & Cohen, 2004).

Iron supplements are often required by pregnant women to meet their high iron requirements (Milman et al., 2017), with an estimated 71% of NZ women using iron supplements during pregnancy (Morton et al., 2010). A Cochrane review of 61 randomised and quasi-randomised trials investigating the effect of daily iron supplementation in pregnancy found ID and IDA to be reduced by 57% and 67%, respectively, at term (Peña-Rosas, De-Regil, Garcia-Casal, & Dowswell, 2015). Similarly, a systematic review also found a 67% reduction in IDA prevalence at term in those using iron supplements (Yakoob & Bhutta, 2011). Hence, there is good evidence that using iron supplements during pregnancy will help to maintain iron status and reduce the prevalence and likely severity of maternal prenatal ID.

There is conflicting evidence regarding the requirement of iron supplements in the postpartum period. Baykan et al. (2006) found no difference in iron status between supplemented and unsupplemented women at four months postparum. However, for ethical reasons anaemic women were excluded from this study and therefore, women may not have needed additional iron, especially considering the low iron requirements during this time. A RCT comparing the change in iron status in women given either a placebo or an iron supplement for 3.5 months found the iron supplemented group to have greater increases in ferritin and haemoglobin concentrations when measured at two weeks and four months postpartum (Jorgensen, Yang, Lönnerdal, Chantry, & Dewey, 2017); anaemic mothers were also excluded from this study. Nevertheless, in the placebo group, only one mother developed mild anaemia at four months postpartum, showing that most women, who are not initially anaemic, are able to maintain their haemoglobin concentration without supplementation. An earlier RCT found that anaemia was reduced in women who used iron supplements during pregnancy and for eight weeks postpartum (8% vs 16% unsupplemented) (Milman et al. 1991, as cited in Milman et al. 2011). These results indicate that iron supplements are benefical in women experiencing postpartum IDA but appear unneccessary in the absence of anaemia.

2.12.6 Ethnicity

A child's risk of developing ID may differ between ethnic groups. Several studies have found children of minority ethnicities, such as Māori, Pasifika, North African, South Asian and Blacks, to have the greatest risk of ID or anaemia, while European infants often have the lowest risk (Gunaseelan et al., 2020; Leslie et al., 2020; Morton et al., 2014; Wall, Brunt, & Grant, 2009). It is likely the cultural differences in infant feeding practices (i.e., tea drinking among Pasifika or low meat consumption among South Asian ethnicities) that may contribute to this ethnic disparity (Grant, Wall, Brunt, et al., 2007; Gunaseelan et al., 2020).

Similar ethnic variations in iron status between women are seen. In America, white women typically have higher SF and haemoglobin concentrations (Miller, 2014) and lower rates of postpartum anaemia (Bodnar et al., 2001). In NZ, Beck et al. (2014) found Asian females were nearly five times as likely to develop ID than European women (OR 4.84, $P<0.001$). Conversely, the 2008/9 NZ Adult Nutrition Survey found no difference in the prevalence of ID between Māori and non-Māori (University of Otago and Ministry of Health, 2011).

2.12.7 Interval Between Pregnancies and Parity

An inadequate interval between pregnancies can result in poor iron status in subsequent pregnancies if iron repletion is inadequate (Coad & Conlon, 2011; Kilbride et al., 2000). Increasing parity is associated with reduced haemoglobin, SF and transferrin saturation, and increased sTfR; indicating that women with high parity are at an increased risk of poor iron status (Miller, 2014). As the average number of children per woman in NZ is 1.6 (Stats NZ, 2021), this may not contribute significantly NZ women's iron status.

2.12.8 Socioeconomic Status and Food Security

Food security is closely related to socioeconomic status. Having a low household income, receiving a government benefit (income supplementation) and living in the most deprived neighbourhoods are risk factors for being food insecure (Ministry of Health, 2019). Additional risk factors include identifying as with a minority ethnic group

(Metallinos-Katsaras, Colchamiro, Edelstein, & Siu, 2016), including Māori or Pasifika, household overcrowding (Ministry of Health, 2019; Schlichting, Hashemi, & Grant, 2019) and lower educational attainment (Kazemi, Ghaemmaghami Hezaveh, Nikniaz, & Nikniaz, 2020; Metallinos-Katsaras et al., 2016).

Food insecurity is defined as an unreliable or limited access to food of sufficient quantity and quality (Kazemi et al., 2020; Ministry of Health, 2019). Food insecurity may increase the risk of ID as diets are more likely to be nutritionally inadequate (Parnell, 2005), be less diverse (Lynch, 2011) and may lack iron-rich foods, such as meat (Kazemi et al., 2020). New Zealand is a developed country and yet, 14% of the total population (FAO, 2019) and 13.5% of infants, at nine months old, are estimated to be moderately food insecure (Schlichting et al., 2019). An increasing trend according to the 2008/9 NZ Adult Nutrition Survey, with 10% more people now being classified as moderately food insecure than in 1997 (University of Otago and Ministry of Health, 2011).

There appears to be a trend toward food insecure or low socioeconomic status women and children having a lower iron status than their counterparts. This has been found in premenopausal women (Kazemi et al., 2020), pregnancy (Park & Eicher-Miller, 2014), postpartum women (Bodnar et al., 2002), infants (Metallinos-Katsaras et al., 2016), and children (Skalicky et al., 2005). An Iranian study found that food insecure premenopausal women were more likely to be anaemic than food secure women (P=0.01) (Kazemi et al., 2020). There was also a trend towards a greater likelihood of ID in food insecure women (P=0.07). An American study found food insecure pregnant women to have a lower total daily iron intake, compared to those who were food secure, due to a significantly lower iron intake from supplements (10 mg/day lower; P=0.02) (Park & Eicher-Miller, 2014). Hence, food insecure pregnant women were 2.9 times more likely to be ID (P<0.05). Low-income postpartum women in the US were more likely to be ID than higher income women, 30% vs 7% at 0-6 months postpartum (Bodnar et al., 2002). Metallinos-Katsaras et al. (2016) found that infants living in food insecure households were 42% more likely to develop anaemia by 18 months of age than those in food secure households. However, not all studies have found this (Heath et al., 2001; Skalicky et al., 2005; Szeto, Harrison, & Innis, 2012). A NZ study by Heath et al. (2001) did not find an association between socioeconomic status and mild ID in premenopausal women. Although, food security was not measured, most participants

were European (95%) and of average socioeconomic status hence, food insecurity was likely to be low.

Parnell (2005), found that in food insecure households, women's dietary intake was often compromised, whereas children's was not, suggesting that women prioritise feeding their children above themselves. However, she did not find a difference in dietary iron intake between food security status. Similarly, the 2008/9 NZ Adult Nutrition Survey found the prevalence of ID did not differ based on an individual's level of deprivation (University of Otago and Ministry of Health, 2011).

2.12.9 Other Risk Factors

The following are also common risk factors for infant and/or maternal ID but were outside the scope of this review: Timing of umbilical cord clamping (Ashish et al., 2017); intake of iron-rich foods such as meat, fish and poultry or iron fortified cereals (Heath et al., 2001; Makrides, Leeson, Gibson, & Simmer, 1998); cow's milk intake <12 months of age; consumption of tea in infancy (Grant, Wall, Brunt, et al., 2007); infant sex (Yang et al., 2009); infant growth rate (Ziegler et al., 2014); maternal BMI (Dumrongwongsiri et al., 2021): and blood loss during childbirth (Butwick & McDonnell, 2021).

2.13 Summary

The iron status of NZ infants under six months age and their mothers, remain largely unknown. Currently, no NZ studies have investigated the iron status of term infants between 3-6 months of age and only one study has reported maternal iron status at six months postpartum.

This research will endeavour to determine if young infants and their mothers living in Auckland, New Zealand have an adequate iron status before they commence complementary feeding at around six months of age. The results of this research will add evidence regarding the relationship between maternal and infant iron status and determine if infant iron status differs between modes of milk feeding.

Chapter 3: Manuscript

3.0 Abstract

Background: Iron deficiency (ID) in infancy may cause irreversible deficits in cognitive functioning that can persist into adulthood. Maternal postpartum anaemia is associated with a reduced quality of life and can adversely affect the bond between mother and child and consequently, child development; hence it is critical that ID is avoided during these vulnerable periods of life.

Objectives: To determine the iron status of infants and mothers, to investigate the relationship between maternal and infant iron status and to determine the differences in infant iron status according to mode of milk feeding, prior to commencing complementary feeding.

Methods: This study reports the baseline iron status of 133 mother-infant pairs from a randomised controlled trial. Healthy, term infants, 3-6 months of age that had not yet started solids, and their mothers, were included in the analysis. Haemoglobin and serum ferritin concentrations were measured to determine iron status. C-reactive protein was measured to determine the presence of inflammation. Infant anthropometric measures were taken. Demographic and dietary information was collected via questionnaires. Pearson's and Spearman's rho correlations were used to determine the relationship between maternal and infant iron status. One-way ANOVA and Kruskal-Wallis tests determined the differences in infant iron status according to mode of milk feeding.

Results: Most infants (93.2%; SF ≥10 µg/L, Hb ≥110 g/L) and mothers (80.5%; SF ≥15 µg/L, Hb ≥120 g/L) had sufficient iron stores. No infants or their mothers had iron deficiency anaemia (infant: SF <10 µg/L, Hb <110 g/L; maternal: SF <15 µg/L, Hb <120 g/L). No infants had ID (SF <10 µg/L, Hb ≥110 g/L) but 6.8% had anaemia without ID (SF ≥10 µg/L, Hb <110 g/L). One mother had ID (0.8%; SF <15 µg/L, Hb ≥120 g/L), 9.8% had mild ID (SF <30 µg/L, Hb ≥120 g/L), 7.5% had serum ferritin ≥150 µg/L indicating iron overload and 1.5% had anaemia without ID (SF ≥15 µg/L, Hb <120 g/L). There was a weak positive relationship between maternal and infant serum ferritin (r=0.19, P(two-tailed)=0.03), but no relationship between maternal and infant haemoglobin (P=0.91). There were no significant differences in either serum ferritin

(*P*=0.92) or haemoglobin (*P*=0.50) concentrations between milk feeding modes (breast milk vs. infant formula vs. mixed feeding).

Conclusion: Most infants and their mothers were iron-replete prior to starting complementary feeding and their iron stores were weakly related at 3-6 months postpartum. Additionally, infant iron status did not differ by the type of milk infants were fed.

Key words: iron status, infant, mothers, maternal, postpartum, relationship, milk feeding.

3.1 Introduction

Nutrition during the first 1000 days influences health outcomes later in life (Burke et al., 2014). Iron is particularly important for the growth and development of infants and deficiencies may cause irreversible damage to cognitive functioning (Coad & Pedley, 2014).

