Iron Status of Preterm Infants after Hospital Discharge

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Abstract

Background: Preterm infants are at an increased risk of developing iron deficiency (defined in paeditaric populations as a ferritin value <12 μg/L or a serum transferrin receptor concentration >2.4 mg/L) after discharge due to their shortened gestational length, increased requirements for rapid growth, and excessive blood losses through phlebotomy. Optimising preterm infant iron status after discharge is important as poor iron status has been associated with negative health and neurodevelopmental outcomes later in life. Only preterm infants born before 32 weeks gestation or with a birth weight less than 1800 g currently receive routine iron supplementation after discharge from Auckland City Hospital; however there is paucity of evidence to determine whether this is best practice.

Objective: To investigate the iron status of preterm infants in Auckland, New Zealand at four months after discharge from hospital.

Methods: Sixty one preterm infants were recruited through Auckland City Hospital. At four months after discharge infant haemoglobin, serum ferritin and soluble transferrin receptor (sTfR) concentrations were measured to assess iron status. Weight, length and head circumference were also measured. Information about iron supplementation and mode of feeding was collected using an online questionnaire. Statistical analysis using independent *t*-tests, Mann-Whitney tests and bivariate correlations were performed.

Results: 16.4% of preterm infants had iron deficiency anaemia (defined in paediatric populations as a haemoglobin less than 110 g/L in conjunction with low iron stores) at four months after discharge, with an additional 6.6% of preterm infants classified as having iron deficiency. No infant had iron overload. Iron supplementation was associated with significantly higher haemoglobin (P<0.001) and serum ferritin (P<0.001) concentrations along with lower sTfR concentrations (P=0.005) at four months after discharge. Iron supplementation was also protective against suboptimal iron status at four months after discharge (P=0.018). Mode of feeding, introduction of

solids, intrauterine growth restriction, and maternal iron status had no effect on infant iron status at four months after discharge. There was also no relationship between growth and iron supplementation or iron status at four months after discharge.

Conclusion: Preterm infants who did not receive iron supplements after discharge had an increased risk of developing iron deficiency and iron deficiency anaemia at four months after discharge. Routine iron supplementation for all preterm infants combined with screening for iron deficiency anaemia after discharge appears to be a safe and effective way to reduce the risk of iron deficiency and iron deficiency anaemia at four months after discharge.

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Table of Contents

Abstract ..... **iii**

Acknowledgements ..... **v**

Table of Contents ..... **vi**

List of Tables ..... **ix**

List of Figures ..... **xi**

Abbreviations ..... **xii**

Chapter 1 Introduction **1**

1.1 Background 1

1.2 Purpose of the Study 6

1.3 Aim 7

1.4 Thesis Structure 8

1.5 Researchers' Contributions 9

Chapter 2 Literature Review **10**

2.1 Preterm Birth: Definitions, Causes and Consequences 10

2.2 Iron 14

2.3 Iron Deficiency and Iron Deficiency Anaemia 18

2.4 Iron Overload 20

2.5 Biomarkers of Iron Status 22

2.6 Maternal and Infant Factors Affecting Iron Status 30

2.7 Feeding Protocol in the Neonatal Intensive Care Unit 34

2.8 Effect of Breast and Formula Feeding on Infant Iron Status 36

2.9 Iron Supplementation 38

2.10 Care of the Preterm Infant after Discharge 42

2.11 Introduction of Solids 44

2.12 Summary 50

Chapter 3 Methods **51**

3.1 Study Design 51

3.2 Ethical Approval 51
3.3 Study Population 51
3.4 Measures to Assess Infant Iron Status and Growth at Four Months after Discharge 53
3.5 Standard Operating Procedure for Booking 61
3.6 Standard Operating Procedure for Appointment 61
3.7 Standard Operating Procedure for Data Collection from Medical Notes 63
3.8 Standard Operating Procedure for Informing Participants of Results 63
3.9 Statistical Analysis 63

Chapter 4 Results 65
4.1 Description of Participants 65
4.2 Preterm Infant Iron Status after Discharge 66
4.3 Iron Supplementation and Iron Status 67
4.4 Feeding Practices after Discharge and Iron Status 72
4.5 Pre-Discharge Characteristics and Iron Status 74
4.6 Maternal Characteristics and Iron Status 75
4.7 Iron Status and Infant Growth 76

Chapter 5 Discussion 78
5.1 Study Population Characteristics 78
5.2 Iron Status of Preterm Infants at Four Months after Discharge 78
5.3 Iron Supplementation and Preterm Infant Iron Status at Four Months after Discharge 83
5.4 Infant Feeding Practices after Discharge and Iron Status at Four Months after Discharge 88
5.5 Pre-Discharge Characteristics and Iron Status 90
5.6 Maternal Characteristics and Iron Status 92
5.7 Iron Status and Infant Growth 93

Chapter 6 Conclusion 94
6.1 Summary of the Study 94

6.2 Conclusion 96
6.3 Strengths 97
6.4 Limitations 99
6.5 Recommendations for Future Studies 101

References 103

Appendices 123
Appendix A. *Study Poster* 123
Appendix B. *Contact Letter* 125
Appendix C. *Information Sheet* 126
Appendix D. *Contact Details Slip* 131
Appendix E. *Data Collection Sheet* 132
Appendix F. *Serum Ferritin Data Sheet.* 135
Appendix G. *Serum Soluble Transferrin Receptor Data Sheet* 138
Appendix H. *C-Reactive Protein Data Sheet* 141
Appendix I. *Demographic Questionnaire* 144
Appendix J. *Feeding Questionnaire* 146
Appendix K. *Supplement Questionnaire* 154
Appendix L. *Eligibility Screening* 157
Appendix M. *Infant Informed Consent Form* 158
Appendix N. *Maternal Informed Consent Form* 159
Appendix O. *Medical Notes Questionnaire* 160
Appendix P. *Letter to GP* 165

List of Tables

Chapter 1 1

Table 1.1. *Researchers' Contributions to this Study* 9

Chapter 2 10

Table 2.1. *Advantages and Disadvantages of Common Biomarkers of Iron Status* 27

Table 2.2. *Summary of the Characteristics and Results of Studies Exploring the Effects of Iron Supplementation on Haematologic Parameters in Infants* 39

Chapter 3 51

Table 3.1. *Biomarkers and Respective Cut-off Values to Determine Iron Status in Preterm Infants and Female Adults* 58

Chapter 4..... 65

Table 4.1. *Characteristics of Preterm Infants (<37 weeks gestation) in the Study at Four Months after Hospital Discharge* 66

Table 4.2. *Iron Status of All Preterm Infants in Study at Four Months after Discharge* 67

Table 4.3. *Incidence of Iron Deficiency and Iron Deficiency Anaemia in Preterm Infants at Four Months after Discharge* 67

Table 4.4. *Characteristics of Preterm Infants Who Received Iron Supplements after Discharge Compared to Preterm Infants Who Did Not* 69

Table 4.5. *Iron Status of Preterm Term Infants Who Received Iron Supplements after Discharge and Those Who Did Not* *70*

Table 4.6. *Incidence and Characteristics of Preterm Infants with Suboptimal Iron Status at Four Months after Discharge* 71

Table 4.7. *Incidence of Suboptimal Iron Status at Four Months after Discharge in Preterm Infants Born after 32 Weeks Gestation Receiving Iron Supplements Compared to Those Not Receiving Supplements* 72

Table 4.8. *Combined Effect of Iron Supplementation and Feeding Method on Preterm Infant Iron Status at Four Months after Discharge* 73

Table 4.9. *Correlation Coefficients Showing the Relationship between Maternal Iron Status and the Iron Status of Preterm Infants at Four Months after Discharge* 76

Table 4.10. *Relationship between Iron Status at Four Months after Discharge and Changes in Growth Z-Scores between Birth and Home Visit*..... 76

Table 4.11. *Relationship between Iron Supplementation after Discharge and Changes in Growth Z-Scores between Birth and Home Visit* 77

List of Figures

Chapter 1 1

Figure 1.1. *Definitions of Preterm Birth and Related Survival Rates* 2

Figure 1.2. *Overview of the Stages of Iron Deficiency* 3

Chapter 2 10

Figure 2.1. *International Rates of Preterm Birth by Gestational Age and Country* 11

Figure 2.2. *Postulated Method of Iron Absorption in the Duodenum of Neonates* 16

Figure 2.3. *Enteral Feeding Guidelines at Auckland City Hospital NICU* 35

Chapter 4 65

Figure 4.1. *Flow Diagram detailing Recruitment and Final Number of Participants for Data Analysis* 65

Abbreviations

AAP	American Academy of Pediatrics
BMI	Body Mass Index
CHr	Reticulocyte Haemoglobin Content
CRP	C-Reactive Protein
CV	Coefficient of Variance
EDTA	Ethylenediaminetetraacetic Acid
ELBW	Extremely Low Birth Weight
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
HIC	High Income Country
LIC	Low Income Country
LBW	Low Birth Weight
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IUGR	Intrauterine Growth Restriction
IVF	In Vitro Fertilisation
IVN	Intravenous Nutrition
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MUHEC	Massey University Human Ethics Committee
NHI	National Health Index
NICU	Neonatal Intensive Care Unit
PCV	Packed Cell Volume
RCT	Randomised Control Trial
RDI	Recommended Dietary Intake
sTfR	Soluble Transferrin Receptor
TfR	Transferrin Receptor
TIBC	Total Iron Binding Capacity
VLBW	Very Low Birth Weight

WHO	World Health Organisation
ZnPP	Zinc Protoporphyrin

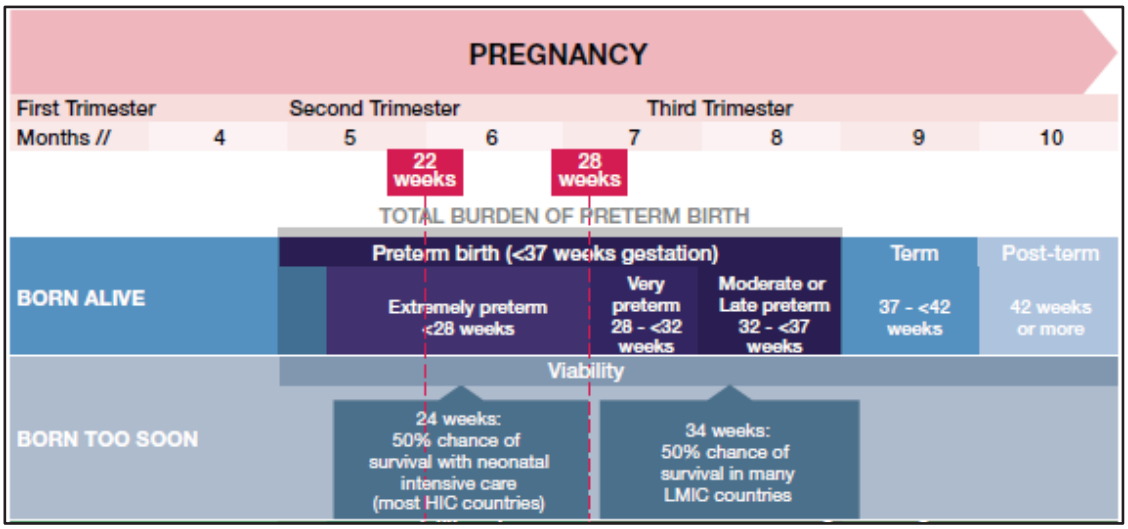
1. Introduction

1.1. Background

Preterm birth, which is defined as any birth before 37 weeks gestation, is a significant global problem with approximately 15 million infants worldwide being born too soon each year (WHO, 2012). In Auckland, New Zealand alone, 820 infants were born preterm in the year ending December 2012, accounting for 10.4% of all births (Pot, Sadler, McDougall, Harilall, & Battin, 2012). Being born preterm is the leading cause of death in infants less than four weeks of age and is a major determinant of long term health complications (Beck et al., 2010; WHO, 2012). The World Health Organisation (WHO) is currently calling for urgent attention to be given to the rising rates of preterm birth, with particular emphasis on the prevention of preterm birth and optimising the treatment of these infants once they have been born (WHO, 2012).

Preterm infants are a unique population with medical, developmental and nutritional needs which cannot be compared to any other group (Behrman & Butler, 2007). There is also immense variation between preterm infants, with preterm birth being further classified as extremely preterm (born before 28 weeks gestation), very preterm (born between 28 to 32 weeks gestation) and moderate to late preterm (32 to less than 37 weeks gestation) (Figure 1.1) (WHO, 2012). At present, it is generally accepted that the lower limits of viability are 22 to 24 weeks gestation although this depends on the economic environment and resuscitation policy of the country; with preterm infants being born in lower income countries fearing far worse (Behrman & Butler, 2007; WHO, 2012). For infants born between 22 and 24 weeks gestation, aggressive resuscitation and intensive neonatal care is usually required (Behrman & Butler, 2007). There are concerns about the survival of these infants into adulthood and the likelihood of medical complications and associated disabilities which need to be considered (Beck et al., 2010; Behrman & Butler, 2007; WHO, 2012). While survival of very and extremely preterm infants is associated with major sequalae, it is not an inevitable consequence of being born at the lower limits of viability (Behrman & Butler, 2007). Likewise, being born closer to term does not completely protect an

infant from medical, cognitive and developmental complications; highlighting the heterogeneous nature of this population (Behrman & Butler, 2007).



HIC = High Income Countries; LMIC= Low and Middle Income Countries

***Figure 1.1**: Definitions of Preterm Birth and Related Survival Rates (adapted from WHO, 2012).*

Once the respiration status of the newborn preterm infant has been stabilised and haemodynamic homeostasis reached, nutrition becomes the most pressing issue for clinicians working in the Neonatal Intensive Care Unit (NICU) (Groh-Wargo & Sapsford, 2009). Preterm infants are at particular risk of nutrient deficiencies, both during hospitalisation and post-discharge (Long et al., 2012). Infants with shortened gestational length miss out on critical in utero accretion periods and are more likely to be born growth-restricted, leading to greater utilization of nutrient stores for accelerated postnatal growth (Long et al., 2012; Rao & Georgieff, 2007). Preterm infants are also more likely to have medical problems or developmental delay which can complicate the introduction of solids (Shah & Shah, 2009). The result is that many preterm infants will continue to be at risk of nutrient deficiencies after discharge from the NICU. Nutritional support is often still required beyond hospital discharge but is not necessarily available.

A nutrient of particular importance for preterm infants is iron; an essential micronutrient which plays a critical role in erythropoiesis, oxidative metabolism and

cellular immune function (Munoz, Villar, & Garcia-Erce, 2009). It also plays a significant role in growth, neurodevelopment, myelination of neurons, and neuronal energy metabolism (Collard, 2009; Mukhopadhyay et al., 2011). As the majority of iron accretion occurs during the last trimester, many preterm infants, especially those born extremely and very preterm, will have inadequate stores at birth (Amin, Scholer & Srivastava, 2012). Uncompensated phlebotomy losses during hospitalization and increased iron requirements needed for rapid growth also predispose preterm infants to iron deficiency (ID), a state characterised by a reduction in the body's iron stores (Figure 1.2) (Amin, Scholer & Srivastava, 2012; Baker & Greer, 2010; Mukhopadhyay et al., 2011; Thom, Parnell, Broadbent & Heath, 2003; WHO, 2010). For example, Amin, Scholer and Srivastava (2012) found that in a population of extremely preterm infants, as many as 23% had latent ID prior to discharge from hospital. If left untreated and the iron stores exhausted, ID may progress to iron deficiency anaemia (IDA), where functional impairment along with a reduction in haemoglobin is observed (Figure 1.2) (Amin, Scholer & Srivastava, 2012; Baker & Greer, 2010; Mukhopadhyay et al., 2011; Thom et al., 2003; WHO, 2010). A study conducted in New Zealand by Thom et al. (2003) found that in a population of low birth weight infants (<2500g) 15% suffered from IDA at 9 months of age. This study is pertinent to preterm infants as preterm birth is a major cause of low birth weight (Meis, Ernest & Moore, 1987).