Unfortunately, iron deficiency (ID) and iron deficiency anaemia (IDA) are common in women and children with 800 million women and children estimated to have IDA worldwide (WHO, 2017). The World Health Organisation (WHO) estimates that 16% and 15% of New Zealand (NZ) pregnant women and children, respectively, have anaemia (WHO, 2021b), approximately half of which may be caused by ID (WHO, 2017). Iron deficiency, with or without anaemia, can adversely affect psychomotor development and cognitive function (Coad & Pedley, 2014). These effects may persist into adolescence, even after iron repletion (Georgieff, 2011). Additionally, postpartum anaemia has been associated with a reduced quality of life and poor child development (Beard et al., 2005; Milman, 2011; Perez et al., 2005).

Iron requirements are higher during infancy than at any other stage of life due to their rapid growth and development (Cormack, 2013; Grant, Wall, Brewster, et al., 2007), leaving infants vulnerable to developing ID (Moy, 2006). New Zealand studies have found ID and IDA in children under two years of age, to range between 6-22% and 4-7%, respectively (Grant, Wall, Brunt, et al., 2007; Heath et al., 2002; Lovell et al., 2018; Soh et al., 2004); highlighting that suboptimal iron status is common in young NZ children. Healthy term infants are born with a store of iron that is estimated to cover their requirements for the first 4-6 months of life (Domellöf et al., 2014). In Europe, <2% of infants have IDA before six months of age (Domellöf et al., 2014). Research on the iron status of young infants, particularly in NZ, is scarce. The most recent study investigating young NZ children's iron status is now 14 years old (Grant, Wall, Brunt, et al., 2007). The average age of children in this study was 15 months and therefore, it is unlikely to accurately represent the iron status of young infants.

The postpartum period is an opportunistic time to replenished iron stores lost during pregnancy and childbirth. Iron requirements during this time are the lowest of any time in a women's life as iron losses are minimal due to postpartum amenorrhea and the low iron content of breast milk (~0.3 mg/d). Studies have shown that rates of ID

progressively declined throughout the first postpartum year (30% at 6 weeks to 10% at 11 months) (Bjørke-Monsen et al., 2012; Finkelstein et al., 2013). A recent NZ study found that most (90%) women were iron replete six months postpartum and that rates of ID were low at 4.2% (Jin et al., 2021). Worldwide, studies investigating the iron status of postpartum women, especially late postpartum, are lacking.

The relationship between maternal iron status in pregnancy or at delivery and that of her newborn child has been well investigated. However, the relationship between maternal and infant iron status at mid-infancy is not as well understood. The current evidence is limited and results have been inconsistent when investigating the relationship between iron biomarkers such as serum ferritin (SF) and haemoglobin (Hb) (Quezada-Pinedo et al., 2021). However, several studies have found maternal IDA in pregnancy to be related to ID, IDA, or anaemia in their infants at six-month-old (Abioye et al., 2019; Kilbride et al., 2000; Leslie et al., 2020; Liu et al., 2015).

Although breast milk is the optimal food for babies, studies have found breastfed infants to have a lower iron status (Chen et al., 2020; Dube et al., 2010) and an increased risk of ID (Burke et al., 2018; Clark et al., 2017; Grant, Wall, Brunt, et al., 2007; Hirata et al., 2017), when compared to formula fed infants. This is of particular importance if the iron endowment from birth was poor, as this is a major determinant of the degree of protection infants have against ID (Beard et al., 2007). Grant, Wall, Brunt, et al. (2007) found that the risk of developing ID increased 2.5-fold if breastfeeding was continued beyond seven months.

The lack of studies investigating iron status in infants under six months of age may be due to the assumption that the birth iron endowment is sufficient until this age. However, international studies have shown that ID and IDA can occur in the first six months of life and hence it is important that we understand the iron status of young infants in NZ. Additionally, the relationship between maternal and infant iron status prior to complementary feeding is not well understood. Therefore, this study aims to determine the iron status of infants and their mothers prior to starting complementary feeding. It will also investigate the relationship between maternal and infant iron status and determine if infant's iron status varies according to their mode of milk feeding.

3.2 Method

3.2.1 Study Design

A cross sectional, observational study investigating the iron status of infants and their mothers living in Auckland, New Zealand. This study reports baseline iron status from a randomised, controlled trial, as described below.

3.2.2. Participants and Recruitment

Participants were recruited between May 2019 and January 2020, by advertising on radio, social media, email, community notice boards and word of mouth (Appendix 1). The participants in this study were recruited to assess the impact of a “vegetables first” approach to complementary feeding on later intake and liking of vegetables in infants: a randomised controlled trial (Rapson, von Hurst, Hetherington, & Conlon, 2021). A minimum of 130 participants (including a 20% dropout rate), were needed for the primary study to reach a clinically significant difference at 80% power and 5% statistical significance (Rapson et al., 2021). Mother-infant pairs were included in the study if the infant was aged between 3-6 months at the time of blood sampling, had not started complementary feeding, was born at term (≥37 weeks gestation), were of normal growth/birth weight (≥2500 g) and had no known food allergies, chronic diseases or medical conditions (Appendix 2). Prior to participation in the study, written informed consent was provided by mothers for themselves and their infant to participate.

Ethical approval was provided by the Massey University Human Ethics Committee: Southern A, Application SOA 18/56. This study was registered with the Australian New Zealand Clinical Trial Registry, ACTRN12619000737134.

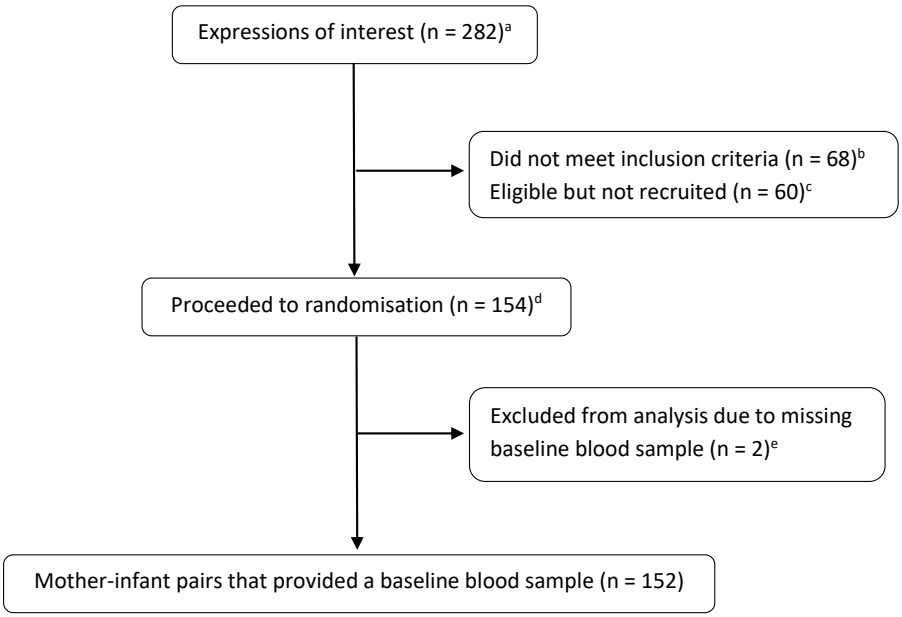


Figure 3.1 Flow diagram of recruitment.

[a]Mothers expressed their interest by registering on the study website (www.vegesfirststudy.co.nz). [b]Inclusion criteria: Infant 3-6 months at the time of blood sampling, had not started complementary feeding, was born at term (≥37 weeks gestation), normal growth/birth weight (≥2500 g), no known food allergies, chronic diseases or medical conditions, complete questionnaires (eligibility, demographics, food frequency questionnaire (pregnancy and lactation), baby frequency questionnaire). [c]Declined to participate or no longer eligible for the primary study. [d]Mother-infant pairs from both intervention and control groups were included in this study. [e]No baseline blood sample from either mother or her infant.

3.2.3 Data Collection

Participant information was collected via an emailed link to the online survey tool, Qualtrics™, or via phone call. An Eligibility Questionnaire was completed to ensure infants meet the inclusion criteria. A Demographic Questionnaire (Appendix 3) collected information regarding mothers' age, ethnicity, medical history during pregnancy including if they had been diagnosed with anaemia or iron deficiency, geographical location, educational attainment, parity and food security status and infant age and sex. Food Frequency Questionnaires (FFQ) (Appendix 4 & 5) collected dietary data, of which only the information regarding red meat intake during pregnancy and lactation was analysed in this study. Mothers who answered yes to eating red meat most days or at least once a week were defined as 'eats red meat at least once a week', and mothers who answered yes to eating red meat once a month, once during their pregnancy/lactation or never, were defined as 'eats red meat less than once a week'. The FFQ's were adapted from an existing New Zealand validated FFQ (Beck,

Kruger, et al., 2012). Questionnaires were peer-reviewed by three nutrition and dietetic experts and pilot tested by 14 new mothers. A Baby FFQ (Appendix 6) provided information about infant milk feeding practices including if infants were breastfed and/or formula fed and the duration of exclusive breastfeeding. Infants were put into the following milk feeding categories based on their milk intake at the time of blood sampling: breast milk only, infant formula only, mixed feeding (breast milk and infant formula) or any infant formula (i.e., any infant that was not exclusively breastfed). The pregnancy, lactation and baby FFQ's were completed by mothers when they joined the primary study. An Iron Questionnaire (Appendix 7) was completed by mothers at the commencement of this study to collect information about the factors that may have influenced maternal and infant iron status. This included whether mothers followed a vegetarian diet or used iron supplements during pregnancy or lactation, and infant iron supplement use.

Participants visited the Human Nutrition Research Unit at Massey University in Auckland where a Massey University researcher took infant anthropometric measures, and a registered phlebotomist took blood samples from mothers and infants, as per standardised protocols outlined in Rapson et al. (2021). Weight (naked) was measured on Sensortronic (New Zealand) electronic baby scales. Length was measured supine on a Seca 416 (IN) infant meter (length board). For head circumference, a paper tape measure was used to take three measures of the widest part of the head, then the largest measure, to the nearest 0.1cm, was selected.

3.2.4 Blood Sampling and Analysis

A venous blood sample was taken from mothers and a non-fasting capillary 'heel prick' blood sample from infants to assess iron status. Haemoglobin was measured immediately on a HemoCue® Hb 201+ System, if haemoglobin measures were outside the normal range (Infant: Hb <110 g/L, Mother: Hb <120 g/L) (WHO, 2011a), participants were informed and given a referral letter see their doctor. Aliquots of serum were stored at -80°C until subsequent analysis of serum ferritin and C-reactive protein at LabTests (Auckland).

Established cut-off values were used to determine ID, IDA and anaemia status of mothers and infants (Baker et al., 2010; WHO, 2011a, 2011b), as well as mild ID

(Milman et al., 2017) and iron overload for mothers (WHO, 2011b) (Table 3.1 & 3.2). The presence of infection or inflammation was defined as CRP ≥10 mg/L, these participants were excluded from analysis (Pepys, 1981; Starship, 2019; WHO, 2011b).

Table 3.1 Cut-off values for defining infant iron status

Infant					
		Optimal Iron Status	ID	IDA	Anaemia
6-59 months	SF (µg/L)	≥10*	<10*	<10*	-
	Hb (g/L)	≥110ǂ	≥110ǂ	<110ǂ	<110ǂ

ID - Iron deficiency; IDA - Iron deficiency anaemia; SF - serum ferritin; Hb - Haemoglobin.
*Baker at al. (2010); ǂWHO (2011a).

Table 3.2 Cut-off values for defining maternal iron status

Maternal							
		Optimal Iron Status	Mild ID	ID	IDA	Iron Overload	Anaemia
20-59 years	SF (µg/L)	≥15†	<30‡	<15†	<15†	≥150†	-
	Hb (g/L)	≥120ǂ	≥120‡	≥120ǂ	<120ǂ	-	<120ǂ

ID - Iron deficiency; IDA - Iron deficiency anaemia; SF - serum ferritin; Hb - Haemoglobin.
†WHO (2020); ǂWHO (2011a); ‡Milman, Taylor, Merkel, and Brannon (2017).

3.2.5 Statistical Analysis

Statistical analysis was conducted using IBM SPSS statistics package version 27 (IBM corporation, New York, USA). Data was tested for normality using Kolmogorov-Smirnov tests and Shapiro-Wilk tests and normality plots. Parametric data was reported as mean ± standard deviation and non-parametric data was reported as median (25th and 75th percentiles). Non-parametric data was log transformed and re-tested for normality. Log transformed data that was normally distributed was reported as geometric mean and 95% confidence interval. If transformations did not correct normality of distribution, it was reported as median (25th and 75th percentiles). Categorical data was reported as number of participants and percentage.

Correlations between maternal and infant variables were determined using Pearson's correlation for parametric data and Spearman's rho for non-parametric data. When correlating continuous and categorical variables, normality of distribution was tested to ensure each group was normally distributed. Partial correlation was used to

determine if there was a relationship between maternal and infant serum ferritin. This correlation controlled for the following variables that may also have had an impact on maternal and infant iron status: infant age and sex, maternal ID during pregnancy and maternal iron supplement use during breastfeeding. For all tests, a *P*-value <0.05 was considered statistically significant.

To determine if there was a difference in infant iron status between different modes of infant milk feeding, one-way ANOVA was used for log infant serum ferritin (parametric) and Kruskal-Wallis was used for infant haemoglobin (non-parametric) when comparing breast milk only, infant formula only and mixed feeding. When comparing breast milk only with infants fed any infant formula, Independent samples t-test was used for log infant serum ferritin and Mann-Whitney was used for infant haemoglobin.

3.3 Results

3.3.1 Participants

One hundred and fifty-two mother-infants pairs provided a blood sample for analysis. Nineteen were excluded (Figure 3.2). Blood samples were analysed for 133 mother-infant pairs.

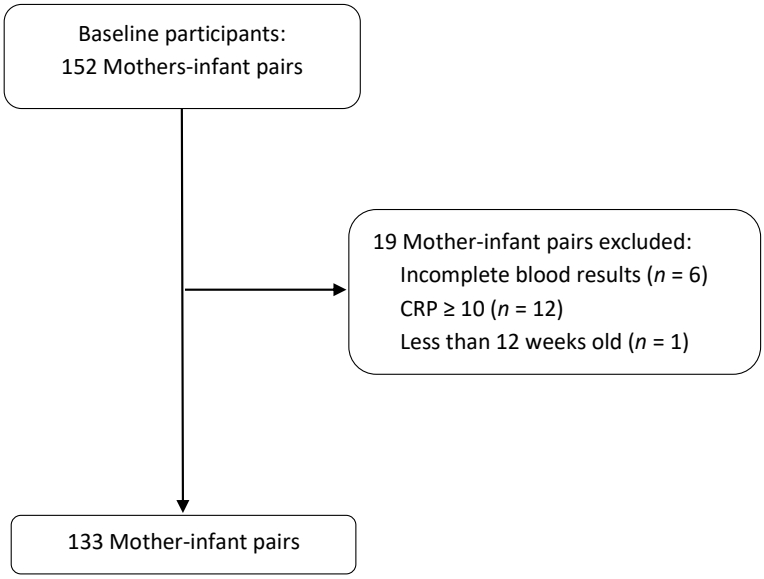


Figure 3.2 Flow diagram of excluded participants.

At the time of blood sampling, the median age of infants was four months and over half of infants were fed breast milk only (61.3%) (Table 3.3). The median length of time that mothers exclusively breastfed their infant was three months.

Table 3.3 Infant characteristics (n=133)

Age (months), median (25th, 75th percentile)	4 (3, 5)
Females, n (%)	66 (49.6)
Anthropometrics	
Weight (kg), geometric mean (95% CI)	7.0 (6.8, 7.1)
Length (cm), mean ± SD	64.1 ± 3.0
Head circumference (cm), mean ± SD	42.5 ± 1.4
Milk feeding mode (at time of blood sampling), n (%)‡	
BM only	76 (61.3)
IF only	13 (10.5)
Mixed fed (BM & IF)	35 (28.2)
Duration of EBF (months), median (25th, 75th percentile)‡	3.0 (0.8, 4.0)
Iron supplement use, n (%)‡	1 (1.0)

BM – breast milk; CI - confidence interval; EBF - exclusive breastfeeding; IF - infant formula.
‡The following data is missing: milk feeding mode n=9; duration of EBF n=9; iron supplement use n=35.

The mean age of mothers was 32.6 years, most mothers identified as being of European ethnicity (90.9%) and were highly educated (87%) (Table 3.4). Red meat intake was common with 87-88% of mothers eating red meat at least once a week whilst pregnant and breastfeeding. Over half of mothers were diagnosed with iron deficiency while pregnant (57.1%), while few mothers became anaemic (5.3%); the cause of anaemia is unknown. Many women used iron-containing supplements while pregnant and breastfeeding (88.8% and 49.0%, respectively).

Table 3.4 Maternal characteristics (n=133)

Age (years), geometric mean (95% CI)	32.6 (31.9, 33.4)
Ethnicity, n (%)§‡	
European	120 (90.9)
NZ Māori and Pacific Islander	15 (11.4)
Other (i.e. Chinese, Indian)	13 (9.8)
Educational attainment, n (%)‡	
Tertiary or higher	114 (87.0)
Below tertiary	17 (13.0)
Food secure, n (%)	127 (96.2)
Primipara, n (%)‡	92 (69.7)
Vegetarian, n (%)*‡	4 (4.0)
Pregnancy, n (%)	
Red meat intake†‡	

At least once a week	110 (88.0)
Less than once a week	15 (12.0)
Used iron containing supplement‡	87 (88.8)
Diagnosed with iron deficiency	76 (57.1)
Diagnosed with anaemia	7 (5.3)
Breastfeeding, n (%)	
Red meat intake†‡	
At least once a week	107 (87.0)
Less than once a week	16 (13.0)
Used iron containing supplement‡	48 (49.0)

BM – breast milk; CI - confidence interval; EBF - exclusive breastfeeding; IF - infant formula.
*No meat consumption while pregnant or BF.
§Multiple choices were possible.
†Beef, lamb or pork.
‡The following data is missing: ethnicity n=1, educational attainment n=2, food secure n=1, primipara n=1, vegetarian n=34, red meat intake (pregnancy) n=8, red meat intake (breastfeeding) n=10, iron supplement use (pregnancy) n=35, iron supplement use (breastfeeding) n=35.

3.3.2 Iron Status

The median haemoglobin for infants was 126 g/L and the mean serum ferritin was 103.2 µg/L (Table 3.5). Nine infants were anaemic but had normal serum ferritin concentrations therefore, indicating that the anaemia was unlikely to be caused by iron deficiency (WHO, 2017). Thus, based on serum ferritin concentrations, all infants had sufficient iron stores.

Table 3.5 Infant iron status (n=133)

Haemoglobin (g/L)**	Serum ferritin (µg/L)*	Iron replete† n (%)	ID/IDA†† n (%)	Anaemia††† n (%)
126.0 (115.0, 137.0)	103.2 (90.9, 117.2)	124 (93.2)	0	9 (6.8)

Iron replete status when anaemia without ID††† is excluded: n=133 (100%).
ID - Iron deficiency; IDA - Iron deficiency anaemia; SF - Serum ferritin.
**Median (25^{th}, 75^{th} percentile); *Geometric mean (95% CI).
†Iron replete: Hb≥110g/L + SF≥10µg/L.
††ID: Hb≥110g/L + SF<10µg/L; IDA: Hb<110g/L + SF<10µg/L.
†††Anaemia: Hb<110g/L + SF≥10µg/L.

The mean maternal haemoglobin was 138.9 g/L and mean serum ferritin was 60.6 µg/L (Table 3.6). No mothers had IDA however, two mothers were anaemic but had normal iron stores, indicating that anaemia was unlikely to be caused by iron deficiency (WHO, 2017). Therefore, when anaemia by other causes, such as vitamin

B_{12} or folate deficiency, is excluded, most (81.9%) mothers had sufficient iron stores, based on their serum ferritin concentrations. Thirteen mothers had mild ID (SF <30 µg/L) and one had ID (SF = 14 µg/L). Ten mothers had a serum ferritin ≥150 µg/L (SF range 168-314 µg/L), indicating iron overload; of which, one also had a marginally low haemoglobin concentration (SF = 196 µg/L, Hb = 119 g/L).

Table 3.6 Maternal iron status (n=133)

Haemoglobin (g/L)*	Serum ferritin (µg/L)*	Iron replete† n (%)	Mild ID†† n (%)	ID/IDA††† n (%)	Iron overload‡ n (%)	Anaemia[Ø] n (%)
138.9 (137.0, 140.8)	60.6 (54.4, 67.6)	107 (80.5)	13 (9.8)	1 (0.8)	10 (7.5)	2 (1.5)

Iron replete status when anaemia without ID[Ø] is excluded: n=109 (81.9%).
ID - Iron deficiency; IDA - Iron deficiency anaemia; SF - Serum ferritin.
*Geometric mean (95% CI).
†Iron replete: Hb≥120g/L + SF≥30µg/L.
††Mild ID: Mothers - Hb≥120g/L + SF 15-29µg/L.
†††ID: Hb≥120g/L + SF<15µg/L; IDA: Hb<120g/L + SF<15µg/L.
‡Iron overload: SF≥150µg/L; NB: mother with SF indicating iron overload and anaemia (SF=196µg/L, Hb=119g/L) only counted once under 'Iron overload'.
[Ø]Anaemia: Hb<120g/L + SF≥15µg/L.