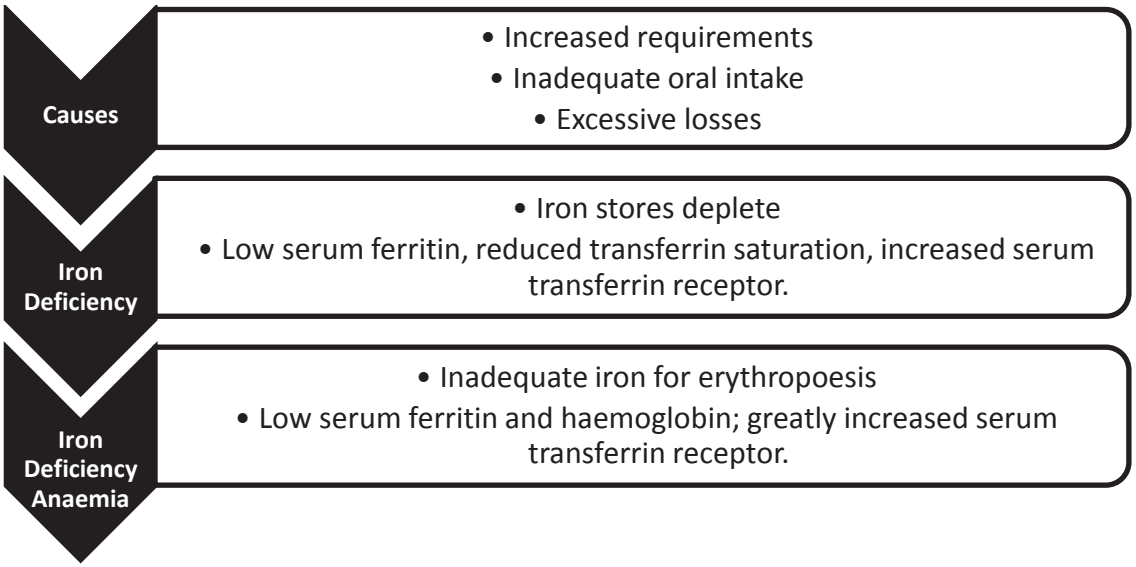


Figure 1.2: *Overview of the Stages of Iron Deficiency (adapted from Baker & Greer, 2010).*

Prevention and early treatment of ID and IDA is essential for preterm infants because reduced erythropoiesis, poor oxygen carrying capacity and altered cellular metabolism associated with ID can result in a myriad of poor health outcomes (Collard, 2009; Rao & Georgieff, 2009). These include gastrointestinal disturbances, immune and thyroid dysfunction, and altered temperature regulation (Collard, 2009; Rao & Georgieff, 2009). Peri- and postnatal ID can also affect neurodevelopment with studies showing that both ID and IDA during infancy can compromise recognition memory, language ability, fine motor skills and tractability later in life (Georgieff & Innis, 2005; Lozoff, Beard, Connor, Felt, Georgieff, & Schallert, 2006; Tamura et al., 2002).

While it is vital that preterm infants are protected from ID and IDA, there is mounting evidence that these infants are also at risk of developing iron overload (Baker & Greer, 2010; Kirpalani et al., 2006; Rao & Georgieff, 2009). Approximately 80% of very low birth weight infants and 95% of extremely low birth weight infants receive erythrocyte transfusions to treat overt IDA whilst in the NICU (Rao & Georgieff, 2009). Preterm infants who receive multiple transfusions can maintain adequate iron stores for up to six months (Rao & Georgieff, 2009). Therefore administering supplemental iron to these infants can predispose them to iron overload. Just as ID can cause neurodevelopmental deficits, iron overload has been associated with ischaemic brain injury and neurodegenerative disorders later in life (Amin, Myers & Wang, 2012). Iron overload may also be associated with chronic lung disease, decreased vitamin E absorption, and retinopathy of prematurity; although a causative relationship has been hard to elicit due to the multi-factorial nature of these conditions (Cooke, Drury, Yoxall & James, 1997; Inder, Clemett, Austin, Graham & Darlow, 1997; Rao & Georgieff, 2009).

Despite the risk of iron overload, current research supports the use of iron supplements to prevent ID and IDA in preterm infants. The American Academy of Pediatrics (AAP) and European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) currently recommend that all preterm infants, especially those with a birth weight below 1800 g, should be supplemented with 2-3 mg/kg/day of supplemental iron, commencing within the first month of life and continuing until 12

months of age (Agostoni et al., 2009; Baker & Greer, 2010). The AAP specifically recommend that all preterm infants who are breastfed after discharge, even those born moderately and late preterm, should receive 2 mg/kg/day of supplemental iron until one year of age, with the exception of those infants who received multiple erythrocyte transfusions who may not need supplementation (Baker & Greer, 2010). Both guidelines recommend ferrous sulphate as the preferential iron supplement due to its acceptability, efficacy, and ability to be administered as a once daily dose (Agostoni et al., 2009; Baker & Greer, 2010).

Auckland City Hospital NICU currently base their iron supplementation protocols on the guidelines set by ESPGHAN rather than the AAP. All preterm infants born before 32 weeks gestation or with a birth weight less than 1800 g are routinely treated with 3-6 mg/kg/day of ferrous sulphate whilst in the NICU, beginning at four weeks of age (Cormack, 2012). The current protocol does not recommend that preterm infants born after 32 weeks gestation receive supplemental iron unless they have been diagnosed with ID (Cormack, 2012). In addition, only infants born before 32 weeks gestation are discharged with prescriptions for iron supplements (Cormack, 2012). While these practices may improve the nutrition of extremely and very preterm infants, those infants born between 32 and 37 weeks gestation may be at risk of developing ID post-discharge. Little is known about the morbidity and iron status of moderate to late preterm infants after discharge, although they account for approximately 70% of preterm births (Tomashek et al., 2006). In clinical practice, moderate to late preterm infants are often treated as if they were fully functional term infants, especially if they have a normal birth weight, despite the fact that they are more vulnerable to complications and nutrient deficiencies than full term infants (Behrman & Butler, 2007, Tomashek et al., 2006). In addition, in a somewhat paradoxical manner, those infants who are born most preterm receive routine iron supplementation whilst in hospital and after discharge, but are also most likely to receive erythrocyte transfusions whilst in the NICU, predisposing them to iron overload. A recent study by Amin, Scholer and Srivastava (2012) found that of 131 infants, 19% had iron overload at 35 weeks post menstrual age (defined as serum

ferritin >400 μg/L), calling for immediate research into whether iron supplementation in preterm infants should be reviewed.

In addition to supplementation, feeding practices may also impact on a preterm infant's iron status. Breast milk is the preferred method of feeding preterm infants due to its immune properties and its protective effect against necrotizing enterocolitis (Shah & Shah, 2009; Schurr & Perkins, 2008). However the iron content of unfortified breast milk is less than infant formula, despite having a higher bioavailability, and may be insufficient to meet the nutrient requirements of preterm infants (Shah & Shah, 2009). Although there is paucity of data, the few small studies available suggest that preterm infants exclusively breastfed after discharge are most at risk of ID (Shah & Shah, 2009; Thom et al., 2003). In addition, late introduction of iron rich solids, such as iron-fortified cereals, meat and poultry, and early introduction of cow's milk can further predispose preterm infants to ID (Rao & Georgieff, 2007).

While the iron status of preterm infants may be monitored closely in the hospital after birth, there is currently no routine screening in the community after discharge. This means that preterm infants who do not receive supplements post-discharge may be at risk of developing ID unbeknownst to the parents and medical professionals caring for the infant. In addition, preterm infants who are discharged from the NICU with iron supplements may be receiving inadequate or excessive doses at four months after discharge which would be going undetected as iron status is not usually monitored.

1.2. Purpose of the Study

The post-discharge nutrition of preterm infants is an understudied area. To provide light on this issue an observational study has been designed to determine the iron and vitamin D status of preterm infants at four months after discharge from hospital. The study has also been designed to present a situational analysis of current feeding and supplement practices in preterm infants at four months after discharge and at one year corrected age. This thesis presents the iron status of preterm infants born at

Auckland City Hospital at four months after discharge, along with factors which may affect this status.

As previously mentioned, not all preterm infants discharged from Auckland City Hospital will receive iron supplements. Infants who meet the criteria for supplementation will receive a prescription for a three month supply of Ferro-Liquid upon discharge home (Grant, 2005). Anecdotal evidence indicates that compliance with iron supplementation can vary especially beyond three months after discharge when an additional prescription must be sought (B. Cormack, personal communication, 2012). In addition, as red blood cells have a lifespan of approximately 120 days, reductions in haemoglobin levels as a result of iron depletion are unlikely to be observed until at least three months post-discharge (Ullrich et al., 2005). For these reasons four months after discharge is an appropriate time point to assess iron status in preterm infants.

The findings from this study will guide future clinical practice and will identify whether current supplementation and feeding practices are optimal for preterm infants after hospital discharge. If a significant number of the infants are found to have ID there are easy and cost effective strategies which could be implemented such as routine monitoring after discharge or routine iron supplementation for all preterm infants. Alternatively if the study finds that the iron status of infants who take iron supplements during the first four months after discharge are comparable to those who do not, this could change prescribing practice, lead to cost savings and make life easier for parents/caregivers of preterm infants.

1.3. Aim

To investigate the iron status of preterm infants in Auckland, New Zealand at four months after discharge from hospital.

1.3.1. Objectives

1. To describe the iron status of preterm infants in Auckland, New Zealand at four months after discharge.

2. To compare the iron status at four months after discharge between preterm infants who receive iron supplements and those who do not.
3. To compare the iron status between preterm infants who have been breast fed until at least four months after discharge and preterm infants who have been predominately formula fed.
4. To assess the effect of pre- and post-discharge factors and feeding practices on iron status in preterm infants at four months after discharge.
5. To assess the relationship between iron status and growth in preterm infants at four months after discharge.

1.3.2. Hypotheses

1. Preterm infants in Auckland, New Zealand will have a lower than recommended iron status due to poor accrual of iron stores in utero.
2. The iron status at four months after discharge will be higher in preterm infants who receive iron supplements (thus those born before 32 weeks gestation) compared to those infants who do not (those born between 32 and 37 weeks gestation).
3. Preterm infants who have been breastfed until at least four months after discharge will have a poorer iron status than those who have been predominately formula fed.
4. Pre- and post-discharge factors and feeding practices will affect iron status in preterm infants at four months after discharge.
5. There will be no relationship between iron status and growth in preterm infants at four months after discharge.

1.4. Thesis Structure

This study is structured into six chapters. The first chapter contextualises the study and highlights the importance of conducting this research. The literature is then reviewed in Chapter 2 and covers the aetiology of preterm birth, iron status of preterm infants, and factors contributing to the iron status of these infants at four months after discharge from hospital. Chapter 3 details and justifies the methods used to investigate iron status of preterm infants in this study. This is followed by Chapter 4 which reports the results of this study. The findings from this study are discussed in Chapter 5.

Finally, Chapter 6 summarises this study, reflecting on its strengths and limitations and makes recommendations for future research.

1.5. Researchers' Contributions

Table 1.1: *Researchers' Contributions to this Study*

Author	**Contributions to Thesis**
Charlotte Moor	Led the research, applied for ethics, designed research including questionnaires, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted results, main author of thesis
Dr Cath Conlon	Academic supervisor, applied for ethics, designed research, assisted with development of questionnaires and protocols, assisted with interpretation of results, revised and approved the thesis
Barbara Cormack	Professional supervisor, designed research, aided in the recruitment of participants, revised and approved the thesis
Professor Frank Bloomfield	Designed research, revised results section of thesis
Owen Mugridge	Phlebotomist, recruited participants, conducted research
Briar Emmett	Applied for ethics, designed research including questionnaires, recruited participants, conducted research
Dr Pamela von Hurst	Assisted with statistical analysis
Cheryl Gammon	Assisted with statistical analysis
PC Tong	Processing of blood samples

2. Literature Review

2.1. Preterm Birth: Definitions, Causes and Consequences

2.1.1. Definition of Preterm Birth

Preterm birth is defined as any birth before 37 weeks gestation (WHO, 2012). Within this it can be can be further classified as extremely preterm (born before 28 weeks gestation), very preterm (born between 28 to 32 weeks gestation) and moderate to late preterm (32 to less than 37 weeks gestation) (WHO, 2012).

2.1.2. Classification by Birth Weight

In addition to the degree of prematurity, preterm infants are often classified depending on their birth weight. Due to their shortened length of gestation, preterm infants comprise a large percentage of infants born with a low birth weight (Meis et al., 1987). Infants are deemed to have a low birth weight (LBW) if their weight at birth is less than 2500g, very low birth weight (VLBW) if their birth weight is less than 1500g and extremely low birth weight (ELBW) if they are born at less than 1000g (Robison, TeKolste, & Wakeley, 2005).

2.1.3. Rates of Preterm Birth in New Zealand

Advances in both perinatal and neonatal care have meant that more preterm infants are being discharged from hospital each year (Darlow, Cust & Donoghue, 2003). Darlow et al. (2003) found that there was a significant increase in the survival rate of VLBW infants to discharge, increasing from 81.8% in 1986 to 90.3% in 1999. More research into the wellbeing of preterm infants living in the community and life-long consequences of being born preterm is therefore required.

In the year ending December 2012 there were 7863 infants born at National Women's Hospital in Auckland, New Zealand (Pot et al., 2012). Of these infants 121 (1.5%) were born between 20 and 27 weeks gestation, 107 (1.4%) were born between 28 and 31 weeks gestation and 592 (7.5%) were born between 32 and 36 weeks gestation, which is significantly higher than the national preterm birth rate of 6.1%; reflecting the tertiary level of care offered at this hospital (Ministry of Health, 2012;

Pot et al., 2012). The greater percentage of moderate to late preterm infants compared to very and extremely preterm is reflected amongst other District Health Boards in New Zealand (National Maternity Monitoring Group, 2013). On average, between, 5-7% of all births each year are classified as moderate to late preterm while only 1-3% are very or extremely preterm (National Maternity Monitoring Group, 2013). The findings of this study will therefore also be relevant to other neonatal units which may not offer a tertiary level and only treat moderate to late preterm infants.

2.1.4. International Rates of Preterm Birth

International rates of preterm birth are unacceptably high and are on the rise (WHO, 2012). Approximately 15 million infants are born preterm each year; accounting for more than 1 in 10 births worldwide (WHO, 2012). On average, there has been an annual global increase in preterm births of 0.8% between 1990 and 2010 (Blencowe et al., 2012). Low income countries are disproportionately affected by preterm birth, with approximately 12% of infants being born preterm in the poorest countries compared to an average of 9% in high-income countries (Figure 2.1) (WHO, 2012). Inequalities in the accessibility to health care, warmth, breastfeeding support, and basic care for infections means that the survival rates of preterm infants in low income countries are also drastically lower compared to rates in higher income countries (WHO, 2012).