3.3.3 Relationship Between Maternal and Infant Iron Status

Infant log serum ferritin was positively associated with maternal log serum ferritin (small effect size; r=0.19, n=133, P(two-tailed)=0.03). The strength of this relationship increased when infant age, infant sex, maternal ID in pregnancy and maternal iron supplement use during breastfeeding were controlled for in a partial correlation (r=0.253, r^2=0.064, n=92, P(two-tailed)=0.01). Maternal log serum ferritin explained 6% of the variance in infant log serum ferritin. Conversely, there was no significant relationship between log maternal haemoglobin and infant haemoglobin (r=0.01, n=133, P(two-tailed)=0.91).

3.3.4 Infant Iron Status by Mode of Milk Feeding

There was no significant difference in the means (95% CI) of log infant serum ferritin between infants who were fed breast milk only (99.6 µg/L (83.9, 118.3)), infant formula only (97.8 µg/L (63.5, 150.7)) or were mix fed (105.6 µg/L (81.5, 136.8)), F=0.09, (2, 121) df, P=0.92. Similarly, the median (25^{th}, 75^{th} percentile) of infant haemoglobin was not significantly different between infants who were fed breast milk only (124 g/L (121,

130), infant formula only (128 g/L (125, 144) or were mix fed (123 g/L (116, 130), $H(2)=1.40$, $P=0.50$. When comparing infants fed breast milk only or who were fed any formula (IF only and mixed fed), there was still no statistically significant difference in log infant serum ferritin ($t=-0.769$, 122 df, $P=0.79$) or infant haemoglobin ($U=1768.0$, $P=0.77$ (2-tailed)).

3.4 Discussion

This study shows that most infants and their mothers had adequate iron stores prior to commencing complementary feeding. We also determined that there was a weak relationship between maternal and infant serum ferritin at 3-6 months postpartum and that infant iron status did not differ according to the mode of milk feeding.

3.4.1 Infant Iron Status

In this cohort, all infants had sufficient iron stores, based on their serum ferritin concentrations. No infants were identified as ID or IDA and thus the prevalence of ID is lower than previously reported in studies from developed countries. In infants 3-6 months of age, the prevalence of ID and IDA ranges from 0-9% and 0-2.7%, respectively (Chen et al., 2020; Dube et al., 2010; Friel et al., 2003; Libuda, Hilbig, Berber-Al-Tawil, Kalhoff, & Kersting, 2018; Tuthill et al., 2002). These prevalence rates exclude a study by Friel et al. (2003) which had significantly higher rates of ID and IDA at all ages (3-12 months ID: 8-33%, IDA: 0-14%), compared to similar studies. Iron deficiency, particularly progressing to anaemia, is uncommon in young infants living in developed countries. In a study by Tuthill et al. (2002) only one infant had developed ID at three months of age therefore, although our results are low, they are not dramatically lower. Nor are they unexpected as healthy, term infants are estimated to be born with enough iron stores to cover their requirements, along with the small amount of iron supplied by breast milk, for the first four to six months of life (Berglund & Domellöf, 2021).

The prevalence of ID and IDA of NZ infants aged between 6-24 months old is estimated to be between 2.8-22% and 0-7%, respectively (Daniels et al., 2018; Grant, Wall, Brunt, et al., 2007; Heath et al., 2002; Lovell et al., 2018; Soh et al., 2004). Again,

these prevalence rates are greater than that found in the current study. This is unsurprising given that most of these studies looked at infants older than nine months of age and it is known that rates of ID increase after six months old as the birth iron endowment is typically exhausted by this time (Berglund & Domellöf, 2021).

Nine infants (6.8%) in this study had low haemoglobin concentrations but sufficient iron stores (Hb <110 g/L + SF ≥10 µg/L) hence, their anaemia is unlikely to be caused by ID (WHO, 2017). Nevertheless, this remains a concern as anaemia, no matter the cause, can still result in poor cognitive and motor development in infants (WHO, 2020a). It has been suggested that the cut-off value for anaemia in infants <6 months old should be lowered to Hb <105 g/L (Domellöf, Hernell, et al., 2002). If we used this haemoglobin cut-off value, four infants (3.0%) would still be classified as anaemic.

Berglund and Domellöf (2021) stated that infants with a low risk of ID do not need iron supplements in the first six months of life to maintain their iron status. The results from the present study support this conclusion as all infants had a low risk of deficiency and iron supplements were only used by one infant yet, no infants developed ID prior to starting complementary feeding.

3.4.2 Maternal Iron Status

As with their infants, most (80.5%) mothers in this study had sufficient iron stores. Our results are similar to the study by Jin et al. (2021) which is currently, the only other NZ study investigating iron status at six months postpartum. They found 90.5% of women to have sufficient iron stores. In both studies, no women had IDA and rates of ID were low at only 0.8% (n=1; SF <15 µg/L) in the current study, and 4.2% (n=3; SF <12 µg/L) in the study by Jin et al. (2021). In a Norwegian study looking at iron status over the first postpartum year, 19% of women were ID (SF <15 µg/L) at 4 months postpartum (Bjørke-Monsen et al., 2012). One likely explanation for the higher prevalence of ID is that only 17% of mothers in the study by Bjørke-Monsen et al. (2012), used iron supplements in the postpartum period, compared to 49% of women in the current study, this may have resulted in a slower recovery of iron status after pregnancy. However, only 8% (n=6) of mothers used iron supplements in the study by Jin et al. (2021) and yet the prevalence of ID remained low.

An American study by Bodnar et al. (2002) investigating ID among low-income postpartum women, found 12.7% of women 0-6 months postpartum had ID and 4.2% had IDA. Again, the prevalence of ID and IDA is higher than in the current study, one reason for this may be that Bodnar et al. (2002) includes mothers from childbirth through to six months postpartum. Iron deficiency and IDA are known to be highest after childbirth and decrease as mothers recover their iron stores throughout the first postpartum year (Bjørke-Monsen et al., 2012; Finkelstein et al., 2013). Another explanation is the difference in socioeconomic status of mothers, Bodnar et al. (2002) investigated low-income mothers whereas the current cohort includes mothers who are highly educated and food secure, and therefore are likely to be of high socioeconomic status. Although socioeconomic status was not directly assessed, mothers were mostly European (90.9%), highly educated (87.0%), food secure (96.2%), regularly ate red meat (87-88% 'at least once a week') and were high users of iron-containing supplements during pregnancy and breastfeeding (88.8% and 49.0%, respectively); additionally, the primary study was about introducing vegetables as babies first foods and consequently, is likely to have attracted health-conscious mothers. These factors are likely protective of maternal iron status as they likely translate to greater access to iron-rich foods and an overall better health status of mothers in pregnancy, which in turn will provide her infant with adequate iron stores and protection against ID (Beck et al., 2014; Beck et al., 2013; Heath et al., 2001; Kazemi et al., 2020; Miller, 2014; Peña-Rosas et al., 2015). In contrast, mothers from more socioeconomically disadvantaged backgrounds are more likely to be food insecure and have reduced access to iron-rich foods and supplements during pregnancy (Kazemi et al., 2020; Park & Eicher-Miller, 2014); hence, their infants may have a higher risk of ID.

Anaemia in pregnancy is a strong predictor of postpartum anaemia (Bodnar et al., 2001; Grant, Wall, Brewster, et al., 2007). Only 5.3% of women were diagnosed with anaemia in pregnancy therefore, the risk of postpartum anaemia is low. Furthermore, a longer duration of breastfeeding may reduce the risk of postpartum anaemia due to amenorrhoea (Bodnar et al., 2001; Lopez et al., 2016). In the present study mothers breastfed for a median of three months and 89.5% of mothers were fully or partially breastfeeding at the time of blood sampling. Bodnar et al. (2001) found that women who breastfed for ≥7 weeks were 24% less likely to develop postpartum anaemia

compared to women who never breastfed. Hence, the low prevalence of anaemia in pregnancy and the high prevalence of breastfeeding likely contributed to the low rate of postpartum anaemia (1.5%) seen in the present study.

Surprisingly, ten mothers (7.5%) had serum ferritin concentrations >150 µg/L, indicating possible iron overload (WHO, 2020b). To our knowledge, iron overload has not previously been investigated in any studies of postpartum women. However, studies of premenopausal women have excluded women based on serum ferritin concentrations >200 µg/L, defined as iron overload (Beck et al. 2013). If we used this cut-off value, four mothers (3.0%) would still have serum ferritin concentrations indicative of iron overload. Of the ten mothers with SF >150 µg/L, we have iron supplement information for eight of these mothers. Of this group, all eight mothers with high serum ferritin concentrations were also taking iron supplements. This highlights the need to test iron levels before supplementing, especially in low-risk populations such as postpartum women. Unfortunately, we do not know if these women had genetic conditions, such as haemochromatosis, that may have provided another explanation for their high iron status (Heath & Fairweather-Tait, 2003).

We found 9.8% of mothers to have mild ID (SF <30 µg/L), to our knowledge, no other studies investigating iron status in postpartum women have reported mild ID. Our results indicate that the iron stores of postpartum women can vary dramatically, from ID to iron overload.

3.4.3 Relationship Between Maternal and Infant Iron Status

We determined that there was a weak positive relationship (r=0.19, P=0.03) between maternal and infant serum ferritin at 3-6 months postpartum. The fact that this relationship became stronger (r=0.253, P=0.01) when the confounding variables were controlled for supports the existence of this relationship. However, this model indicated that maternal log serum ferritin only explained 6% (r^2=0.064) of the variance in infant log serum ferritin. Therefore, it shows that many other factors can affect an infant's iron store, including the size of iron endowment at birth (Berglund & Domellöf, 2021), which was not able to be measured in the present study.

There is a paucity of evidence regarding the relationship between maternal and infant iron status at mid-infancy, particularly regarding serum ferritin. A recent study by Liu et al. (2015) did not measure infant serum ferritin concentrations at six months of age however, they did find a number of associations that suggest maternal and infant iron status are related at mid-infancy. Firstly, a weak correlation between maternal serum ferritin concentration in pregnancy and the prevalence of anaemia in six-month-old infants (r=0.112, P<0.01). Secondly, haemoglobin concentrations were significantly lower, serum transferrin receptor (sTfR) concentrations were significantly higher (P<0.01) and IDA prevalence was higher (P<0.01) in six-month-old infants whose mother was anaemic in her third trimester of pregnancy (Hb <100 g/L), compared to non-anaemic mothers (Hb >110 g/L). This suggests that depleted iron stores in late pregnancy may comprise the iron status of infants and may be related to a reduced iron endowment. Although 57.1% of mothers in the present cohort had ID during pregnancy, only 5.3% had anaemia. Fetal iron accretion is unlikely to be affected until severe maternal anaemia occurs (Cheng & Juul, 2011; Domellöf et al., 2014) hence, at most, 5.3% of infants could have had a reduced iron endowment at birth. However, as no infants had ID or IDA this is unlikely to have occurred.

Due to the lack of ID and IDA infants and mothers, we were unable to determine if a relationship between these variables exists. Most of the available literature reports finding a significant relationship between maternal and infant IDA at mid-infancy (Abioye et al., 2019; Finkelstein et al., 2013; Leslie et al., 2020; Liu et al., 2015). However, this has not been found in all studies (Marques et al., 2014). Randomised controlled trials are needed to better understand this complex relationship.

We did not find a significant relationship between maternal and infant haemoglobin (P=0.91) at 3-6 months postpartum. This is in contrast to Finkelstein et al. (2013) and Liu et al. (2015) who found significant relationships between 2-6 months postpartum. However, in agreeance with the present study, Amano and Murakami (2019) and Marques et al. (2014) also failed to find a relationship between maternal and infant haemoglobin at mid-infancy. The reason for these differences is unclear as all studies had differences in sample size, timings of blood sampling or average haemoglobin concentrations.

3.4.4 Infant Iron Status by Mode of Milk Feeding

In the present study, infant iron status did not differ according to the mode of milk feeding. This is in contrast to the majority of studies which report breastfed infants to have a lower iron status and greater risk of ID, than formula-fed infants, due to infant formula being fortified with a high concentration of iron (Burke et al., 2018; Chen et al., 2020; Clark et al., 2017; Grant, Wall, Brunt, et al., 2007; Hirata et al., 2017; Maguire et al., 2013). Libuda et al. (2018) also did not find a difference between most biomarkers of infant iron status and milk feeding mode at four and 10 months of age. One explanation for not finding a difference in the present study could be because no infants had ID or IDA and hence likely had a sufficient birth iron endowment. Therefore, the extra exogenous iron provided by infant formula may not have been needed to maintain their iron status. Additionally, this study may have been underpowered to detect a true difference between milk feeding modes, especially due to the low proportion of infants exclusively formula fed (10.5%).

3.4.5 Strengths and Limitations

A strength of the study design was the use of haemoglobin and serum ferritin to determine infant and maternal iron status. These iron biomarkers are widely used in research and hence will aid the comparison of our results with other studies. Additionally, it enabled us to determine the prevalence of IDA. A further strength was measuring iron overload in mothers and determining the relationship between maternal and infant iron status at mid-infancy, as the evidence in these areas is limited. Excluding participants with inflammation, based on elevated CRP results (CRP ≥10 mg/L), is another strength as it provided serum ferritin concentrations more reflective of true iron storage. As serum ferritin is a positive acute phase reactant, it may appear elevated in the presence of inflammation (WHO, 2011b).

A limitation of this study was having a low response rate (74%) to the Iron Questionnaire that asked about infant iron supplement use, maternal iron supplement use in pregnancy and breastfeeding and maternal vegetarian status. This may have biased our results if the mothers who answered the questionnaire were different from those that did not. A higher response rate may have provided more detail about maternal and infant iron intake, which in turn can influence iron status. The low

response rate is likely due to the questionnaire being asked after mothers had completed the primary study, when their infant was ≥12 months old, and hence participants may have been less willing to complete the questionnaire. Additionally, all study questionnaires were completed retrospectively which may have resulted in recall bias. Our sample may have been limited by the inclusion criteria for the primary study and likely resulted in a smaller number of participants which could affect the iron status results. Our participants were not representative of the NZ population therefore limiting the generalisability of our results. Another limitation was not measuring sTfR, this would have shown the demand for iron in tissues, as even if stores are sufficient there may be problems with iron transport.

3.4.6 Conclusion

Although iron status in the current cohort of mothers and infants was mostly adequate, this cohort is not representative of the NZ population and hence this limits the generalisability of our results. However, from these results we can say that mothers who are highly educated, food secure, breastfeeding, and who did not experience IDA in pregnancy will likely restore their iron status by 3-6 months postpartum. Additionally, their infants are unlikely to become iron deficient prior to starting complementary feeding. Further research is required to ascertain the iron status of low socioeconomic status postpartum women and their infants in NZ.

Chapter 4: Conclusions and Recommendations

4.1 Achievement of Aims and Objectives

The overall aim of the research was to determine the iron status of infants and their mothers prior to commencing complementary feeding. We first hypothesised that infants would be iron replete prior to starting complementary feeding. The WHO (2003) and New Zealand Ministry of Health (2008) recommends term infants to be exclusively breastfeed for around the first six months of life. This recommendation is firstly to ensure infants are provided with safe and nutritionally adequate food for optimal growth and development. Secondly, an infant's iron stores from birth will become depleted at approximately six months of age, hence infants need to begin eating iron-rich complementary foods at this time. Therefore, as the infants in this cohort were all between 3-6 months old and had not yet started complementary feeding, they should still have had sufficient iron stores from birth. We found this to be true as no ID or IDA was present. A small percentage of infants did have anaemia (6.8%), however, as their iron stores were adequate it was unlikely to be caused by ID.

Secondly, we hypothesised that mothers would be iron replete prior to their infants starting complementary feeding. The postpartum period is a time of very low iron requirements (Bothwell, 2000) and hence by 3-6 months postpartum mothers would likely have been able to replace their iron stores after pregnancy. This was true for most mothers (80.5%) however, a small proportion of mothers were not able to restore their iron status by this time, as 9.8% were mildly ID (SF <30 µg/L) and one mother had ID (SF <15 µg/L). On the other hand, a few mothers (7.5%) had iron stores indicative of iron overload (SF ≥150 µg/L). This highlights the wide range of iron stores possible in postpartum mothers and emphasises the need for postpartum screening of iron status, particularly as the use of iron supplements was high in this cohort and likely contributed to the very high serum ferritin concentrations seen. Furthermore, the signs and symptoms of ID, such as fatigue and reduced work performance may be hard to distinguish from the normal demands of motherhood hence, objective measures of iron status are needed. Mothers of low socioeconomic status may have a greater need for iron screening as they have a higher risk of postpartum ID (Bodnar et al., 2002).

Thirdly, we hypothesised that there would be a relationship between maternal and infant iron status. This was partially true as maternal and infant serum ferritin, but not haemoglobin, were related. The literature in this area is limited and inconsistent however, most of the available studies have found a relationship between ID and IDA in pregnancy and the prevalence of ID and IDA at mid-infancy. This would indicate that some degree of relationship exists however, more research is needed to better understand this complex relationship.

Finally, we hypothesised that infants fed breast milk only will have a lower iron status than those fed infant formula (exclusively and in combination with breast milk). Most studies have found breastfed infants to have lower iron stores and a greater risk of developing ID and IDA in late infancy than those fed infant formula (Burke et al., 2018; Chen et al., 2020; Grant, Wall, Brunt, et al., 2007). Even though iron in breast milk is highly bioavailable, the high level of iron added to infant formula means that overall, infants absorb more iron from formula than breast milk (Lönnerdal, 2017a). Hence, formula fed infants may use less of their birth iron endowment as they have more exogenous iron. However, we did not find a difference in infant serum ferritin or haemoglobin levels between infants fed breast milk or infant formula. This may have been due to the lack of ID and IDA found in this cohort.

4.2 Research Impact

The results of this study should be interpreted with caution. Most participants were highly educated, of European ethnicity and food secure and hence are likely to be of high socioeconomic status. These participant characteristics mean that the results of this study are not generalisable to New Zealand's diverse population or to people experiencing food insecurity. Over emphasising the results may increase health inequities. However, the learnings from this study could be used to design further large-scale iron studies.

This study did not find any infants to have ID or IDA prior to starting complementary feeding. Thus, highly educated and food secure mothers can be reassured that exclusive breastfeeding is unlikely to have a negative effect on their infants' iron status. However, the introduction of iron-rich solids should continue to be strongly encouraged

to ensure iron status is maintained once the birth iron endowment is depleted in mid to late infancy.

4.3 Strengths

To our knowledge, this is the first New Zealand (NZ) study to investigate iron status in infants and mothers between the age of 3-6 months postpartum. Previous NZ studies have focused on the iron status of infants between 6-24 months of age. Investigating the iron status of infants prior to starting complementary feeding is especially important due to the serious effects that ID can have on cognitive development if experienced during infancy and hence, identifying ID early in infancy may prevent these detrimental effects. Information about the iron status of postpartum mothers prior to their infant starting complementary feeding is also lacking. Hence, this study adds valuable information about maternal iron status.

Most studies investigating the relationship between maternal and infant iron status focus on maternal iron status in pregnancy and the iron status of her child at birth. However, there is limited evidence about the relationship between maternal and infant iron status at mid-infancy, hence this study adds to our current knowledge.

Another strength of this study was measuring both ID and iron overload. There is a paucity of evidence surrounding iron overload in postpartum women (WHO, 2016). Hence, this study adds to our knowledge of the prevalence and risk of iron overload in the postpartum period, especially among high users of iron supplements. However, larger studies and randomised controlled trials are needed to further explore this.

A strength of the study design was the use of haemoglobin and serum ferritin to determine infant and maternal iron status. These iron biomarkers are widely used in research and hence will aid the comparison of our results with other studies. It also enabled us to determine if anaemia was caused by ID. We also used C-reactive protein (CRP) to measure inflammation and excluded participants with CRP ≥10 mg/L. This provided serum ferritin levels that were more reflective of true iron storage levels, as serum ferritin is a positive acute phase reactant, it may appear elevated in the presence of inflammation (WHO, 2011b).

4.4 Limitations

This study had several limitations. Firstly, as this study is a secondary analysis of a randomised controlled trial some participants, who would have otherwise been eligible for this study, were excluded based on the criteria of the primary study. This has led to a smaller sample size and may have affected the iron status results. Unfortunately, it also meant that some questions regarding the factors that can influence iron status were not asked such as, mother's amenorrhoeic status and whether mother's had heredity conditions such as haemochromatosis. Women who are amenorrhoeic are likely to have higher iron stores than those who have begun menstruating again due to the significant decrease in iron losses. This may explain some of the high serum ferritin concentrations identified. Participants with conditions such as haemochromatosis, should have been excluded.