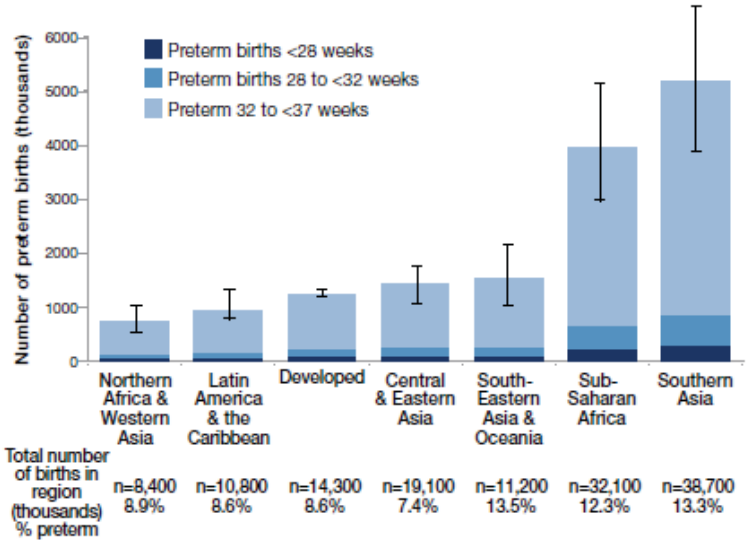


Figure 2.1: *International Rates of Preterm Birth by Gestational Age and Country (WHO, 2012).*

For the year ending 2009, the national preterm birth rate in New Zealand was 6.1% (Ministry of Health, 2012). This is comparable to Australia and Europe, but much lower than the average of 9% for high-income countries reported by the World Health Organisation (Beck et al., 2010; WHO, 2012)

2.1.5. Causes of Preterm Birth

The aetiology of preterm birth is varied and complex. The most common causes of preterm birth are multiple pregnancy, pre-eclampsia, young or advanced maternal age, in vitro fertilisation (IVF), short duration between pregnancies, low maternal body-mass index (BMI), intrauterine infection, uteroplacental haemorrhage, uterine distension, and stress (Blencowe et al., 2012, Goldenberg, Culhane, Iams, & Romero, 2008; Goldenberg et al., 2012; Ministry of Health, 2012; WHO, 2012). Family history of preterm birth is also a strong predictor of preterm birth (Blencowe et al., 2012). Whilst there has been significant research into how to reduce preterm birth, the varied and complex aetiology of prematurity means that there will always be infants being born preterm and thus research into how to best optimise the wellbeing of preterm infants after both birth and discharge is essential (Czeizel, Puho, Langmar, Scs, & Banhidy, 2010; da Fonesca, Bittar, Carvalho, & Zugaib, 2003; Norman et al., 2009; WHO, 2012)

Interestingly, women with lower serum iron, folate or zinc during pregnancy are more at risk of preterm birth (Goldenberg et al., 2008). This may be due to decreased blood volume or uterine blood flow resulting from nutrient deficiencies, causing maternal placental thinness and spontaneous preterm birth (Goldenberg et al., 2008). A study by Beck et al. (2012) found that 9.4% of women aged 18 to 44 years living in New Zealand had sub-optimal iron status (serum ferritin <20μg/L). Little is known however about the iron status of pregnant women living in New Zealand although it is reported that on average they consume 11-14 mg of iron per day, far less than the Recommended Dietary Intake (RDI) of 27 mg/day (Houghton, 2008; NHMRC, 2006). As infants born to mothers with ID and IDA also have lower iron stores at birth compared to infants of iron replete mothers, it begs the question as to whether New Zealand should follow the likes of the United States of America and implement routine pre-natal iron supplementation (UNICEF, UNU & WHO, 2001).

2.1.6. Complications Associated with Preterm Birth

Despite improvements in perinatal and neonatal care and a reduction in preterm infant mortality, the prevalence of medical complications in this population is still exceedingly high (Behrman & Butler, 2007). Developmental immaturity affects a wide range of organ systems including the respiratory system, gastrointestinal system, cardiovascular system, immune system, nervous system, hearing and sight (Behrman & Butler, 2007). The complex aetiology of preterm birth, including inflammation and cytokine injury, is thought to be implicated in the pathogenesis of many medical complications associated with prematurity (Behrman & Butler, 2007). The most common complications associated with preterm birth are anaemia of prematurity, respiratory distress syndrome, chronic lung disease, necrotizing enterocolitis, retinopathy of prematurity, brain white matter injury, bradycardia, hypotension, and patent ductus arteriosus (Behrman & Butler, 2007; Blencowe et al., 2012; Chen & Smith, 2007; Fraser, Walls, & McGuire, 2004; Peterson et al., 2000; Strauss, 2010; WHO, 2012). Although critical illness may be less common in moderate to late preterm infants, this population have an increased risk of hypothermia, hypoglycaemia, respiratory distress, delayed lung fluid clearance, jaundice, infection, feeding intolerance, and greater rates of readmission after hospital discharge compared to term infants (Dani et al., 1999; Engle, Tomashek, & Wallman, 2007; Lapillone, O'Connor, Wang, & Rigo, 2013; Rubaltelli, Bonafe, Tangucci, Spagnolo, & Dani, 1998). While these conditions are not always immediately life threatening, they impact on an infant's progression to full oral feeds and can predispose moderate to late preterm infants to nutrient deficiencies.

In addition to the above medical complications, there is evidence indicating that preterm infants are at a greater risk of being born with medical disabilities which may impact their cognitive development (Blencowe et al., 2012; Moster, Lie, & Markestad, 2008; Robison et al., 2005). A study by Moster et al. (2008) found that 11% of individuals aged 19-35 years who were born between 23 and 27 weeks gestation lived with some form of disability, while only 8.3% of those born at 28 to 30 weeks gestation, 4.2% of those born at 31 to 33 weeks gestation, 2.4% of those born at 34 to 36 weeks gestation, and 1.7% of those born full term were living on a disability benefit

(P<0.001). The same study found that preterm infants had an increased risk of cerebral palsy; mental retardation; disorders of psychological, behavioural and emotional development; epilepsy; and autism-spectrum disorders (Moster et al., 2008).

Compared with their full-term counterparts, preterm infants are more likely to experience delays in fine and gross motor skills, sensory integration, cognitive function, feeding, and communication (Kerstjens et al., 2011). In conjunction, preterm infants are also more likely to have behavioural and socio-emotional problems both in early childhood and in later life (Kerstjens et al., 2011). A recent study by Kerstjens et al. (2011) found that at preschool age, both extremely and moderately preterm infants experienced developmental delays, with the prevalence of developmental delays in moderately preterm infants 2-fold higher than term infants, and extremely preterm infants faring even worse.

2.1.7. Preterm Birth and Nutrient Deficiencies

Preterm infants are also at particular risk of developing nutrient deficiencies, both during hospitalisation and post-discharge (Long et al., 2012). Due to a shortened gestational length, preterm infants miss out on vital periods of nutrient accretion in-utero, often resulting in these infants being born with lower nutrient stores than their term counterparts (Long et al., 2012; Rao & Georgieff, 2007). In addition preterm infants may utilize these stores at an accelerated rate compared to term infants due to their rapid growth after birth (Long et al., 2012; Rao & Georgieff, 2007). Uncompensated phlebotomy losses during hospitalisation and complications delaying the introduction of solids will also predispose preterm infants to nutrient deficiencies (Rao & Georgieff, 2007, Shah & Shah, 2009). Studies indicate that preterm infants are at particular risk of developing deficiencies in fat soluble vitamins, vitamin C and iron (Amin, Scholer & Srivastava, 2012; Dawodu & Nath, 2011; Shah & Shah, 2009).

2.2. Iron

2.2.1. Iron Biochemistry

Iron is an essential micronutrient which plays a critical role in erythropoiesis, oxidative metabolism and cellular immune function (Munoz, Villar, & Garcia-Erce, 2009). Of

particular importance is its role in growth, neurodevelopment, myelination of neurons, and neuronal energy metabolism (Collard, 2009; Mukhopadhyay et al., 2011). It exists in two states, the reduced ferrous form (Fe^{2+}) and the oxidised ferric form (Fe^{3+}), with the bioavailability of ferrous iron far superior to that of ferric iron (Geissler & Singh, 2011; Santiago, 2012). Iron is also present in the diet in two forms, haem iron and inorganic, non-haem iron (Anderson, Frazer, McKie, Vulpe & Smith, 2005). Non-haem iron is the most abundant in the diet but is poorly absorbed compared to haem iron, which is derived primarily from haemoglobin and myoglobin and thus primarily associated with meat intake (Anderson et al., 2005).

2.2.2. Iron Absorption in the Neonate

In the first six months of life the main sources of iron in an infant's diet are breast milk or infant formula; both of which consist mostly of non-haem iron (Collard, 2009). Dietary non-haem iron is predominately in the insoluble Fe^{3+} form and prior to absorption, must be reduced to Fe^{2+} (Cheng & Juul, 2011). This occurs at the apical membrane of enterocytes by ascorbic acid and duodenal cytochrome B (Cheng & Juul, 2011; Collard, 2009). Once reduced, Fe^{2+} iron is transported into the duodenal enterocytes via the divalent metal transporter 1, a process which requires protons as co-transporters (Collard, 2009). A labile pool of Fe^{2+} forms within the enterocyte from where it can be utilised by the enterocyte for cellular metabolism or stored as ferritin before the enterocyte is sloughed off (Cheng & Juul, 2011). Conversely the iron can be transported across the basolateral membrane via the ferroportin transporter into the bloodstream (Collard, 2009). At this point Fe^{2+} iron is converted back to Fe^{3+} by hephaestin before it binds to transferrin to be transported around the body (Collard, 2009). Figure 2.2 below graphically depicts this method of iron absorption.

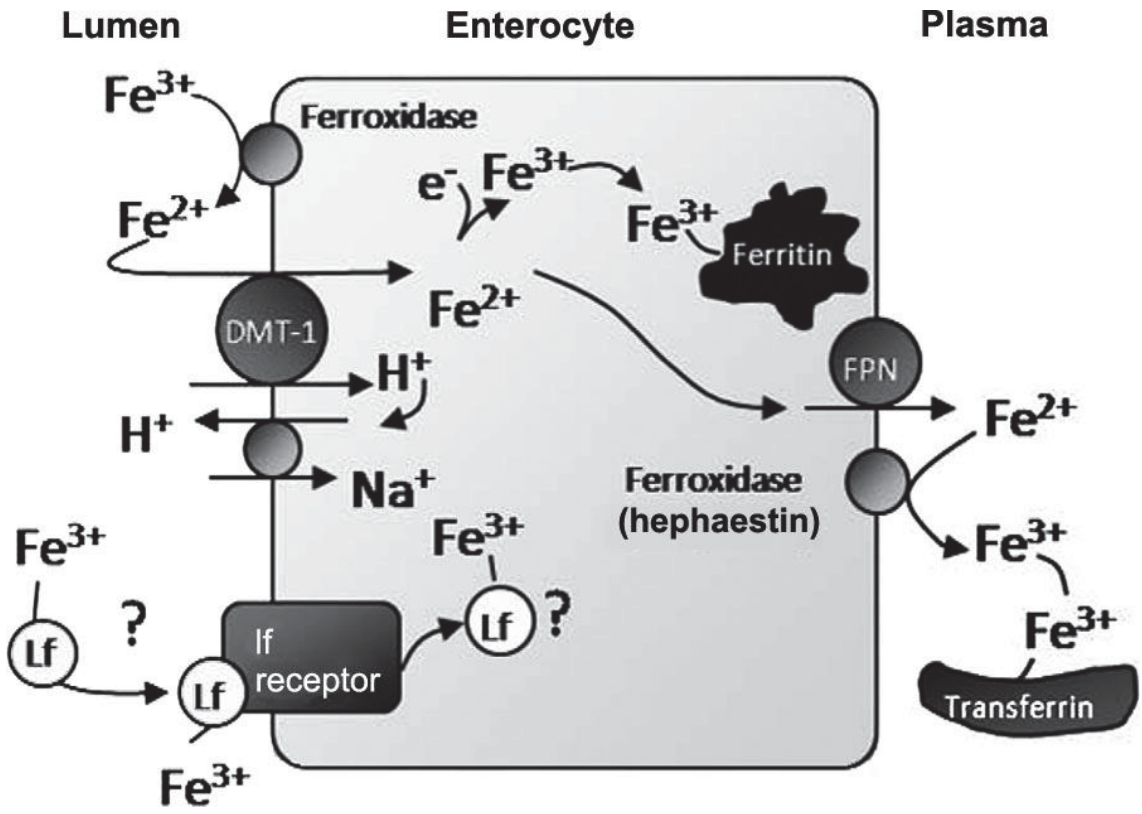


Figure 2.2: *Postulated Method of Iron Absorption in the Duodenum of Neonates (Collard, 2009).*

2.2.3. Iron Transport in the Neonate

Once absorbed into the blood stream, iron is transported to various organs and muscles around the body via the iron binding protein, transferrin (Collard, 2009). Mounting evidence also suggests that albumin binds iron in healthy individuals, preventing it from forming free radicals and causing oxidative damage (Collard, 2009). Several studies however have shown that concentrations of transferrin and albumin are low in infancy, particularly in preterm infants (Collard, 2009; Galinier et al., 2005; Scott, Berger, Kennard, Scott & Wharton, 1975). Preterm infants with low transferrin and albumin levels may not be able to utilize their absorbed iron as effectively as term infants (Collard, 2009). In addition, lower albumin levels may contribute to the increased prevalence of oxidative damage in this population; leading to complications such as chronic lung disease and retinopathy of prematurity (Cheng & Juul, 2012; Collard, 2009).

2.2.4. Iron Storage in the Neonate

Iron is stored as ferritin which is mainly found in the reticuloendothelial cells of the liver, spleen and bone marrow (Cheng & Juul, 2011). There are also small amounts of ferritin in serum, which accurately correlates with storage iron (Cheng & Juul, 2011).

2.2.5. Iron Homeostasis in the Neonate

For iron homeostasis there needs to be tight systemic regulation of iron absorption and transport. This is regulated mostly by hepcidin, an antimicrobial peptide which is released by hepatocytes when serum iron levels are high (Collard, 2009). It subsequently binds to the ferroportin transporters on enterocytes causing its internalisation and degradation (Collard, 2009). Iron remains within the enterocytes stored as ferritin, which will eventually be excreted as the enterocytes are sloughed off (Cheng & Juul, 2011). The result of this is that less dietary iron is transported into the bloodstream.

While iron status is tightly regulated throughout most of an individual's life, recent animal studies reveal that during early infancy there appears to be little or no ability to homeostatically regulate iron uptake (Collard, 2009). It is not until approximately 9 months of age that infants appear to be able to regulate iron absorption in response to iron supplementation (Collard, 2009). In addition, the blood-brain-barrier, which homeostatically regulates brain iron stores throughout adult life, appears to be incomplete until 6 months of age and therefore lacks the ability to respond to low and high serum iron levels (Collard, 2009). As a result, studies have found iron supplementation to treat overt IDA during the first six months of life may not be able to correct functional defects in the brain (Collard, 2009; Georgieff & Innis, 2005; Rao & Georgieff, 2007).

This inability to homeostatically regulate iron absorption is particularly pertinent to preterm infants. Healthy term infants are likely to have adequate iron stores over their early postnatal life despite the lack of homeostatic regulation as they are generally born with reasonable iron stores (Collard, 2009). Preterm infants, however, are born with compromised iron stores due to a shortened accretion period

and are at risk of developing iron deficiency within the first few months of life due to the inability to up-regulate iron absorption (Amin, Scholer & Srivastava, 2012; Collard, 2009). Young infants are also unable to down-regulate their iron absorption when iron intake is high (Collard, 2009). As preterm infants may receive iron supplements after birth, this puts them at risk of iron overload.

2.3. Iron Deficiency and Iron Deficiency Anaemia

2.3.1. Iron Deficiency

Iron deficiency (ID) is the most common micronutrient deficiency, affecting approximately two billion people worldwide (WHO, 2007). A study conducted in Auckland, New Zealand found that 14% of infants aged six to 23 months had ID, highlighting that ID is not just a problem of the developing world (Grant, Wall, Brunt, Crengle, & Scragg, 2007a). Iron deficiency is characterized by a state in which there is a reduction in iron stores and a disparity between serum iron levels and cellular requirements, but adequate iron for erythropoiesis (WHO, 2010). Iron deficiency may result from inadequate dietary iron intake, increased requirements (for example during growth) or increased losses (for example gastrointestinal bleeding or excess phlebotomy) (Baker & Greer, 2010).

2.3.2. Iron Deficiency Anaemia

Iron deficiency anaemia (IDA) occurs when there is inadequate iron available for erythropoiesis (Baker & Greer, 2010). Iron deficiency anaemia is officially defined as a haemoglobin concentration two standard deviations below the mean haemoglobin for a normal age and sex matched population (Baker & Greer, 2010). In the study by Heath et al. (2002) researchers found that 7% of infants aged nine months were classified as having IDA.

2.3.3. Prematurity and Iron Deficiency/ Iron Deficiency Anaemia

Preterm infants have an increased risk of developing ID and IDA as they have lower iron stores at birth as a result of missing out on the critical accretion period (Long et al., 2012). The majority of iron is accrued during the third trimester, meaning that most preterm infants, especially those born very and extremely preterm, are born with

inadequate iron stores (Amin, Scholer & Srivastava, 2012). Preterm infants also utilise their stored iron faster than their term counter parts due to the need for rapid growth (Rao & Georgieff, 2007). Phlebotomy in the first weeks and months of life can also put these infants into negative iron balance (Georgieff & Innis, 2005).

2.3.4. Anaemia of Prematurity

Anaemia of prematurity is a condition characterised by normochromic, normocytic red blood cells and reduced production of erythropoietin; the hormone involved in stimulating red blood cell synthesis in response to hypoxia (Cheng & Juul, 2011). Anaemia of prematurity is common in preterm infants as foetal and preterm infant erythropoietin is produced in the liver which is relatively insensitive to hypoxia, whereas at gestation, erythropoietin is generally produced by the kidney (Cheng & Juul, 2011). In addition, as the infant transitions from the hypoxic uterus to the hyperoxic environment, erythropoietin production is decreased, resulting in reduced red blood cell synthesis (Cheng & Juul, 2011).

2.3.5. Clinical Significance of Iron Deficiency/ Iron Deficiency Anaemia

For many years it has been known that IDA causes reduced erythropoiesis and poor oxygen carrying capacity resulting in a myriad of poor health outcomes (Collard, 2009; Rao & Georgieff, 2009). These include gastrointestinal disturbances, immune and thyroid dysfunction, and cognitive abnormalities (Collard, 2009; Rao & Georgieff, 2009). However recent research has discovered that the negative consequences of inadequate iron during infancy can occur even before clinical IDA (Rao & Georgieff, 2007). Perinatal ID negatively impacts greatly on the growth and functioning of many organ systems, especially the heart, skeletal muscle, gastrointestinal tract and the brain (Rao & Georgieff, 2007). It also has a detrimental effect on immune function and temperature stability, further reducing the wellbeing of these infants (Rao & Georgieff, 2007). Evidence also suggests that infants with perinatal ID have a greater risk of developing ID later in life with studies showing that in developing countries, infants who have low serum ferritin levels at birth develop ID three months earlier than those with adequate stores at birth (Kilbride et al., 1999; Rao & Georgieff, 2007). This is

particularly important in preterm populations due to their reduced ability to accrue iron stores during gestation.

The most devastating effects of ID are on neurodevelopment and cognition (Georgieff & Innis, 2005; Lozoff et al., 2006; Tamura et al., 2002). Iron deficiency affects several developmental brain processes including myelination, energy and monoamine metabolism, and hippocampal dendritic growth (Georgieff & Innis, 2005). These brain processes are dependent on haemoproteins and iron-sulphur compounds which are compromised during ID and IDA (Georgieff & Innis, 2005). Hypomyelination as a result of ID or IDA causes slower nerve conduction velocity; affecting cognition and development (Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998). In human infants, early ID also results in altered motor movement patterns and long-term striatal frontal cognitive changes (Georgieff & Innis, 2005). Iron deficiency and IDA in infancy can predispose children to learning difficulties later in life, with studies showing that low serum ferritin levels at birth are linked with impaired language ability, fine motor skills and tractability at five years of age (Tamura et al., 2002). Unfortunately, both human and animal studies have shown that the negative effect of ID and IDA on neurodevelopment cannot be reversed following iron repletion by supplementation (Georgieff & Innis, 2005; Rao & Georgieff, 2007).

2.4. Iron Overload

2.4.1. Iron Overload

Iron overload is defined as an excess of storage iron or serum ferritin (Amin, Myers & Wang, 2012). In preterm infants, iron overload usually develops because of inherent defects in iron metabolism, such as hereditary haemochromatosis, or secondary to multiple erythrocyte transfusions (Amin, Myers & Wang, 2012). Approximately 80% of VLBW infants and 95% of ELBW infants receive erythrocyte transfusions as part of their treatment in the NICU (Rao & Georgieff, 2007). Preterm infants who have received multiple transfusions can maintain adequate iron stores for up to six months (Rao & Georgieff, 2007). Unlike most other nutrients, there are no regulatory mechanisms to excrete iron from the human body (Agostoni et al., 2009). Therefore administering supplemental iron to infants receiving multiple erythrocyte transfusions will

predispose them to iron overload. While the cut-off for iron overload in the preterm infant population is not clearly defined, recent studies have used a serum ferritin of 300 µg/L to indicate moderate iron overload (Barrett, Freyne, & Molloy, 2011). A recent study by Amin, Scholer and Srivastava (2012) found that 19% of infants born before 32 weeks gestation had iron overload prior to discharge and were at risk of associated complications.

2.4.2. Clinical Significance of Iron Overload

Iron overload affects multiple organs around the body including the heart, liver, endocrine system and the brain (Mir et al., 2012). While excess iron in itself does not have a deleterious effect on an individual's wellbeing per-se, excessive non-protein-bound-iron is able to produce free oxygen radicals via Fenton and Haber-Weiss reactions which cause significant damage to cells (Amin, Myers & Wang, 2012). Agostoni et al. (2009) reported that preterm infants with high iron intakes (≥5.09 g/kg/day) had increased glutathione peroxidise concentrations, which is a marker of oxidative stress. Iron overload and associated oxidative damage have been linked with chronic lung disease, decreased vitamin E absorption, and retinopathy of prematurity in preterm infants (Mills & Davies, 2012).

While adequate iron is essential for proper neurodevelopment, there is mounting evidence that iron overload can be just as detrimental to the development of a young brain (Amin, Scholer & Srivastava, 2012). Excess free iron has been linked to periventricular white matter injury and hypoxic ischaemic injury in preterm infants (Amin, Scholer & Srivastava, 2012). As preterm infants have immature antioxidant systems they are particularly vulnerable to the deleterious effects of iron overload on neurodevelopment (Rao & Georgieff, 2007). Fortunately, a recent study by Amin, Myers and Wang (2012) found that modest neonatal iron overload (serum ferritin less than 400 µg/L) was not associated with an increased risk of neurodevelopmental impairment during infancy, indicating that a higher cut-off for iron overload may be required, for example greater than 1000 µg/L (Amin, Myers & Wang, 2012).

2.5. Biomarkers of Iron Status

The optimal method of assessing iron status is currently unclear; complicated by the existence of multiple biomarkers of iron status, each reflecting slightly different aspects of iron metabolism (Table 2.1). The following is an outline of the key biomarkers of iron status currently available and the optimal combination of tests to assess status in a neonatal population.

2.5.1. Bone Marrow Aspirate

The gold standard for assessing iron status is the examination of bone marrow aspirates stained with Prussian blue for iron (Koulaouzidis, Said, Cottier, & Saeed, 2009). This method however is invasive, has large operator variability, and is not feasible to collect outside of a hospital setting (Koulaouzidis et al., 2009).

2.5.2. Serum ferritin

Serum ferritin has traditionally been used to assess iron status and has gained the widest acceptance in both clinical and population based settings (Lynch, 2010). Serum ferritin is a storage protein which accurately reflects body stores, even in the early stages of negative iron balance, with high specificity and moderate sensitivity (Cameron & Neufeld, 2011). Despite its wide use, serum ferritin is an acute phase protein which means that it is elevated during infection and inflammation (Lynch, 2010). When either inflammation or infection is suspected, it is essential that results are interpreted together with markers of acute-phase proteins, for example C-reactive protein (Cameron & Neufeld, 2011).

2.5.3. Haemoglobin

Haemoglobin is an iron containing metalloprotein which transports oxygen in the blood from the respiratory system to other organs and tissues of the body (Cameron & Neufeld, 2011). As the abundance of free and stored iron decreases, the ability for the body to produce haemoglobin also decreases (Cameron & Neufeld, 2011). Haemoglobin levels only fall once iron stores have vanished, and therefore is only an indicator of IDA, limiting its use as a sole indicator of iron status (Siddappa, Rao, Long, Widness & Georgieff, 2007). Studies have consistently shown that even transient ID

without anaemia can cause detrimental effects to the neonate, necessitating the use of haemoglobin in combination with other biomarkers of iron status (Amin, Scholer & Srivastava, 2012; Baker & Greer, 2010; Collard, 2009; Georgieff & Innis, 2005; Shah & Shah, 2009; Siddappa, et al., 2007).

As a biomarker of iron status, haemoglobin lacks sensitivity and specificity (Lynch, 2010). It has poor sensitivity as the cut-offs to define IDA overlap with the levels used to define iron-sufficient individuals (Lynch, 2010). Sensitivity is especially poor when cut-off values are not adjusted for age, gender, pregnancy, ethnicity, smoking and altitude (Lynch, 2010). Specificity is poor because there are many other causes of anaemia such as endemic infections, HIV disease, tuberculosis, and folate and B12 deficiency (Lynch, 2010). In addition, haemoglobin levels are derived from the entire population of red blood cells which have a life span of approximately 120 days, and therefore may take some time to respond to IDA (Ullrich et al., 2005). Haemoglobin concentrations also change during the first few postnatal months with erythropoiesis dropping after birth (Cheng & Juul, 2011). This phenomenon is more pronounced in preterm infants than term infants and therefore some caution must be taken when using cut-off values from the general infant population (Cheng & Juul, 2011).

2.5.4. Haematocrit

Haematocrit or packed cell volume (PCV) measures the volume fraction of packed red blood cells (Gibson, 2005; WHO, 2007). As erythropoiesis becomes impaired by ID, the number of red blood cells decreases, thus reducing the packed red blood cell volume (Gibson, 2005). Despite wide use in the clinical setting, haematocrit lacks sensitivity, with haematocrit levels only falling with severe IDA (Gibson, 2005). In addition, haematocrit lacks specificity as it is affected by the same factors which affect haemoglobin (Gibson, 2005; WHO, 2007). Haematocrit also lacks precision, especially when capillary bloods are taken (Gibson, 2005). Dilution by alcohol wipes, poorly packed red blood cells, and elevated white blood cells causing a poorly defined boundary between erythrocytes and plasma can all affect the validity of haematocrit readings (Gibson, 2005).

2.5.5. Mean Cell Volume

Mean cell volume (MCV) measures the average size of red blood cells in femtolitres (fL) and is determined by dividing haematocrit by red blood cell count (Gibson, 2005). Abnormally small or microcytic cells are indicative of IDA, allowing MCV to differentiate between the different nutritional anaemias (iron, folate and vitamin B12) (Gibson, 2005; WHO, 2007). Although MCV is a specific measure of IDA, it lacks sensitivity, with low MCV values only occurring with severe ID (Gibson, 2005; WHO, 2007).

2.5.6. Mean Cell Haemoglobin

While MCV is based on the volume fraction of haematocrit, mean cell haemoglobin (MCH) is determined by dividing haemoglobin by red blood cell count (Gibson, 2005). Like MCV, MCH is a more specific indicator than haemoglobin alone, with a low MCH indicating IDA and a high MCH denoting folate or vitamin B12 deficiencies (Gibson, 2005; WHO, 2007).

2.5.7. Mean Cell Haemoglobin Concentration

Mean cell haemoglobin concentration (MCHC) is determined by dividing haemoglobin by haematocrit (Gibson, 2005). It is the least useful indicator of iron status as it is the last red cell index to fall with IDA (Gibson, 2005).

2.5.8. Serum Iron, Total Iron Binding Capacity and Transferrin Saturation

Serum iron, total iron binding capacity (TIBC) and transferrin saturation are interrelated indices of iron status and are most commonly reported together. Serum iron measures the amount of ferric iron (Fe^{3+}) bound to transferrin, excluding iron in haemoglobin (Cameron & Neufeld, 2011). It is therefore an indicator of moderate ID, as levels will only drop once iron stores have been depleted. Unfortunately serum iron levels fluctuate significantly throughout the day, making it an unreliable indicator of iron status (Cameron & Neufeld, 2011). It is also decreased during infection and inflammation (Cheng & Juul, 2011).

Total iron binding capacity (TIBC) estimates the percentage of unoccupied iron-binding sites on transferrin molecules (Cameron & Neufeld, 2011). When iron stores become depleted, the body responds by synthesising more transferrin to compensate for diminished serum ferritin levels (Cameron & Neufeld, 2011). This results in an increase in TIBC during ID as there are more iron-binding sites and fewer are being occupied (Gibson, 2005). While TIBC is not prone to the same diurnal variation as serum iron, Cameron and Neufeld (2011) report that venous blood samples usually need to be taken to measure TIBC, thus limiting its use in paediatric populations.

Transferrin saturation is an indicator of the rate of delivery of iron to bone marrow for erythropoiesis and is calculated by dividing serum iron by TIBC (Gibson, 2005). A transferrin saturation of less than 16% is generally consistent with ID while a transferrin saturation of over 70% is associated with iron overload (Gibson, 2005). Unfortunately, cut-offs are not well defined for infants and children, and results must be based on values for adult populations (Gibson, 2005).

The three interrelated indices, serum iron, TIBC and transferrin saturation are useful in differentiating between ID and IDA associated with chronic disease, inflammation or neoplastic disease (Gibson, 2005). With nutritional iron deficiencies, serum iron is reduced and TIBC is elevated, resulting in a low transferrin saturation (Gibson, 2005). However in the case of anaemia of chronic disease, inflammation or neoplastic disease, both serum iron and TIBC are low, with transferrin saturation usually sitting around the lower end of normal (Gibson, 2005).

2.5.9. Soluble Transferrin Receptor (sTfR) or Transferrin Receptor 1 (TfR1)

Transferrin receptor (TfR or TfR1) is a protein which facilitates the uptake of transferrin iron into cells (Baker & Greer, 2010). The expression of cellular TfRs is proportionate to the body's requirement for iron, with TfR expression being upregulated in ID, enabling the cell to compete for iron more effectively (Baker & Greer, 2010). Transferrin receptor expression therefore reflects the intensity of erythropoiesis and the demand for iron (WHO, 2007). As TfR is also a marker of general erythropoiesis, care must be taken when interpreting results as they can be skewed by malaria infection and

haemolytic diseases (Engle-Stone, Nankap, Ndjebayi, Erhardt, & Brown, 2013). A soluble form of TfR also exists which circulates within serum (sTfR). This is proportionate to cellular TfR expression and is more easily measured (Gibson, 2005).