A limitation of this study was having a low response rate (74%) to the Iron Questionnaire that asked about infant iron supplement use, maternal iron supplement use in pregnancy and breastfeeding and maternal vegetarian status. This may have biased our results if the mothers who answered the questionnaire were different from those that did not. A higher response rate may have provided more detail about maternal and infant iron intake, which in turn can influence iron status. The low response rate is likely due to the questionnaire being asked after mothers had completed the primary study, when their infant was ≥12 months old, and hence participants may have been less willing to complete the questionnaire. Additionally, all study questionnaires were completed retrospectively which may have resulted in recall bias.

Serum transferrin receptor is a measure of iron demand in tissues and would have provided greater clarity about participant's functional iron status. This would have been particularly useful in helping to rule out ID as the cause of anaemia and assist in explaining why one mother had a very high serum ferritin concentration but a marginally low haemoglobin concentration (SF =196 µg/L, Hb =119 g/L). Even though iron stores may be sufficient, iron may not be able to get to bone marrow to produce haemoglobin.

Most participants were likely of a high socioeconomic status hence, this cohort is not representative of the NZ population. This limits the generalisability of the results.

Additionally, this is a cross-sectional study and therefore provides a snapshot of iron status. A longitudinal study would provide more information about iron status over time. This would be particularly useful in determining when most mothers become iron sufficient after childbirth and the prevalence of ID in the second half of infancy.

4.5 Recommendations and Future Directions for Research

- Research is needed to determine appropriate iron biomarker reference values for infants <6 months of age. Currently, researchers extrapolate cut-off values validated for use in older infants but these may not be appropriate for younger infants due to the normal physiological changes that occur in iron biomarkers (Domellöf, Hernell, et al., 2002).
- More research is needed in infants from low socioeconomic groups, food insecure households and of diverse ethnicities, focusing on Māori and Pasifika in NZ. It has been shown in internationals studies that infants from resource-limited settings have high rates of ID and IDA before six months of age (Finkelstein et al., 2013). Hence, researchers should endeavour to recruit participants from low-socioeconomic groups as this will provide a more representative sample of the NZ population.
- The Centers for Disease Control and Prevention (CDC) currently recommends iron screening of infants at-risk of ID between 9 and 12 months of age, then six months later and yearly from 2-5 years of age (CDC, 1998). To our knowledge, there is no iron screening for term infants in NZ. Hence, research should focus on whether increased screening for ID could reduce the prevalence of ID in at-risk NZ infants. Researchers should also assess the risks versus benefits of ID screening. Unnecessary blood sampling may increase ID risk whereas screening may allow ID to be detected early and hence reduce the harm caused by ID or IDA.
- When investigating the iron status of women, including postpartum women, research should investigate both ID and iron overload due to the potential health risks of both deficient and excess iron stores.
- Longitudinal studies of postpartum iron status would be useful to determine the mean length of time it takes women to replenish their iron stores after pregnancy, and the factors that influence this recovery time.

- More research is needed to determine and fully understand the relationship between maternal and infant iron status at mid-infancy.

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Appendices

Appendix 1: Recruitment Poster

Is your baby getting ready to start solids?

We want to invite you to participate in our study!

You will need to:

- Live in Auckland and have a baby 4-6 months of age
- Feed your baby recommended infant foods for 4 weeks
- Fill in simple questionnaires and baby food diary
- Provide blood and stool samples, and child growth measurements
- Video record your baby trying foods for the first time

You will receive FREE:

- Infant feeding and nutrition support from an **NZ registered dietitian**
- **Nutritious baby foods** during the 4-week trial
- **Iron** and **Vitamin D** status assessments for you and your baby
- Infant growth assessments
- **Fun DVD** of your baby's first food experiences

INTERESTED? Please contact:

Jeanette Rapson
0210773419 | vegesfirst@massey.ac.nz
www.vegesfirststudy.co.nz

MASSEY UNIVERSITY TE KUNENGA KI PŪREHUROA UNIVERSITY OF NEW ZEALAND | COLLEGE OF HEALTH TE KURA HAUORA TANGATA

Jeanette Rapson

MUHEC: Southern A, Application SOA 18/56.

Appendix 2: Participation Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

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Vegetables as first foods for babies study

INFORMATION SHEET

You are invited to take part in the *Vegetables as first foods for babies study* which is looking at the impact of a vegetables first approach to complementary feeding on infant food preferences.

The researchers are as follows:

Lead researcher

Supervisor

Supervisor

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Whether or not you take part is your choice. If you do not want to take part, you do not have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you would like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is seven pages long. Please make sure you have read and understood all the pages.

If you have any further question, concerns or complaints about the study at any stage, please do not hesitate to contact our lead researcher:

Jeanette Rapson
Lead researcher

Tel: 0210773419
email: vegesfirst@massey.ac.nz
Website: www.vegesfirststudy.com

What is the study about?

In New Zealand, complementary feeding is recommended to start at around 6 months. Suggested first foods include vegetables, fruits, meat, and commercial infant foods including baby rice.

Introducing vegetables early in life may help children like and eat more vegetables later. It is important that children eat plenty of different vegetables and fruits to provide the vitamins, minerals and dietary fibre needed for their growth and development.

Few studies have investigated different combinations of vegetables and fruits during complementary feeding, especially in New Zealand. The aim of this study is to compare two different feeding regimes which differ in the types of vegetables and fruits they provide. We will also examine the mother's diet during pregnancy and lactation as this may have some impact on the taste preference of their child.

Our results may better inform current infant feeding recommendations in New Zealand and worldwide.

Who can take part?

We are looking for 120 mothers and their baby to volunteer to participate in this study.

To take part in all parts of this study, your baby should be:

- 4-6 months of age
- healthy and born at term gestation (37+ weeks)
- with no known allergies
- with no chronic diseases or medical conditions that may affect abilities to participate in the study
- at a stage where they have not started eating solids
- living in the Auckland region

What will my participation involve?

Your participation involves visiting our Human and Nutrition Research Unit, giving a blood sample, having measurements taken for your baby (growth, heel prick blood sample and faecal) completing dietary and lifestyle questionnaires, and video-recording your infant trying foods for the first time.

If you decide to take part in this study after you have read and had time to consider this information sheet, you will be asked to complete a screening questionnaire to ensure that you meet the study's eligibility criteria. If eligible, you will be invited to take part in the study and asked to sign a consent form. You will then be sent a series of dietary and lifestyle questionnaires to complete at home.

Total time involved for this study is **10 hours over a period of 12 months.** This time is divided as follows:

Each visit takes about 1 hour (total of 4 hours). Questionnaires each take about 10-30 minutes to complete (total of 2.5 hours). It is not anticipated that infant feeding will take any longer than what is usual for you, however, additional time will be needed to complete infant feeding diaries and video recordings (5-10 minutes per day).

When your baby is 4 months old

A researcher will make an appointment with you and your baby to visit the Human Nutrition Research Unit at Massey University in Albany at a time that suits. We will provide reserved free parking, appropriate bathroom facilities and private spaces for feeding. This visit will take about **1 hour.**

During this visit, we will ask you to:

- Meet with a New Zealand registered dietitian to discuss any questions you may have about the study and infant feeding.
- Tell us more information about your diet and lifestyle.
- Have a small blood sample taken from yourself by a qualified phlebotomist (about 20ml which is equivalent to 4 teaspoons) to measure your iron and vitamin D levels.
- Have a small blood sample taken from your baby by qualified phlebotomist using a 'heel prick' test to measure their iron and vitamin D levels (please see what does a heel prick involve?)
- Have your baby's length, weight and head circumference measured.
- Provide a small faecal sample from your baby to assess their faecal microbiota composition (please see what does a faecal sample involve?)
- Collect your infant feeding pack, which includes the foods, equipment and instructions needed during the 4-week trial.
- Discuss with a researcher about video recording your baby's feeding sessions using a device of your choice, e.g. mobile phone, video camera.

You and your baby will be randomly allocated one of two infant feeding regimes that differ by the types of vegetables and fruits they provide. You will not be told which feeding regime you have been assigned to; however, the feeding regimes include vegetables and fruits that are recommended for babies starting solids. All babies will receive approved age-appropriate foods that enable them to meet recommended dietary guidelines (please see 'what will the foods be like?').

During the 4-week trial

When your baby is ready to start solids (around 4-6 months of age), we will ask you to:

- Notify the researcher that you have started/ready to start complementary feeding.
- Feed the allocated foods to your baby for a period of 4-weeks alongside breastmilk or infant formula. You will also be asked to please not provide any other foods or beverages except water and meat (please see what will the foods be like?)
- Video record the first time you feed the allocated foods to your baby, and then at least two additional feeding sessions during the 6-weeks.
- Complete a daily weighed food diary (please see what does a weighed food diary involve?)
- Each week you will be asked to compete an online 2-minute check-in questionnaire to see how you are going.

After the 4-week trial

You will no longer need to feed the allocated foods and can resume your normal family diet. A researcher will contact you to arrange a time to meet you and your baby at the research unit at Massey University Albany (home visits available on request). This visit will take about **30 minutes.**

During this visit, we will ask you to:

- Have a small blood sample taken from your baby by qualified phlebotomist using a 'heel prick' test to measure their iron levels.
- Have your baby's length, weight and head circumference measured.
- Provide a small faecal sample from your baby to assess their faecal microbiota composition.
- Provide your baby's infant feeding diary, videos and return any borrowed equipment.

When your baby is 9 months old

When your baby is 9 months of age, a researcher will contact you to arrange a time to meet you and your baby at the research unit at Massey University Albany (home visits available on request). This visit will take about **30 minutes.**

During this visit, we will ask you to:

- Have a small blood sample taken from your baby by qualified phlebotomist using a 'heel prick' test to measure their iron levels.
- Have your baby's length, weight and head circumference measured.
- Provide a small faecal sample from your baby to assess their faecal microbiota composition.
- Video record your baby trying a vegetable food that we will provide.
- Complete dietary questionnaires for your baby.

When your baby is 12 months old

When your baby is 12 months of age, a researcher will send you online questionnaires to complete then arrange a time to meet you and your baby at the research unit at Massey University Albany (home visits available on request). This visit will take about **30 minutes.**

During the visit, we will ask you to:

- Have your baby's length, weight and head circumference measured.
- Provide a small faecal sample from your baby to assess their faecal microbiota composition.

FAQ

What does the 'heel prick' test involve?

Iron is extremely important for infant brain development and growth. Iron deficiency can also cause taste disturbances, which may affect your baby's liking and intake of foods. It is vital that your baby has good iron status for the study outcomes and their health. Babies also need good levels of vitamin D for growth and development.

The common way to measure and assess iron and vitamin D status in babies is to do a 'heel prick' test. This involves making a pinprick puncture in one heel of your baby to collect a small blood sample. This is completed by a qualified phlebotomist and is then sent to a laboratory to be analysed.