There is growing acceptance of sTfR as a biomarker of ID. A recent study by Lynch (2010) found that raised sTfR was potentially the most useful marker of functionally significant ID. Olivares, Walter, Cook, Hertrampf and Pizarro (2000) also found that the ratio between serum ferritin and sTfR was a better indicator of iron status than serum ferritin alone in the infant population. Research also shows that sTfR is less affected by inflammation than serum ferritin, making it a more useful marker in populations prone to inflammation and infection, such as preterm infants (WHO, 2010). Unfortunately, standard values for infants and children are yet to be set for sTfR and cut-offs often differ depending on what method on analysis is used (Baker & Greer, 2010). Engle-Stone et al. (2013) defined ID as sTfR >8.3 mg/L while Vendt et al. (2009) defined ID as a sTfR >2.4 mg/L using the IDeA method (sensitivity 84%, specificity 94%) and a sTfR >7.4 mg/L using the Tina-quant method (sensitivity 80%, specificity 92%). Cheng and Juul (2011) concur with Vendt et al. that ID should be defined by a sTfR of greater than 2.45 mg/L. Conversely, Gibson (2005) suggests that a cut-off of greater than 11 mg/L should be used for infants aged four to nine months, although they did not specify which method of assessment this cut-off correlates with.

2.5.10. Reticulocyte Haemoglobin Content (CHr)

A more novel method of assessing ID with or without anaemia is reticulocyte haemoglobin content (CHr). Unlike red blood cells, which have a lifespan of approximately 120 days, reticulocytes have a much shorter lifespan of 24-48 hours, making CHr a more accurate “real-time’ indicator of iron status (Ullrich et al., 2005). In addition, CHr is not affected by inflammation or infection like serum ferritin and therefore may be a more appropriate biomarker in a population like preterm infants where inflammation is to be expected (Baker & Greer, 2010). Unfortunately at present there are insufficient studies validating the use of CHr as a measure of ID or IDA in the preterm infant population, although a study by Ullrich et al. (2005) found that a CHr of less than 27.5 picograms (pg) was more accurate at identifying IDA than a haemoglobin

of less than 110 g/L in healthy 9 to 12- month old infants. Further research is needed before CHr can be adopted in universal assessment for IDA. The availability of the CHr assay is also reasonably scarce, limiting its use in this study (Baker & Greer, 2010).

2.5.11. Zinc Protoporphyrin (ZnPP)

Zinc protoporphyrin (ZnPP) is a product of disordered haem synthesis. When there is inadequate iron for erythropoiesis, zinc is incorporated into protoporphyrin IX to form ZnPP instead of haem (Gibson, 2005). Zinc protoporphyrin then accumulates within red blood cells, where it remains for the duration of the cells life (Gibson, 2005). Therefore, like haemoglobin, it may take up to 120 days for erythrocyte ZnPP levels to increase in the face of IDA. In addition, zinc is affected by infection and inflammation which limits the use of ZnPP as a marker of iron status in preterm infants (Clark, 2009).

2.5.12. Hepcidin

There has also been research in recent years into the use of hepcidin as a biomarker of iron status. As previously mentioned, hepcidin is a regulatory protein which inhibits absorption of iron in the intestines when serum iron levels are high (Collard, 2009). The production of hepcidin is decreased in IDA and increases with inflammation and iron overload (Clark, 2009). Hepcidin assays have shown promise as it has good stability and responds rapidly to changes in iron stores (Vermeulen & Vermeersch, 2012). Unfortunately the assay to measure hepcidin is not yet widely available and clear cut-offs for ID and IDA are yet to be defined (Beard, deRegnier, Shaw, Rao, & Georgieff, 2007).

Table 2.1: *Advantages and Disadvantages of Common Biomarkers of Iron Status*

Biomarker	**ID &/or IDA**	**Advantages**	**Disadvantages**
Bone Marrow Aspirate	ID + IDA	Only direct measure of iron status	Invasive; large operator variability; only available in hospital setting
Serum Ferritin	ID	High specificity and moderate sensitivity	Results may be inaccurate during infection and inflammation
Haemoglobin	IDA	Well defined reference values for neonates	Cannot differentiate iron, folate and vitamin B12 anaemia; poor

			sensitivity and specificity
Haematocrit	IDA	Used widely in the clinical setting	Lacks sensitivity, precision and specificity
Mean Cell Volume	IDA	Differentiates between iron, folate and vitamin B12 anaemia	Lacks specificity; values only drop with severe IDA
Mean Cell Haemoglobin	IDA	Differentiates between iron, folate and vitamin B12 anaemia	Not widely used in research setting for paediatric populations
Mean Cell Haemoglobin Concentration	IDA		Least useful indicator of iron status; last red blood cell index to fall with IDA
Serum Iron	ID + IDA	Differentiates between ID and anaemia associated with chronic disease, inflammation or neoplastic disease	Significant diurnal variation
Total Iron Binding Capacity	ID + IDA	Differentiates between ID and anaemia associated with chronic disease, inflammation or neoplastic disease	Requires venous blood sample thus limiting its use in paediatric populations
Transferrin Saturation	ID + IDA	Differentiates between ID and anaemia associated with chronic disease, inflammation or neoplastic disease	Cut-offs not well defined in paediatric populations
Soluble Transferrin Receptor	ID	Less affected by inflammation than serum ferritin; better indicator in preterm infant population than serum ferritin	Cut-offs not well defined in paediatric populations
Reticulocyte Haemoglobin Concentration	ID + IDA	Not affected by inflammation or infection; more accurate “real-time” indicator of iron status than haemoglobin	Assay not widely available; insufficient validation in paediatric population
Zinc Protoporphyrin	IDA	Differentiates between iron, folate and vitamin B12 anaemia	Affected by infection and inflammation
Hepcidin	ID + IDA	Rapid response to changes in iron stores	Assay is not yet widely available; clear cut-offs for paediatric population are yet to be defined

ID = Iron Deficiency; IDA = Iron Deficiency Anaemia

2.5.13. Optimum Method of Assessing Iron Status in Infants

Traditionally haemoglobin and serum ferritin have been used as the sole indicators of iron status in the majority of studies worldwide. There has been call however in recent years to up-date recommendations for iron status assessment due to the consensus that measuring serum ferritin and haemoglobin alone may no longer be sufficient (Clark, 2009). A report published by the World Health Organisation (WHO) in 2010 concluded that the best approach to assess iron status in a population is to report haemoglobin along with serum ferritin and sTfR concentration. This gives an indication of iron stores, transport iron and iron in the erythrocyte pool (Grant et al., 2007b). However due to difficulties in determining cut-off points and paucity of information, the optimal approach to measuring iron status in preterm infants is yet to be determined.

2.5.14. *Diagnosis of Iron Deficiency and Iron Deficiency Anaemia*

It is generally accepted that in paediatric populations, ID is diagnosed by a serum ferritin value <12 μg/L and a sTfR >2.4 mg/L (Cheng & Juul, 2011; Schiza et al., 2007; Vendt et al., 2009). Paediatric IDA is defined as a haemoglobin level <110 g/L in addition to a low serum ferritin and/or high sTfR (Baker & Greer, 2010; Grant et al., 2007a; Friel et al., 2003; Heath et al., 2002, Thom et al., 2003, WHO, 2011a). Conversely, iron overload in paediatric populations is defined as a serum ferritin of >300 μg/L (Barrett et al., 2011; Berglund, Westrup & Domellof, 2010; Cameron & Neufeld, 2011; WHO, 2011b).

As severe maternal IDA can affect breast milk iron concentration and thus the iron status of those infants being breast fed, it is essential to also define iron cut-off values for this population (Kumar, Rai, Basu, Dash, & Singh, 2008; Zavaleta et al., 1995). For this population, ID is defined as a serum ferritin of <15 μg/L and a soluble transferrin receptor >2.4 mg/L (Sampson, 2008; WHO, 2011b). Iron deficiency anaemia is characterised by a haemoglobin of <120 g/L in addition to a low serum ferritin and/or high sTfR (WHO, 2011a). In this population, iron overload is diagnosed by a serum ferritin of >150 μg/L (WHO, 2011b).

2.6. Maternal and Infant Factors Affecting Iron Status

2.6.1. Duration of Gestation

As the majority of iron is accrued during the third trimester, there is a logical link between the length of gestation and iron status at birth and during the first few months of life. In general, extremely and very preterm infants are born with lower iron stores than their moderately preterm or term counterparts (Siddappa et al., 2007). Studies have shown that infants born before 34 weeks gestation have significantly lower serum ferritin concentrations at birth (26-270 μg/L) than those born after 34 weeks gestation (20-600 μg/L) (Jansson, Holmberg, & Ekman, 1979).

2.6.2. Gender

Studies have shown that term female infants have higher serum ferritin levels at birth than males, possibly due to differences in sex hormones (Siddappa et al., 2007). The sex-ferritin link is much less pronounced however for preterm infants and further research is required in this area (Siddappa et al., 2007).

2.6.3. Intrauterine Growth Restriction

Intrauterine growth restriction (IUGR) is generally defined as a birth weight and/or birth length below the 10^{th} percentile for gestational age along with pathologic restriction of foetal growth (Wollman, 1998). As part of the pathophysiology of IUGR these infants may have impaired placental function resulting in an increased risk of developing ID and IDA due to impaired iron transport and chronic foetal hypoxemia (Siddappa et al., 2007). In an effort to increase oxygen saturation in the blood, foetal erythropoiesis is up-regulated so that more oxygen binding haemoglobin can be synthesised (Siddappa et al., 2007). This causes an increase in iron requirements which is mirrored by a subsequent decrease in serum ferritin (Siddappa et al., 2007). Studies show that serum ferritin levels are decreased and sTfR concentrations are increased in IUGR infants at birth (Siddappa et al., 2007). As IUGR is a major cause of preterm birth, it is an important factor to consider in this population (Siddappa et al., 2007).

2.6.4. Multiparity

Pregnancy and childbirth place significant physiological stress on the body and are costly in terms of maternal iron stores. There is an increased demand for iron during pregnancy in order to expand erythrocyte mass and to fulfil iron requirements of the growing foetus (Farooq, Rauf, Hassan, & Sadiq, 2011). Childbirth can also be costly in terms of maternal iron stores due to the blood loss associated with both vaginal and caesarean labour (Farooq et al., 2011). A recent study by Farooq et al (2011) investigating the prevalence of ID in nulliparous, primiparous and multiparous women found that only 16% of nulliparous women had ID after birth, while 36% of primiparous and 72% of multiparous women had low serum ferritin levels (Farooq et al., 2011).

2.6.5. Maternal Hypertension and Pre-Eclampsia

Maternal hypertension is common during pregnancy and is characterised by a blood pressure of ≥140/90mmHg or ≥160/110mmHg in the case of severe hypertension (Tranquilli, Brown, Zeeman, Dekker, & Sibai, 2013). Pre-eclampsia differs from maternal hypertension in that it is also classified by the presence of proteinuria (Tranquilli et al., 2013). Both conditions can affect foetal iron status as they can result in placental vascular insufficiency and chronic foetal hypoxemia, leading to increased foetal erythropoiesis (Siddappa et al., 2007). This impairment of placental function and altered foetal iron metabolism predisposes the foetus to ID at birth and in early life (Siddappa et al., 2007). As pre-eclampsia has a role in the aetiology of preterm birth, it is essential to take note of pre-eclampsia with regards to infant iron status (Goldenberg et al., 2012).

2.6.6. Diabetes Mellitus

Gestational, Type One and Type Two diabetes are all risk factors for foetal and infant ID (Siddappa et al., 2007). Hyperglycaemia during pregnancy increases foetal metabolic rate, thus increasing oxygen consumption and eventually resulting in foetal hypoxemia (Siddappa et al., 2007). As mentioned above, hypoxemia results in increased erythropoiesis at the expense of foetal iron stores. Diabetes during pregnancy is also a risk factor for IUGR, further increasing the risk of foetal and infant ID and IDA (Rao and Georgieff, 2007).

2.6.7. Maternal Smoking

Infants born to mothers who smoked during pregnancy have elevated foetal erythropoietin, cord haemoglobin, sTfR concentrations and decreased cord ferritin levels at birth; consistent with foetal hypoxemia (Siddappa et al., 2007). Foetal hypoxemia in these infants is likely due to decreased placental blood flow and increased placental vasoconstriction caused by nicotine from the cigarettes (Siddappa et al., 2007). Although these infants are born with higher cord haemoglobin levels, they are predisposed to ID and IDA during the first year of life as their iron stores have been compromised. Maternal smoking is also a risk factor for IUGR and preterm birth (Rao & Georgieff, 2007).

2.6.8. Maternal Iron Status

Maternal iron status may affect iron stores at birth, but only during severe deficiency (Cheng & Juul, 2011). The placenta is able to adapt to low maternal iron status to protect the foetus for a period of time, ensuring adequate placental transfer at the expense of maternal iron stores (Cheng & Juul, 2011; Siddappa et al., 2007). However some studies have shown that infants of anaemic and iron deficient mothers have lower cord serum levels at birth compared to infants of iron replete mothers (Cheng & Juul, 2011; Siddappa et al., 2007). In addition, treatment of iron deficient mothers with supplementation during pregnancy has been shown to increase infant serum ferritin levels at birth and at three months of age (Rao & Georgieff, 2007).

Interestingly, studies show that there is no correlation between maternal ID and the iron content in breast milk (Domellof, Lonnerdal, Dewey, Cohen & Hernell, 2004; Kumar et al., 2008; Zavaleta et al., 1995). This suggests that iron accretion in breast milk is regulated by active transport mechanisms in the mammary gland and that the iron content of breast milk will be conserved at the detriment of maternal stores (Domellof et al., 2004). There is however a relationship between severe maternal IDA and lower iron concentrations in breast milk suggesting that active iron uptake into breast milk can be continued for a period of time at the expense of maternal iron stores, however once maternal iron stores are exhausted, breast milk iron concentration decreases (Kumar et al., 2008; Meinzen-Derr et al., 2006).

2.6.9. Cord Clamping

Cord clamping refers to the time at which the umbilical cord is clamped after delivery (Andersson, Hellstrom-Westas, Andersson, & Domellof, 2011). Delaying cord clamping has been proposed as a cost effective way of reducing infant IDA as it allows for extra transfer of foetal blood from the placenta to the infant, resulting in 20-60% more erythrocytes in the infant (Collard, 2009). This practice however is fairly controversial as some studies suggest that delayed cord clamping may increase the risk of postnatal respiratory symptoms, polycythaemia and hyperbilirubinaemia (Andersson et al., 2011). A recent study by Andersson et al. (2011) found that at four months of age, infants subjected to delayed cord clamping had 45% higher mean serum ferritin levels (117 µg/L v 81 µg/L, $P<0.001$) and a lower risk of developing ID. There was also no significant difference in the risk of developing respiratory symptoms, polycythaemia or hyperbilirubinaemia (Andersson et al., 2011).

2.6.10. Erythrocyte Transfusions

Erythrocyte transfusions are administered to infants in the NICU as a treatment for severe IDA. Each erythrocyte transfusion typically supplies the infant with 8 mg/kg of iron, leaving infants who receive multiple erythrocyte transfusions at risk of iron overload if iron supplements are subsequently prescribed (Agostoni et al., 2009; Griffin & Cooke, 2010). Infants born most preterm are likely to have accrued the least iron and thus are most likely to require erythrocyte transfusions during their NICU admission.