You can ease any distress for your baby by cuddling and feeding them and making sure they are warm and comfortable. Your baby's foot will also be warmed by a sock prior to the heel prick test to minimise discomfort. There is a small risk of infection at the puncture site, however strict hygiene and safety procedures will be followed to minimise this risk.

What does a faecal sample involve?

The human microbiota consists of a wide variety of microorganisms such as bacteria, viruses and fungi. The common way to measure the composition of an infant's microbiota is to collect a small faecal (stool) sample and then analyse it in a laboratory. We will ask you to use a collection kit that we provide with easy to follow instructions.

What will the foods be like?

You will receive freeze-dried vegetables and fruit powders that are conveniently made into a smooth puree by adding water. These have been commercially prepared, packaged, and developed especially for this study by a food manufacturer. There are no preservatives, gluten, dairy or nuts added to these foods. They are easy to store and travel with. All foods meet recommended guidelines and safe for baby.

The types of vegetables include: spinach, beetroot, green bean, broccoli, pumpkin and potato. The types of fruits include: apple and pear.

Foods do not contain meat. However, meat is encouraged as an iron-rich food during the study. Support will be given on how to prepare and provide meat foods.

What does a weighed food diary involve?

It is important for us to understand how much of the foods your child eats and their liking of it. An easy way to do this is to weigh (in grams) the amount of leftover food using food kitchen scales, then record this in the diary. We will provide a set to use during the trial. Alternatively, you can freeze the leftover foods and we will collect this from you to complete the measurement.

In this diary, you will need to rate your baby's liking of the given food using a simple rating scale (1=likes very much to 9=dislikes very much), then describe anything that might have affected you baby's feeding that day. You will be given training and support on how to complete this.

What are the possible benefits and risks of this study?

Direct benefits of participating in this study include:

- Free infant nutrition and feeding support from a registered dietitian throughout the study period.
- Free nutritious infant foods during the 4-week trial.
- You will receive feedback on you and your baby's individual blood results, and your baby's growth measurements. You will be advised if any of the blood results are outside of a normal range and advised to seek advice from your general medical practitioner (GP). We will provide you with a copy of your results to give to your GP.
- An increased awareness and knowledge of the processes involved in research by actively participating in it, and a satisfaction in knowing that you are contributing to nutrition knowledge in the community.
- At the end of the study you will receive a DVD with memorable video clips of your child that were made during the research.
- You will receive a brief report summarising the main findings of the project via mail or email.

Foreseeable risks, adverse-effects and discomforts that you may encounter by taking part in this study are minimal but could include possible infection from the site in which blood is drawn and there may be some minor bruising at this site as well. Your baby may also feel some discomfort from the heel prick test. These discomforts will be managed by the presence of a qualified phlebotomist who will be available to assist you should you require it. While all foods provided by the study will be safe, age-appropriate and approved first foods for babies, it is possible that your baby may not like or tolerate the food. A record of all adverse events will be monitored and maintained throughout the course of the study.

Unless otherwise arrange, all follow up visits will be conducted at your home with consent and if deemed safe for the researcher and participant. We will ask if you have any cultural issues or concerns around taking these measurements before they are taken. For example, if you are Maori and consider the head as tapu (sacred), we will ask for your permission before touching the infant's head.

Does it cost money to participate?

There is no monetary cost to you, the participant, for taking part in this study. You will be reimbursed for travelling costs with a $20 voucher following your first visit to the Massey University Human and Nutrition Research Unit (Albany). If you choose to visit our research unit for the follow-up visits, you will be given a $20 voucher following each visit.

What are my rights?

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study (specify timeframe);
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded;
- ask for the recorder or video to be turned off at any time during the interviews or video recordings.

What if something goes wrong?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Data management

The data will be used only for the purposes of this project and no individual will be identified. Only the investigators and administrators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number.

Results of this project may be published or presented at conferences or seminars. No individual will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 16 years, after which it will be destroyed.

Samples will be stored separately and only Dr Cath Conlon, Owen Mugridge and Jeanette Rapson will have access to these records. You will be given the option for samples donated by yourself or your child to be stored for use in future research studies.

What happens if I change my mind?

You are able to stop participating in the study at any time and will be compensated accordingly for your time. Further you are welcome to discuss any concerns you have with the research team at any time, and you have free access to your data. If you withdraw from the study all of the data that was related to you will be destroyed.

The foods and support from a dietitian will not be available to any participant after the study. The outcomes of this study may inform future recommendations enable further investigation in a long-term intervention study.

The study data will be stored at a secure location at Massey University Albany Campus. Electronic data and records will be the responsibility of the Principal investigator. All data will be kept for 10 years, at which point it will be destroyed using University Security methods for removal of confidential material. At the completion of the study all biological samples collected will be disposed of using established methods for discarding biological waste. Any participant can request to have their remaining blood sample returned to them.

You may hold beliefs about a sacred and shared value of all or any tissue samples removed. The cultural issues associated with storing your tissue should be discussed with your family/whanau as appropriate. There are a range of views held by Maori around these issues; some iwi disagrees with storage of samples citing whakapapa and advise their people to consult prior to participation in research where this occurs. However, it is acknowledged that individuals have the right to choose.

We anticipate that the results of this study will be published in a peer-reviewed journal within 12 months of completing the study. Participants are welcome to discuss the findings of this study with the researchers at any time.

Who do I contact for more information or if I have concerns?

If you have any questions, concerns or complaints about the study at any stage, you can contact researchers in the study.

You can contact the following researchers:

> Jeanette Rapson, Dr Cath Conlon or Associate Professor Pam von Hurst. Contact details are at the beginning of this information sheet.

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, *Application SOA 18/56. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone + 64 6 356 9099 x 85094, email humanethicsoutha@massey.ac.nz .*

Appendix 3: Demographic Questionnaire

Finding out about you

Thank you for taking the time to complete this questionnaire.
It should take approximately **10-15 minutes** to complete.

This questionnaire is divided into three sections; Health, Lifestyle, and About you.
Please answer all questions yourself. This is **not a test**.

If you have any questions, please contact:
Jeanette Rapson on 0210773419 | vegesfirst@massey.ac.nz
or Dr Cath Conlon on 09 4140800 ext 43658 | C.Conlon@massey.ac.nz

Website: www.vegesfirststudy.co.nz

All information in this questionnaire will be kept confidential.

Committee Approval Statement

Please enter your **three digit** participant number:

Note: this number was sent to you by email. It will be used to match up data sets, **not** to personally identify you.

Choose one of the following options that best describes you.

- ○ I am currently pregnant
- ○ I have a baby less than six months of age

Section A: Health (had baby)

Your Health

The following questions will ask about your health before and during your pregnancy.

During your pregnancy, were you diagnosed with any of the following? *Please tick all that apply.*

- ☐ Anaemia
- ☐ Iron deficiency
- ☐ High blood pressure
- ☐ Type 2 diabetes
- ☐ Gestational diabetes
- ☐ Heartburn
- ☐ Choose not to answer
- ☐ Other (please specify):

Before your pregnancy, in general, how would you describe your health?

- ○ Poor
- ○ Fair
- ○ Good
- ○ Very Good
- ○ Excellent
- ○ Choose not to answer
- ○ Other (please specify) _____

Did this description change during your pregnancy?

- ○ Yes
- ○ No
- ○ Choose not to answer

How did your health change?

- ○ Worse
- ○ Better
- ○ Choose not to answer
- ○ Other (please specify) _____

Is this your first baby?

- ○ Yes
- ○ No
- ○ Choose not to answer

How many other children do you have?

- ○ One
- ○ Two
- ○ Three
- ○ Four
- ○ Five
- ○ More than five (please specify) _____
- ○ Choose not to answer

Section B: lifestyle (had baby)

Your Lifestyle

The following questions will ask about your lifestyle choices before and during your pregnancy.

Over the 6 months prior to becoming pregnant were you actively dieting or trying to lose weight?

- ○ Yes
- ○ No
- ○ Choose not to answer

Did you lose any weight?

- ○ Yes
- ○ No
- ○ Unsure
- ○ Choose not to answer

Approximately how much weight did you lose? *Please write in the text box.*

_____

Over the 6 months prior to becoming pregnant, were you actively trying to gain weight?

- ○ Yes
- ○ No
- ○ Choose not to answer

Did you gain any weight?

- ○ Yes
- ○ No
- ○ Unsure
- ○ Choose not to answer

Approximately how much weight did you gain? *Please write in the text box.*

Section A: Health (pregnancy)

Section A: Health

The following questions will ask about your current and past health.

Have you been diagnosed with the following during your pregnancy?

	Yes	No	Yes Choose not to answer
Anaemia	○	○	○
Iron deficiency	○	○	○
Other mineral deficiency	○	○	○
High blood pressure	○	○	○
Gestational diabetes	○	○	○
Heart burn	○	○	○

Before your pregnancy, in general, how would you describe your health?

- ○ Poor
- ○ Fair
- ○ Good
- ○ Very Good
- ○ Excellent
- ○ Choose not to answer
- ○ Other (please specify in text box below)

Has this description changed since becoming pregnant?

- ○ Yes
- ○ No
- ○ Choose not to answer

How has your health changed?

- ○ Worse
- ○ Better
- ○ Choose not to answer
- ○ Other (please specify)

Is this your first baby?

- ○ Yes
- ○ No
- ○ Choose not to answer

How many other children do you have?

- ○ One
- ○ Two
- ○ Three

- ○ Four
- ○ Five
- ○ More than five (please specify) _____
- ○ Choose not to answer

Lifestyle (pregnancy)

Section B: Lifestyle

The following questions will ask about your lifestyle choices.

Over the 6 months prior to becoming pregnant, were you actively dieting or trying to lose weight?

- ○ Yes
- ○ No
- ○ Choose not to answer

Did you lose any weight?

- ○ Yes
- ○ No
- ○ Unsure
- ○ Choose not to answer

Approximately how much weight did you lose? *Please write in the text box.*

_____

Over the 6 months prior to becoming pregnant, were you actively trying to gain weight?

- ○ Yes
- ○ No
- ○ Choose not to answer

Did you gain any weight?

- ○ Yes
- ○ No
- ○ Unsure
- ○ Choose not to answer

Approximately how much weight did you gain? *Please write in the text box.*

Personal information (pregnancy)

About you

We would like to learn more about you.

What is your date of birth?

	Day	Month	Year
Please Select:			

What is the gender of your baby?

- ○ Male
- ○ Female
- ○ Other
- ○ Not sure yet
- ○ Choose not to answer

Which ethnic group(s) do you belong to? *Please select all that apply.*

- ☐ New Zealand European/ Pakeha

- [ ] New Zealand Maori
- [ ] Cook Island Maori
- [ ] Fijian
- [ ] Samoan
- [ ] Tongan
- [ ] Other Pacific Island
- [ ] Other European
- [ ] Chinese
- [ ] South East Asian
- [ ] Other Asian
- [ ] Choose not to answer
- [ ] Other (please specify) _____

What is your highest qualification? *Please select one.*

- ○ School certificate/ NCEA level 1
- ○ 6th form certificate/ NCEA 2
- ○ NCEA level 3
- ○ Graduate degree/diploma
- ○ Masters
- ○ PhD
- ○ None of the above
- ○ None, yet (still in school)
- ○ Choose not to answer
- ○ Other (please specify) _____

What is your postcode? *Please write in the text box.*

If you do not know your postcode, click here.

Please note: your postcode is for the area you live in, not your house. This is so we cannot identify who you are from your postcode5

How would you describe your household's food availability? *Please select one.*

- ○ Limited
- ○ At times limited
- ○ Adequate
- ○ Choose not to answer
- ○ Other (please specify)

Is there anything else that you would like to tell us about yourself? *Please comment in the text box below.*

Appendix 4: Pregnancy FFQ

Note: only the relevant pages of the FFQ have been included.

Pregnancy Food Frequency Questionnaire

Thank you for taking the time to complete this questionnaire.
It should take approximately **20 minutes** to complete.

This is **not a test**. Your answers will help us learn about food choices during pregnancy.

If you have any questions, please contact:
Jeanette Rapson on 0210773419 | vegesfirst@massey.ac.nz
or Dr Cath Conlon on 09 4140800 ext 43658 | C.Conlon@massey.ac.nz

Website: www.vegesfirststudy.co.nz

All information in this questionnaire will be kept confidential.

Pregnancy

Please enter your **three digit** participant number:

Note: this number was sent to you by email. It will be used to match up data sets, **not** to personally identify you.

Choose one of the following that best describes you.

- ○ I am pregnant
- ○ I have a baby less than six months old

Lean meats, poultry

How often have you eaten **lean meats and poultry** during pregnancy?

	Never	Most days	At least once a week	At least once a month	At least once during my pregnancy
Red meat (e.g. beef, lamb or pork)	○	○	○	○	○
Poultry (e.g. chicken, turkey or duck)	○	○	○	○	○

Appendix 5: Breastfeeding FFQ

Note: only the relevant pages of the FFQ have been included.

Breastfeeding Food Frequency Questionnaire

Thank you for taking the time to complete this questionnaire.
It should take approximately **20 minutes** to complete.

This is **not a test**. Your answers will help us learn about food choices since giving birth.

If you have any questions, please contact:
Jeanette Rapson on 0210773419 | vegesfirst@massey.ac.nz
or Dr Cath Conlon on 09 4140800 ext 43658 | C.Conlon@massey.ac.nz

Website: www.vegesfirststudy.co.nz

All information in this questionnaire will be kept confidential.

ID

Please enter your **three digit** participant number:

Note: this number was sent to you by email. It will be used to match up data sets, **not** to personally identify you.

Lean meats, poultry

How often have you eaten **lean meats and poultry** since giving birth?

	Never	Most days	At least once a week	At least once a month	At least once since giving birth
Red meat (e.g. beef, lamb or pork)	○	○	○	○	○
Poultry (e.g. chicken, turkey or duck)	○	○	○	○	○

Appendix 6: Baby FFQ

Note: only the relevant pages of the FFQ have been included.

Baby Food Frequency Questionnaire

Thank you for taking the time to complete this questionnaire.
It should take approximately **10 minutes** to complete.

This questionnaire asks about how your baby has been fed over the last 4 days. There are no "right" or "wrong" answers.

If you have any questions, please contact:
Jeanette Rapson on 0210773419 | vegesfirst@massey.ac.nz
or Dr Cath Conlon on 09 4140800 ext 43658 | C.Conlon@massey.ac.nz

Website: www.vegesfirststudy.co.nz

All information in this questionnaire will be kept confidential.

We would like to learn more about your baby's **milk feeds.**

Was your baby ever breastfed?

- ○ Yes
- ○ No

Are you still breastfeeding?

- ○ Yes
- ○ No

If no longer breastfeeding, how old was your baby when you stopped?

- ○ Less than 1 week
- ○ 1 week
- ○ 2 weeks
- ○ 3 weeks
- ○ 1 month
- ○ 2 months
- ○ 3 months
- ○ 4 months
- ○ 5 months
- ○ 6 months
- ○ 7 months
- ○ 8 months
- ○ 9 months
- ○ Still breastfeeding

If breastfeeding, how many minutes has baby usually sucked at

each feed in the last 4 days?

- ○ Less than 1 minute
- ○ 1 minute
- ○ 2 minutes
- ○ 3 minutes
- ○ 4 minutes
- ○ 5 minutes
- ○ 6 minutes
- ○ 7 minutes
- ○ 8 minutes
- ○ 9 minutes
- ○ 10 minutes
- ○ More than 10 minutes

Has your baby had infant formula at any stage?

- ○ Yes
- ○ No

How old was your baby when formula was first introduced?

- ○ Less than 1 week
- ○ 1 week
- ○ 2 weeks
- ○ 3 weeks
- ○ 1 month
- ○ 2 months
- ○ 3 months
- ○ 4 months
- ○ 5 months

- ○ 6 months
- ○ 7 months
- ○ 8 months
- ○ 9 months
- ○ No formula introduced

Is your baby still having infant formula?

- ○ Yes
- ○ No

For bottle/cup feeds (any milk or formula), how much does baby usually drink at **each feed** in the last 4 days?

- ○ 50 ml
- ○ 100 ml
- ○ 150 ml
- ○ 200 ml
- ○ 250 ml
- ○ More than 250 ml
- ○ Fully breastfed
- ○ Other, please specify amount: _____

Appendix 7: Iron Questionnaire

Iron and Vitamin D Survey

This should take about **5 minutes** to complete.

We would like to learn more about the factors that may have impacted you and your baby's iron and vitamin D levels. There are no "right" or "wrong" answers.

All information in this questionnaire will be kept confidential.

Thank you for your time!

Please enter your **three digit** participant number:

Note: this number was given to you by email. It will be used to match up data sets, **not** to personally identify you.

Block 1

Did you follow a vegetarian diet whilst pregnant or breastfeeding?

A vegetarian diet means you did not eat any animal meat (beef, chicken, pork, lamb, or fish), but may, or may not, have eaten dairy products (milk, yoghurt or cheese) or eggs.

- ○ Yes, I followed a vegetarian diet whilst pregnant
- ○ Yes, I followed a vegetarian diet whilst breastfeeding
- ○ Yes, I followed a vegetarian diet whilst pregnant and breastfeeding
- ○ No, I did not follow a vegetarian diet whilst pregnant or breastfeeding

○ Other, please state

Block 2

The following questions relate to your supplement use whilst pregnant.

Did you take any supplements that contained **vitamin D** whilst pregnant?

○ Yes
○ No

Did you take any supplements that contained **iron** whilst pregnant?

○ Yes
○ No

Which supplements did you take whilst pregnant? Please select all that apply:

☐ Ferrograd
☐ Ferrograd C
☐ Ferrotab
☐ Maltofer
☐ Elevit
☐ Blackmores Pregnancy and Breastfeeding Gold
☐ Cholecalciferol/Vitamin D
☐ Other, please state.
☐ I don't remember
☐ I did not take any supplements whilst pregnant

At any stage, did your baby receive breast milk?

○ Yes
○ No

The following questions relate to your supplement use whilst breastfeeding.

Did you take any supplements that contained **vitamin D** whilst breastfeeding?

- ○ Yes
- ○ No

Did you take any supplements that contained **iron** whilst breastfeeding?

- ○ Yes
- ○ No

Which supplements did you take whilst breastfeeding? Please select all that apply:

- ☐ Ferrograd
- ☐ Ferrograd C
- ☐ Ferrotab
- ☐ Maltofer
- ☐ Elevit
- ☐ Elevit Breastfeeding
- ☐ Blackmores Pregnancy and Breastfeeding Gold
- ☐ Cholecalciferol/Vitamin D
- ☐ Other, please state.
- ☐ I don't remember
- ☐ I did not take any supplements whilst breastfeeding

Were you taking any other supplements or multivitamins not previously mentioned whilst you were pregnant or breastfeeding?

- ○ Yes, whilst pregnant
- ○ Yes, whilst breastfeeding
- ○ Yes, whilst pregnant and breastfeeding
- ○ No, I did not take any other supplements or multivitamins whilst pregnant or breastfeeding
- ○ I don't remember

Please state the **type and brand** of supplement(s) or multivitamin(s) you used, and **how long** you used them for.

The following questions relate to factors that may have affected your child's iron or vitamin D levels, while they were a baby.

Please note: for the purpose of this survey, we will refer to your child as a baby, as this is the time point we are interested in.

Did your baby receive any supplements that contained **iron** during the first 9 months of life?

- ○ Yes
- ○ No

At what age did your baby receive iron containing supplements? Please tick all that may apply.

- ☐ Less than 1 month of age
- ☐ 1 month of age
- ☐ 2 months of age
- ☐ 3 months of age
- ☐ 4 months of age
- ☐ 5 months of age
- ☐ 6 months of age
- ☐ 7 months of age
- ☐ 8 months of age
- ☐ 9 months of age

Did your baby receive any supplements that contained **vitamin D** during the first 9 months of life?

- ○ Yes
- ○ No

At what age did your baby receive vitamin D containing supplements? Please tick all that may apply.

- [ ] Less than 1 month of age
- [ ] 1 month of age
- [ ] 2 months of age
- [ ] 3 months of age
- [ ] 4 months of age
- [ ] 5 months of age
- [ ] 6 months of age
- [ ] 7 months of age
- [ ] 8 months of age
- [ ] 9 months of age

Did your baby take any of the following supplements during the first 9 months of life?

- [ ] Vitadol C
- [ ] Puria vitamin D
- [ ] Nordic Naturals Baby's Vitamin D3
- [ ] Ferrodan
- [ ] Other, please specify
- [ ] My baby did not take any supplements

Was your baby taking any other supplements or multivitamins not previously mentioned during the first 9 months of life?

- ○ Yes
- ○ No
- ○ I don't remember

Please state the **type and brand** of dietary supplement(s) or multivitamin(s) used

At what age did your baby take these supplements or multivitamins? Please tick all that may apply.

- [ ] Less than 1 month of age
- [ ] 1 month of age
- [ ] 2 months of age
- [ ] 3 months of age
- [ ] 4 months of age
- [ ] 5 months of age
- [ ] 6 months of age
- [ ] 7 months of age
- [ ] 8 months of age
- [ ] 9 months of age