2.6.11. Erythropoietin Administration

Administration of recombinant human erythropoietin may be used in the NICU in place of erythrocyte transfusions to treat IDA (Rao & Georgieff, 2009). Erythropoietin stimulates erythropoiesis, inhibiting hepcidin and increasing the availability of iron from the gut and internal stores (Collard, 2009). Subsequently, erythropoietin administration requires increased iron supplementation as it depletes the body's iron stores (Rao & Georgieff, 2009). While the American Academy of Paediatrics (AAP) recommend that infants undergoing recombinant human erythropoietin therapy receive 6 mg/kg/day of iron, simultaneous enteral and intravenous administration at

even higher doses may be required to support erythropoiesis and maintain iron stores (Rao & Georgieff, 2009). Administration of recombinant human erythropoietin in the NICU without adequate supplementation may therefore result in ID or IDA.

2.7. Feeding Protocol in the Neonatal Intensive Care Unit

2.7.1. Feeding Protocols at Auckland City Hospital Neonatal Intensive Care Unit

Auckland City Hospital NICU follows nutrition guidelines to make sure nutritional intakes are as close as possible to internationally recommended intakes, ensuring the best possible outcomes for the infants in their care. The nutrition guidelines are based on gestational age and birth weight; with younger, smaller infants generally receiving the most intensive nutrition.

2.7.2. Intravenous Nutrition and Iron Status

Intravenous nutrition (IVN) is indicated for those infants born before 37 weeks gestation and/or with a birth weight of less than 1500 g and who are unable to meet their daily feed requirements (Cormack, 2012). It is also indicated for infants when enteral feeding is contraindicated (Battin & Cormack, 2008). As per the Auckland City Hospital NICU protocol, IVN should be commenced within the first 12 hours of life (Cormack, 2012). Standard IVN does not routinely contain iron therefore prolonged IVN without parenteral iron or enteral/oral feeding can therefore result in ID or IDA (ESPGHAN, 2005).

2.7.3. Enteral Feeding and Iron Status

In addition to IVN, many preterm infants will also require enteral feeding, be it via an orogastric tube, nasogastric tube or trans-pyloric feeding tube (Svirskis, 2010). Figure 2.3 details the enteral feeding protocols currently in place at Auckland City Hospital. Infants can be enterally fed with breast milk, infant formula or a combination depending on maternal supply and wishes.

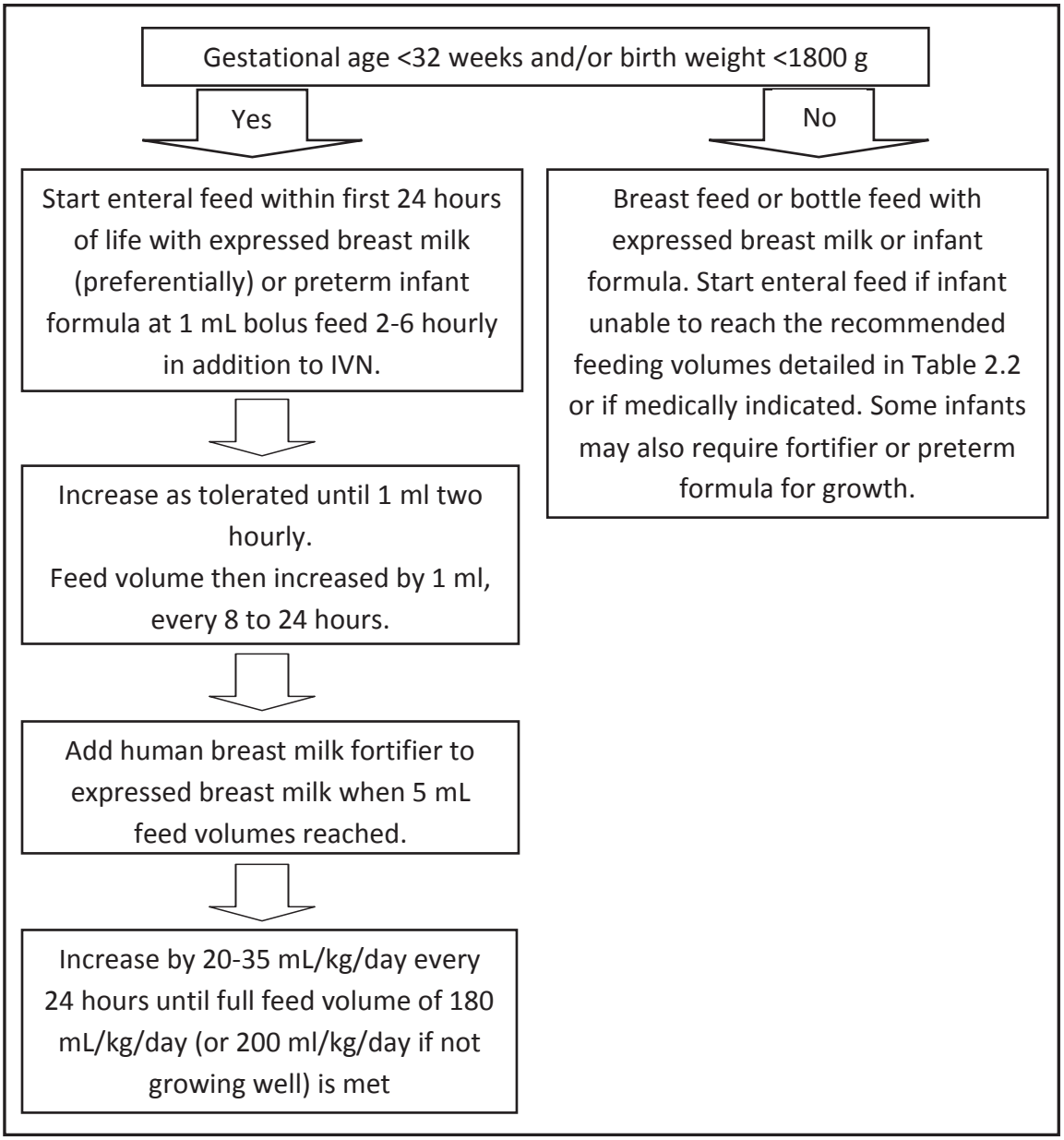


Figure 2.3: *Enteral Feeding Guidelines at Auckland City Hospital NICU (Cormack, 2012)*

If an infant is enterally fed breast milk whilst in the NICU, this can affect their iron status, because while breast milk contains very little iron, it is very well absorbed (Rao & Georgieff, 2009). While breastfeeding is the preferred feeding method for preterm infants, it is not usually possible until 34 weeks gestation due to developmental immaturity (Buckley & Charles, 2006). In addition, only expressed breast milk has the ability to be fortified with human breast milk fortifier. Human breast milk fortifiers supply additional energy, protein, minerals and vitamins to assist growth and development, and are especially useful when only small fluid intakes can be achieved

(Cormack, 2013). As shown in Figure 2.3, all preterm infants in Auckland City Hospital NICU that are fed expressed breast milk enterally or via a bottle and who are born before 32 weeks gestation or below 1800 g are given human breast milk fortifier once feed volumes reach 5 mL per feed (Cormack, 2012). Preterm infants receiving expressed breast milk who do not meet the criteria for routine fortification with human breast milk fortifier may still receive fortifier, although this is left to the discretion of the medical and dietetic team (Cormack, 2012). The human breast milk fortifiers available in New Zealand are Nutricia Breast Milk Fortifier, Nestle FM 85 and Wyeth S-26 Human Milk Fortifier, however only FM 85 contains iron, adding 1.5 mg per 100 mL of breast milk (Cormack, 2013). Thus those preterm infants who are enterally or bottle fed expressed breast milk and who meet the criteria for fortification may have improved iron status during hospitalisation and upon discharge, but only if the FM 85 fortifier is used.

Withholding enteral feeds is a major decision in the NICU (Kuschel, Cormack, & Morreau, 2005). Prolonged periods without feeding may predispose infants to ID and IDA due to the large demands on iron stores for rapid growth and to replace phlebotomy losses. It can also contribute to poor growth and gut immaturity; problems which are particularly detrimental to very and extremely preterm infants (Kuschel et al., 2005).

2.8. Effect of Breast and Formula Feeding on Infant Iron Status

2.8.1. Breast Milk

Breast milk is the recommended source of nutrition for term infants for their first six months of life as it provides optimum nutrition, assists in their physical and emotional development, decreases the incidence of childhood infectious diseases, is associated with reduced infant mortality and hospitalisation, and reduces the risk of chronic disease (Ministry of Health, 2008). Despite this, studies have shown that breast milk may contain insufficient nutrients required for rapid growth and that preterm infants who are exclusively breastfed are at a higher risk of developing ID or IDA during their first year of life (Hall, Wheeler, Benson, Harris, & Rippetoe, 1993; Olivares et al., 1992; Rao & Georgieff, 2009). This is because breast milk only contains approximately 0.5

mg/L of elemental iron, albeit highly bioavailable iron (Mills & Davies, 2012; Rao & Georgieff, 2007; Rao & Georgieff, 2009). While the iron content of breast milk is adequate to meet the requirements of full-term infants during the first four to six months of life, preterm infants require additional iron to compensate for inadequate accretion during gestation, increased utilisation for rapid growth and excessive iron losses due to phlebotomy (Rao & Georgieff, 2009).

2.8.2. Infant Formula

If a mother is unable or wishes not to breastfeed then preterm infants should be fed infant formula for the first year of life (Ministry of Health, 2008). While infant formula is designed to have a similar composition as breast milk, it often has higher concentrations of some nutrients, in particular iron, to compensate for their lower bioavailability (Ministry of Health, 2008). Studies have shown that infant formulas containing 5-9 mg/L of iron are adequate to meet the iron needs of preterm infants for the first six months of life, although only 4-20% of the iron in infant formula is able to be absorbed (Rao & Georgieff, 2007; Rao & Georgieff, 2009). In New Zealand, the commonly available starter infant formulas contain between 5.5 and 9 mg/L of iron while follow-on formulas contain between 8.4 and 12 mg/L (Gillanders & Sloan, 2013).

Low birth weight and preterm formulas are also available in New Zealand and are specifically formulated to meet the additional requirements of preterm infants. They are designed to promote growth rates without disrupting metabolic homeostasis and contain higher concentrations of vitamins and minerals to account for the smaller intake by preterm infants (Cormack, 2013). At the Auckland City Hospital NICU only infants born before 32 weeks gestation or below 1800 g are given preterm formula (Cormack, 2013). This is subsequently stopped before discharge when the baby is fully breastfed (Cormack, 2013). Three LBW and preterm infant formulas are available in New Zealand which contain between 12 and 14 mg/L of iron (Cormack, 2013).

2.8.3. Post-Discharge Formula

At Auckland City Hospital NICU, preterm infants who are born before 33 weeks gestation and who are not breastfeeding may be discharged home on post-discharge

formula (Cormack, 2012; Cormack, 2013). Like preterm formula, this contains additional nutrients for growth, including 12 mg/L of iron (Cormack, 2013). Whether an infant born before 33 weeks gestation is discharged on this formula is up to the clinical judgement of the medical and dietetic team.

2.9. Iron Supplementation

2.9.1. Iron Requirements

Numerous expert panels have made recommendations with regards to iron requirements in preterm infants based on intervention studies and theoretical calculations (Griffin & Cooke, 2010). The AAP recommend that all preterm infants should receive 2 mg/kg/day of supplemental iron starting by one month of age and extending through to 12 months (Baker & Greer, 2010; Kleinman, 2009). The European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee (ESPGHAN) recommend that all preterm infants born less than 1800 g should be supplemented with 2-3 mg/kg/day of supplemental iron, commencing within the first month after birth and continuing until 6-12 months of age (Agostoni et al., 2009). The ESPGHAN have based their recommendations on infants born less than 1800 g as they believe there is insufficient literature to provide recommendations for infants born with a heavier birth weight (Agostoni et al., 2009).

2.9.2. Iron Supplement

Ferrous sulphate is the most commonly used form of iron in paediatric iron supplements (Rao & Georgieff, 2009). It is inexpensive, widely available and can be administered once daily thus increasing compliance (Rao & Georgieff, 2009). Iron absorption from supplements administered between feeds is approximately 25-40% for preterm infants, with 10-25% being incorporated into erythrocytes within two weeks (Agostoni et al., 2009; Rao & Georgieff, 2009).

2.9.3. Iron Supplementation Intervention Trials

2.9.3.1. Iron Supplementation and Iron Status

Table 2.2 details the characteristics and results of a number of studies exploring the relationship between iron supplementation and iron status in preterm and LBW infants.

Table 2.2: *Summary of the Characteristics and Results of Studies Exploring the Effects of Iron Supplementation on Haematologic Parameters in Infants.*

Author(s)	**Study Design**	**Characteristics of Population**	**Biomarkers**	**Results**
Franz, Mihatsch, Sander, Kron, and Pohlandt (2000)	RCT	Infants with a birth weight of less than 1301g (n=153)	Serum ferritin, transferrin saturation, serum iron, haematocrit	2-4 mg/kg/day of enteral iron improved rates of ID from 40% to 14.7%
Friel et al. (2003)	Double-blind RCT	Healthy term breast-fed infants (n=77)	Haemoglobin, MCV, Serum ferritin	Supplementation with 7.5 mg of iron increased MCV at 3.5 months and haemoglobin and serum ferritin at six months. No increase in haematological parameters at other time points
Aggarwal, Sachdev, Nagpal, Singh, and Mallika (2005)	Double-blind RCT	Predominantly breast fed term LBW (n=62)	Haemoglobin, serum ferritin	There were no significant differences in serum ferritin between infants receiving 3 mg/kg/day iron and those receiving placebo. However adjusted haemoglobin concentration was significantly higher in the supplementation group at four and eight weeks

Sankar et al. (2009)	RCT	Preterm infants with birth weight <1500g (n=46)	Haemoglobin, haematocrit, serum ferritin	Early iron supplementation (starting at two weeks of age) did not improve iron status compared to starting supplements at eight weeks of age
Berglund, Westrup, and Domellof (2010)	Double-blind RCT	Term and preterm, marginally LBW infants (2000-2500g)	Haemoglobin, serum ferritin, MCV, and transferrin saturation	The mean serum ferritin for the placebo group was 18.6±2.1 µg/L, which increased to 34.0±2.1 µg/L in the group given 1 mg/kg/day of supplemental iron, and 49.3±2.2 µg/L in the group given 2 mg/kg/day. Supplementation also reduced the risk of IDA in a dose response manner
Long et al. (2012)	Systematic Review	LBW infants (14 studies)	Haemoglobin, haematocrit, serum ferritin, MCV, MCH, TIBC, serum iron, zinc protoporphyrin ratio, sTfR	Majority of studies reported that iron supplementation significantly improved haematologic measures of iron status
Mills and Davies (2012)	Cochrane Review	Preterm and LBW infants (n=2726)	Haemoglobin, serum ferritin, MCV, transferrin saturation, TIBC	Small improvement in haemoglobin at 3-4 months of age with iron supplementation, but no improvement in other biomarkers

ID= Iron Deficiency; IDA= Iron Deficiency Anaemia; LBW= Low Birth Weight; MCH= Mean Cell Haemoglobin; MCV=Mean Cell Volume; RCT= Randomised Control Trial; sTfR= Soluble Transferrin Receptor; TIBC= Total Iron Binding Capacity.

After reviewing the literature it appears that supplementation with 2-4 mg/kg/day of iron can lead to a significant improvement in the rates of IDA in LBW and preterm infants (Aggarwal et al., 2005; Friel et al., 2003; Long et al., 2012; Mills &

Davies, 2012). The effect of iron supplementation on rates of ID in LBW and preterm infants is however less clear (Aggarwal et al., 2005; Berglund et al., 2010; Franz et al., 2000; Long et al., 2012; Mills and Davies, 2012). Further research is required in moderate to late preterm infants and marginally LBW infants.

2.9.3.2. Iron Supplementation and Growth

At present there is limited data on the consequences of ID and IDA on infant growth and even fewer intervention trials assessing the effect of iron supplementation on growth (Sichieri Fonseca, Hoffman, Trugo & Moura, 2006). Of the few studies which are available, Aggarwal et al. (2005), Berglund et al. (2010) and Friel et al. (2003) all failed to find an association between iron supplementation and any growth related parameters including growth rate, length, head circumference and weight. A recently published Cochrane review also found a lack of association between iron supplementation and preterm infant growth (Mills & Davies, 2012).

2.9.3.3. Iron Supplementation and Neurodevelopment

As previously mentioned, ID and IDA have a detrimental effect on neurodevelopment and cognition (Rao & Georgieff, 2007). Despite this there is paucity of research regarding iron supplementation and neurodevelopment and the few studies that are available have conflicting results (Long et al., 2012). Friel et al. (2003) found that infants supplemented with 7.5 mg of iron per day scored on average seven points higher than the control group on the Bayleys Psychological Developmental Index at 12 to 18 months of age. There was no difference however with regards to Mental Development Indices and only a non-significant trend for increased visual acuity in the treatment group (Friel et al., 2003). An earlier study by Friel et al. (2001) found no cognitive improvement at 12 months of age with iron supplementation as measured by the Griffiths' Development Assessment.

2.9.3.4. Iron Supplementation and Negative Health Outcomes

Intervention studies have shown that there is no increase in the risk of chronic lung disease, retinopathy of prematurity, necrotising enterocolitis, invasive infection or rehospitalisation rates with 4 mg/kg/day of supplemental iron in preterm infants

(Sankar et al., 2009; Taylor & Kennedy, 2013). Prophylactic enteral iron doses over 5 mg/kg/day should be avoided in preterm infants due to the increased risk of oxidative damage and retinopathy of prematurity (Agostoni et al., 2009).

2.9.4. Current protocol at Auckland City Hospital Neonatal Intensive Care Unit

The current protocol at Auckland City Hospital NICU states that all preterm infants born before 32 weeks gestation or below 1800 g should be supplemented with 3 mg/kg/day of elemental iron in the form of ferrous sulphate solution starting at four weeks of age and until they are well established on solids (Cormack, 2012). Infants with malabsorptive diseases, long term IVN, fluid restriction, gastrointestinal losses, and infants of mothers known to be at risk of nutritional deficiencies will also be given a prophylactic dose of iron at 3 mg/kg/day or 0.5 ml/kg/day (Cormack, 2012). All preterm infants, regardless of their gestational age or birth weight, who are found to have clinical ID or IDA are supplemented with 6 mg/kg/day of iron (Cormack, 2012).

2.9.5. Current Protocol in Australasia and Worldwide

Iron supplementation practices vary significantly between the units in Australasia. While the majority of units (77%) stated that they commenced iron supplements at four weeks of age, 22% waited until six weeks of age (Cormack, Sinn, Luis, & Tudehope, 2013). In addition, 42% of units reported that they would tell parents to stop giving the supplements once their child reached six months corrected age, 25% when the child was eating solids, 17% when they were fully fed on infant formula, 13% when the child reached six months chronological age (defined as the time elapsed since birth), and 4% at discharge (Committee of Fetus and Newborn, 2004; Cormack et al., 2013).

2.10. Care of the Preterm Infant after Discharge

2.10.1. Early Discharge of Preterm Infants

Early discharge of infants, term or otherwise, is a contentious area. While a Cochrane review on the effect of early discharge, defined as a hospital stay of less than two nights after delivery, on the morbidity of term infants found that there was no significant increase in morbidity, they also concluded that due to methodological limitations, adverse outcomes could not be ruled out (Brown, Small, Faber, Krastev &

Davis, 2002). While most extremely and very preterm infants will have a relatively long stay in hospital after birth, those born closer to term may be treated as if they are term and discharged from hospital within two days of birth (Tomashek et al., 2006). This practice may be detrimental to moderate and late preterm infants as evidence shows that after vaginal birth, late preterm infants who are discharged early are 1.5 times more likely to be readmitted to hospital after birth than their term counterparts, with the most common reasons for readmission being jaundice and infection (Tomashek et al., 2006). The study by Tomashek et al. (2006) also found that early discharge of breastfeeding preterm infants was associated with an increased risk of neonatal morbidity after discharge compared to those infants who were formula fed. While initially a surprising result considering the protective properties of breast milk, Tomashek et al. concluded that this increased risk of morbidity was possibly due to the fact that breastfeeding may not have been fully established at time of discharge. Further research is required to identify evidence-based recommendations for discharge and post-discharge follow up of late preterm infants, particularly those who are breastfeeding.

2.10.2. Post-discharge Nutrition and Growth

Many preterm babies will also suffer growth restriction after hospital discharge (Cooke, 2011). This is largely due to delay in reaching optimal feeding rates after birth, as it is difficult to establish adequate intake in very sick infants (Cooke, 2011). In addition, feeding may be disrupted whilst in the NICU due to medical complications, further preventing optimal nutrition being achieved. Deficits caused by preterm birth may therefore not be corrected by the time of discharge (Cooke, 2011).

Postnatal growth failure can have a severe impact on the wellbeing of the preterm infant. There is a clear relationship between accelerated postnatal growth and neurodevelopment (Cooke, 2011; Latal-Hainal, von Siebenthanl, Kovari, Bucher & Largo, 2003). Those infants who are able to catch up by six to nine months corrected age (defined as the chronological age reduced by the number of days born before 40 weeks of gestation) have a better neurodevelopmental outcome than those who do not (Committee of Fetus and Newborn, 2004; Cooke, 2011; Latal-Hainal et al., 2003).

Optimum nutrition post-discharge is therefore vital to the wellbeing of preterm infants. Studies have found that feeding VLBW and ELBW preterm infant's nutrient enriched post-discharge formula in preference to term formula can improve growth parameters (Carver et al., 2001; Lucas, Bishop, King, & Cole, 1992; Lucas et al., 2001). Conversely, the use of human breast milk fortifiers after discharge is yet to show an improvement in growth or neurodevelopment (Young, Embleton, McCormick, & McGuire, 2013; Zachariassen et al., 2011), reinforcing current practices at Auckland City Hospital NICU.

2.10.3. Post-discharge Supplement Usage

Compliance with administering iron supplements to preterm infants is an understudied area. Auckland City Hospital currently recommend that iron supplements should be continued preferably until 12 months of age or until the infant is well established on a balanced diet of solids (Cormack, 2012). Anecdotal evidence shows that compliance with iron supplementation can vary and that it is likely to decline beyond three months when an additional prescription must be sought (B. Cormack, personal communication, 2012). Due to the paucity of evidence, more research is required to determine the compliance rates in New Zealand and to identify any barriers which parents have to administering the iron supplements.

2.11. Introduction of Solids

The introduction of solids is a major developmental milestone for all infants (Marriott, Foote, Bishop, Kimber, & Morgan; 2003). The introduction of solids occurs almost exclusively after discharge and is often left to the discretion of the parents or community healthcare workers (Norris, Larkin, Williams, Hampton & Morgan, 2002). The timing of the introduction of solids can greatly impact on the health of an infant; too early and the preterm infant may not be developmentally ready, left too late and the infant will be at risk of nutrient deficiencies (Morgan et al., 2004; Palmer & Makrides, 2012).

2.11.1. Current Recommendations for the Introduction of Solids for Preterm Infants

The WHO currently recommend that solid foods should be introduced to term infants around six months of age to promote exclusive breastfeeding until this time (WHO, 2001). Similar guidelines for preterm infants however do not exist and it is not appropriate to merely adopt the WHO recommendation for this population (Palmer & Makrides, 2012). Preterm infants, especially LBW, VLBW and ELBW infants have increased nutrient requirements compared to term infants, even after discharge, due to reduced accretion of nutrients in utero and increased requirements for rapid growth (Marriott et al., 2003; Palmer & Makrides, 2012). The only relevant guideline pertaining to preterm infants was released by the Department of Health in the United Kingdom in 1994, stating that solids should be introduced to preterm infants once they have reached 5 kg, have lost the extrusion reflex, and are able to eat from a spoon (Department of Health, 1994).

Research by Marriott et al. in 2003 questions whether current recommendations about the introduction of solids produced by the Department of Health are appropriate for preterm infants. Participants in the study were randomised into two groups: the parents in one group received information in line with the Department of Health recommendations and were advised to introduce solids as soon as the infant reached 17 weeks chronological age, providing that they were at least 5 kg (Marriott et al., 2003). Parents in the other group were advised to introduce high energy, high protein solids once the infant was 13 weeks chronological age, providing that they were at least 3.5 kg (Marriott et al., 2003). Results of this study showed that infants who were introduced to solids earlier had higher mean daily energy and nutrient intakes, significantly higher haemoglobin and serum iron levels at six months of age, and faster lengthwise growth (Marriott et al., 2003). However as diets were not isocalorically matched further research would be required before adopting these recommendations.

A recent article by Palmer and Makrides (2012) proposed that based on current available evidence, solids should be introduced to preterm infants at three months corrected age. Using three months corrected age would be appropriate from a

developmental point of view for most preterm infants as infants born at 23 weeks gestation would have a chronological age of seven months while those born at 36 weeks would have a chronological age of four months (Palmer & Makrides, 2012).

2.11.2. Factors Affecting the Introduction of Solids in Preterm Infants

Timing of the introduction of solids is largely determined by developmental cues. Parents are often advised to introduce solids once their infant has lost the extrusion reflex, can sit in a stable supported position, can hold their head up well, and starts to lean towards the spoon (Palmer & Makrides, 2012). In general, preterm infants have been observed to have delayed development of gross motor skills compared to term infants, even after correction for prematurity (Palmer & Makrides, 2012). Most preterm infants do not begin to exhibit these developmental motor cues until they are approximately three months corrected age (Palmer & Makrides, 2012). Preterm infants born with neurological abnormalities and disabilities are likely to exhibit even greater developmental delay, further delaying the introduction of solids (Blencowe et al., 2012; Kerstjens et al., 2011). Therefore the assessment of infant cues is vital and each preterm infant should be treated as an individual with regards to their developmental readiness (King, 2009).

Oral aversion and hypersensitivity may also interfere with the introduction of solids. Enteral feeding is common practice in the NICU, especially for preterm infants born very and extremely preterm and those who are acutely unwell (Cormack, 2012). While this is an effective way of ensuring the infant has adequate nutrition, problems can arise when tube feeding is no longer required (Mason, Harris & Blissett, 2005). Mason et al. (2005) suggest that aversive experiences associated with enteral feeding can delay the introduction of solids. Some infants may become hypersensitive to any stimuli in their mouth as a result of an unpleasant oral procedure such as placing or removing a feeding tube (Mason et al., 2005). In addition, gastroesophageal reflux disease, a common disease in preterm infants which is associated with nausea, vomiting and oesophagitis, can also cause oral aversion leading to feeding difficulties (Mason et al., 2005).

Delayed introduction of solids (generally beyond seven months of age) has also been associated with abnormal feeding habits and aversive feeding behaviours (Palmer & Makrides, 2012). While it is still unknown whether feeding progression is inherently programmed or if it is influenced by oral experiences, evidence shows that infants who are not given the opportunity to practice these skills are more likely to develop feeding problems later in life (King, 2009; Palmer & Makrides, 2012). Infants who are not exposed to solid foods during vital developmental stages are more likely to have feeding problems and are less likely to tolerate textured foods (Palmer & Makrides, 2012).

The duration of breastfeeding also affects the introduction of solids. Hopkins et al. (2007) discovered that exclusively breastfeeding beyond 6 months significantly delayed the introduction of solids, causing nutrient deficiencies. They also found that at eight months of age, infants who were fed more than six breast feeds per day were obtaining less energy from solid foods compared to infants receiving fewer breast feeds or formula fed infants (Hopkins et al., 2007). Hopkins et al. (2007) recommend that once the infant is established on a solid diet that the amount of milk offered to infants should be reduced to less than six breastfeeds or 600 mL of formula/expressed breast milk per day to prevent milk intake replacing consumption of solids.

2.11.3. Effects of the Introduction of Solids on Iron Status

The introduction of solids affects iron status in several key ways. Firstly, delayed introduction of solids can predispose infants to ID and IDA. While breast milk is the preferred source of nutrition for the first few months of life, it contains very little iron (Mills & Davies, 2012; Rao & Georgieff, 2009). There is evidence that delaying the introduction of solids beyond six months of age increases the risk of IDA for all infants, but especially preterm infants who require additional iron to compensate for increased utilisation for accelerated growth (Hopkins et al., 2007; Rao & Georgieff, 2009).

The type of food which an infant is weaned onto may also affect their iron status. A study of New Zealand infants aged 6-24 months old found that consumption of iron fortified cereals, meat, poultry and fish was positively associated with serum

ferritin levels in non-breast fed infants (Soh et al., 2002). This in line with the Ministry of Health (2008) recommendations which state that an infant's first food should be iron-fortified cereal, cooked and pureed meat, pureed rice, age appropriate commercial infant foods, or cooked and pureed fruit or vegetables.

Early introduction of cow's milk is also known to affect an infant's iron status (Hopkins et al., 2007; Leung & Sauve, 2003). Consumption of cow's milk is associated with increased occult blood loss from the gastrointestinal tract both during early and late infancy (Leung & Sauve, 2003). The Ministry of Health (2003) therefore recommend that cow's milk should not be given to infants as a drink until one year of age. The increased risk of ID with early cow's milk consumption is also due in part to its low iron content and bioavailability (Leung & Sauve, 2003). Cow's milk contains between 0.3 and 1 mg/L of iron, with only 10% of that being absorbed (Leung & Sauve, 2003). A recent study of New Zealand infants aged 6-23 months found that daily cow milk consumption was associated with a significantly increased risk of ID (RR 4.71, 95%CI 1.99 to 8.56) (Brunt, Grant, Wall, & Reed, 2012).In addition, cow's milk contains a significant amount of calcium which is known to inhibit iron absorption (Hurrell & Egli, 2010). Hopkins et al. (2007) found that every standard deviation increase in calcium consumption (approximately 250 mg) was associated with a drop in mean serum ferritin concentration of about 20% in eight month old infants.

In addition to calcium, there are other inhibitors of non-haem iron absorption found in the diet which may affect iron status of an infant. Consumption of phytates and other inositol phosphates, commonly found in infant breakfast cereals, whole grains, legumes, nuts and seeds, increases the risk of ID in infants (Hurrell & Egli, 2010). Phytates and inositol phosphate elicit their effect by causing ferric iron to precipitate and form macromolecules which are unable to be absorbed (Conrad & Umbreit, 2000). Phytates and inositol phosphate exhibit their effect on non-haem iron even at very low concentrations, starting at approximately 2-10 mg/meal (Hurrell & Egli, 2010). Hopkins et al. (2007) for example found that infants were twice as likely to have low serum ferritin levels for every 2 g/day increase in non-starch polysaccharide consumption. Polyphenols, commonly found in tea, vegetables, some legumes and

infant cereals exert a similar affect as phytates and can further increase the risk of ID in infants consuming solids (Hurrell & Egli, 2010).

In addition to inhibitors of iron absorption, there are also dietary factors which can increase absorption of iron. Consumption of ascorbic acid with non-haem foods has been found to increase iron absorption by facilitating the reduction of ferric iron to ferrous iron (Conrad & Umbreit, 2000). Soh et al. (2002) reported that each milligram increase of ascorbic acid was associated with a 0.2% increase in serum ferritin, which although it was a small increase, was statistically significant.

2.11.4. Current Practices in Preterm Infants

International research shows that at present, preterm infants are introduced to solids earlier than their term counterparts, with the majority of preterm infants being introduced to solids prior to four months corrected age (Fewtrell, Lucas & Morgan, 2003; Norris et al., 2002; Palmers & Makrides, 2012). In a cohort of 253 preterm infants in the United Kingdom, the average age that solids were introduced was 11.5±0.21 weeks corrected age, with 95% of infants being introduced to solids by 17 weeks corrected age (Norris et al., 2002). As predicted, the study also found that those infants who were breastfed were introduced to solids later than those infants who were formula fed (Norris et al., 2002). In addition, those born extremely and very preterm were introduced to solids later than those born closer to term (Norris et al., 2002).

With regards to the introduction of solids in the New Zealand setting, Heath et al. (2002) found that 45% of all infants in Dunedin had been introduced to solids before four months and more than two-thirds (69%) were given cow's milk as a drink before 12 months of age. As international research suggests that preterm infants are introduced to solids earlier than their term counterparts, one can assume that at least half of preterm infants are currently starting solids in line with the recommendations from Palmer and Makrides (2012).

2.12. Summary

Preterm infants are a unique population with medical, physiological and nutritional needs which cannot be compared to any other group. Due to their shortened gestational length, increased requirements for rapid growth and excessive losses through phlebotomy, preterm infants are more vulnerable than the general infant population to developing nutrient deficiencies after birth. Iron is a nutrient of particular concern for preterm infants as it is mostly accrued during the last trimester and thus often low in preterm infants at birth. Ensuring that preterm infants have a sufficient intake of bioavailable iron after birth is extremely important as poor iron status has been associated with negative health and neurodevelopmental outcomes later in life. Supplementation with 2-4 mg/kg/day of elemental iron has been shown as an effective way of reducing the risk of ID and IDA in preterm infants.

While Auckland City Hospital has protocols in place about iron supplementation of preterm infants, there is currently insufficient evidence to guarantee that these are meeting the needs of all preterm infants after discharge. Currently only infants born before 32 weeks gestation and/or under 1800 g receive iron supplements after discharge, possibly placing less preterm infants at risk of ID or IDA. In addition, as the infants who receive routine supplementation after discharge are also the infants most likely to receive erythrocyte transfusions whilst in the NICU, data needs to be collected about the risk of iron overload in these infants. As mode of feeding also affects the iron status of infants after discharge, this research will endeavour to determine whether current supplementation and feeding practices in preterm infants living in Auckland, New Zealand are sufficient to meet their requirements at four months after discharge.

3. Methods

3.1. Study Design

The 'Post-Discharge Nutrition of Preterm Infants: micronutrient status and feeding practices of preterm infants after hospital discharge' study has been designed as a longitudinal, observational study to determine the micronutrient status of preterm infants living in the Auckland area at four months after discharge from Auckland City Hospital and the feeding practices of these infants up to one year corrected age. This thesis will present data on the iron status of preterm infants at four months after discharge from Auckland City Hospital along with factors which may affect this status.

3.2. Ethical Approval

Ethical approval for this study was gained from the Massey University Human Ethics Committee (MUHEC): Southern A (application 13/06). The research committee at Auckland District Health Board also reviewed the study and gave permission for the study to be carried out and the infants to be recruited through Auckland City Hospital (A+5810).

3.3. Study Population

3.3.1. Setting

Auckland City Hospital is one of only two hospitals in Auckland that offers tertiary level care for preterm infants, with other hospitals not treating infants younger than 32 weeks gestation (Auckland District Health Board, 2013; Kidz First Children's Hospital and Community Health, n.d.; Waitemata District Health Board, n.d.). Recruiting preterm infants discharged from Auckland City Hospital Neonatal Intensive Care Unit (NICU) allows for the assessment of iron status in infants with varying degrees of prematurity.

3.3.2. Eligibility

Infants were eligible for this study if they were born at Auckland City Hospital between 1 October 2012 and 30 April 2013 and were born before 37 weeks gestation. Preterm

infants born at other hospitals but who had been transferred to Auckland City Hospital NICU for more intensive care, or participants who were born preterm at Auckland City Hospital but who had been transferred to other hospitals for continued care prior to discharge home were also eligible for this study provided their stay in Auckland City Hospital was between the above dates. To be eligible for this study, participants also had to be living in a home environment within the Auckland area and had to be discharged from hospital less than four months prior to recruitment. In addition infants were excluded from this study if they had been transferred to a paediatric ward for ongoing specialist care or had anaemia due to a nutrient deficiency other than iron (low haemoglobin but adequate iron stores) as this could confound the results.

3.3.3. Sample Size

Using available data on the iron status of low birth weight (LBW) infants in New Zealand, it appears that a sample size of 76 infants would be required to determine iron status based on mean haemoglobin (confidence interval of 5 g/L and 95% confidence interval) (Thom et al., 2003). Due to concerns about retention of the study population over the course of the study, a goal to recruit 100 infants was set, allowing for a possible attrition rate of 30%.

3.3.4. Recruitment of Participants

Participants were recruited through Auckland City Hospital NICU between February and August 2013. Neonatal Intensive Care Unit staff were informed about the study prior to recruitment through an informative poster (Appendix A). Staff were encouraged to discuss the study with parents whilst on the unit if they deemed it to be appropriate. The recruitment process required some ward staff to invest a small amount of their time, therefore it was important that they were aware of the importance of this research.

Participants were recruited by collecting National Health Index (NHI) numbers from the Auckland City Hospital NICU log book, which is a record of all previous admissions and discharges from the NICU. The NHI numbers of all preterm births between 1 October 2012 and 30 April 2013 were recorded and provided to the ward

clerk. The ward clerk provided the researchers with the contact details for all preterm infants born between these times, provided that the infants were still alive at the time of recruitment.

Parents of the infants identified from the initial step of recruitment were sent a contact letter (Appendix B) inviting them to participate in the study and an information sheet (Appendix C) detailing the importance of conducting the research and what the study would involve. The parents were also sent a contact details slip (Appendix D) and a free, return post envelope which they were asked to complete and return to indicate their interest in enrolling their infant in the study. The contact details slip asked parents to fill in their name, address, telephone number and email address (Appendix D). It was important to have this completed by parents interested in the study as the information provided by the NICU ward clerk did not always include all necessary contact details for each infant.

Parents who returned the contact details slip indicating they were interested in being involved in the study were contacted by telephone and verbal consent for them self and their infant(s) to participate in the study was obtained. Parents who did not return the contact details slip were contacted by telephone one week after the letters were sent. The study was explained to the parents and the opportunity to have questions answered was given. For parents who wanted to have their infant(s) enrolled in the study, verbal consent was obtained and a home visit organised.

3.4. Measures to Assess Infant Iron Status and Growth at Four Months after Discharge

3.4.1. Iron Biomarkers

To determine the iron status of infants at four months after discharge, blood was collected and the following biomarkers measured; haemoglobin, serum ferritin, soluble transferrin receptor (sTfR), and C-reactive protein (CRP). Maternal iron status of breastfeeding mothers was also assessed to determine whether it affected infant iron stores. While studies have shown that only maternal haemoglobin is associated with the iron content of breast milk, both serum ferritin and sTfR were also analysed to

ensure that any anaemia was due to iron deficiency (ID) (Kumar et al., 2008; Meinzen-Derr et al., 2006).

3.4.1.1. Infant Blood Collection

Capillary blood samples were collected from each infant via a heel prick performed by a trained paediatric phlebotomist. Whilst gently squeezing the infant's foot, a puncture was made on the outer side of the heel using a sterilised lancet. The first drop of blood was wiped away. The phlebotomist gently massaged the infant's heel and leg to ensure adequate blood flow.

The infant serum sample was collected prior to the haemoglobin sample in line with the order of draw recommended by BD Diagnostics (BD Diagnostics, 2010a). In addition, as blood collection from infants can be difficult there was concern that both sample tubes would not be able to be filled. Collection of the serum sample was therefore prioritised as a HemoCue was utilised in this study to give an indication of haemoglobin concentration if a blood sample could not be collected. To ensure that there was an adequate serum sample for analysis, the phlebotomist collected 400 µL of blood in a gold topped tube via the heel prick method mentioned above. Once filled, the tube was capped and gently inverted five times as per the manufacturer's recommendations (BD Diagnostics, 2010b). To collect the haemoglobin sample, a lavender topped ethylenediaminetetraacetic acid (EDTA tube) was filled with 250 µL of blood. Once filled, the tube was capped and gently inverted eight times (BD Diagnostics, 2010b). The gold topped tube and the lavender EDTA tubes were then placed in a cool polystyrene box with an ice pack and transported to the Human Nutrition Research Laboratory at Massey University and North Shore Hospital Laboratory respectively.

3.4.1.2. Maternal Blood Collection

Venous blood samples were taken from consenting breastfeeding mothers by a trained phlebotomist. The phlebotomist collected the mother's blood into two vacutainers, a gold topped vacutainer for the assessment of the serum biomarkers and a lavender topped tube containing EDTA for the assessment of haemoglobin. Like the infant

samples, the gold topped tube was then gently inverted five times and the lavender EDTA tube eight times (BD Diagnostics, 2010b). The samples were placed in a cool polystyrene box with ice packs. The lavender EDTA tube was then transported to North Shore Hospital Laboratory for analysis while the gold serum tube was transported to the Human Nutrition Research Laboratory at Massey University for processing.

3.4.1.3. Processing Serum Samples

The serum samples were processed at the Human Nutrition Research Laboratory at Massey University. To ensure that the samples had adequate time to clot, the gold top tubes were left to stand for thirty minutes at room temperature before centrifuging. All serum samples were centrifuged within two hours of blood collection. All samples were centrifuged using the Heracus Labofuge 400R SMOU. Prior to centrifuging, the internal chamber was cooled to 4^{o}C.

The infant samples were placed in the Heracus Labofuge 400R microcentrifuge rotor #3325 and balanced. They were spun at 4000rpm for ten minutes at room temperature. The entire serum sample from the vacutainer was aliquoted into an eppendorf tube. The samples were then stored in a freezer at -80^{o}C and analysed as one large batch at the end of the trial at North Shore Hospital Laboratory. Samples were analysed as one batch to reduce the introduction of measurement bias.

The maternal samples were placed in the Heracus Labofuge 400R swing bucket #8179 and balanced. The vacutainer tubes were spun at 3500rpm for ten minutes at room temperature. A minimum of 250 µL of serum were aliquoted from the vacutainers into eppendorf tubes. The samples were then stored upright in a freezer at -80^{o}C and analysed as one large batch at the end of the trial at North Shore Hospital Laboratory.

3.4.1.4. Haemoglobin

Infant and maternal blood were delivered to the North Shore Hospital Laboratory the same day as they were collected as analysis needed to be performed on fresh blood samples. As abnormal results could indicate overt IDA it was also important that these

samples were analysed as quickly as possible so that abnormal results could be communicated to the parents and their family doctor. Haemoglobin was analysed using the SLS-Hb (sodium lauryl sulphate-Hb) method using the Sysmex XE-5000 at 555nm (Sysmex Corporation, Auckland, NZ). The CV was 1%.

3.4.1.5. Haemoglobin Analysis Using the HemoCue

The HemoCue Hb 201+ Analyser was utilised in this study to ensure that a haemoglobin reading could be obtained for each participant. The HemoCue is a portable device which gives immediate haemoglobin readings and requires only 10 µL of blood compared to at least 250 µL required for traditional assessment of haemoglobin (Nkrumah et al., 2011; Schapkaitz, Mahlangu, Letsoalo, 2012). The HemoCue works by converting haemoglobin to azidemethemoglobin (Nkrumah et al., 2011). This occurs when the erythrocyte membranes in the blood sample react with sodium deoxycholate, releasing the haemoglobin (Nkrumah et al., 2011). Sodium nitrate in the cuvette converts the haemoglobin iron from the ferrous to ferric state, forming methemoglobin (Nkrumah et al., 2011). The methemoglobin subsequently reacts with an azide to form azidemethemoglobin, which can be detected using double wave photometry at 570nm and 880nm (Nkrumah et al., 2011).

The HemoCue was cleaned and calibrated daily to ensure accurate results. Between collecting the serum and the haemoglobin samples, a drop of blood from the heel prick for the infants and a drop from the venipuncture for the mothers was collected using a HemoCue microcuvette. The correct volume of blood was drawn into the microcuvette via capillary action. The microcuvette was then inserted into the HemoCue analyser and the corresponding haemoglobin concentration displayed on the digital screen recorded on the data collection sheet (Appendix E). The coefficient of variance (CV) was 0%.

Haemoglobin concentrations at four months after discharge were primarily determined by the values attained from laboratory analysis as this is the most robust measure of haemoglobin concentration. If a haemoglobin sample for laboratory analysis was unable to be collected then the results from the HemoCue were reported.

3.4.1.6. Serum Ferritin

Serum ferritin was analysed using the FERR method; a homogenous sandwich chemiluminescent immunoassay (Siemens Healthcare Diagnostic Inc, Delaware) (Appendix F) on a Siemens Vista analyzer at 680 and 612nm. The CVs were 2-3%.

3.4.1.7. Serum Soluble Transferrin Receptor

An immunoturbidimetric method (Siemens Healthcare Diagnostic Inc, Delaware) (Appendix G), known as the IDeA method (Vendt et al., 2009) was used to determine soluble transferrin receptor concentration using a Siemens Vista analyzer at 840nm. The CVs were 2.2-3.6%.

3.4.1.8. C-Reactive Protein

An immunoturbidimetric method (Siemens Healthcare Diagnostic Inc, Delaware) (Appendix H) was used to determine CRP using a Siemens Vista analyzer at 840nm. The CVs were 5-6%.

3.4.1.9. Definitions of Iron Status

The participants in this study were classified as having optimal iron status, ID, iron deficiency anaemia (IDA) or iron overload according to the cut-off values displayed in Table 3.1.

Table 3.1: *Biomarkers and Respective Cut-off Values to Determine Iron Status in Preterm Infants and Female Adults.*

Infant Iron Status				
	Optimal Iron Status	**ID**	**IDA**	**Iron Overload**
Serum Ferritin (µg/L)	>12	<12	<12	>300
Soluble Transferrin Receptor (mg/L)	<2.4	>2.4	>2.4	
Haemoglobin (g/L)	>110	>110	<110	
Maternal Iron Status				
	Optimal Iron Status	**ID**	**IDA**	**Iron Overload**
Serum Ferritin (µg/L)	>15	<15	<15	>150
Soluble Transferrin Receptor (mg/L)	<2.4	>2.4	>2.4	
Haemoglobin (g/L)	>120	>120	<120	

ID= Iron deficiency; IDA =Iron Deficiency Anaemia

High CRP concentrations were defined as greater than 5 mg/L and were recorded as an indicator of inflammation and infection. The serum ferritin results for participants with a CRP above 5 mg/L were excluded from analysis as these are likely to be artificially elevated (Lynch, 2010). Haemoglobin and sTfR however are accurate even with an elevated CRP.

3.4.2. Anthropometric Measures

Techniques to obtain the weight, length and head circumference of each infant were based on the protocol set out by the Intergrowth-21st Anthropometry Group (2012). Additional training was provided by dietetic staff at Auckland City Hospital NICU to ensure that measurements were accurate and reliable.

3.4.2.1. Weight

The infants' weights were measured using Atronics sbb-003 Low Profile Digital Baby Scales. Prior to each home visit the digital baby scales were cleaned and calibrated. To ensure accurate results, the infant was weighed naked or in a clean nappy, depending on the preference of the parents. The infant scales were placed on a hard surface and tarred/zeroed. The infant was placed in the middle of the scales and the weight recorded. If the infant was wearing a nappy the parents were asked to provide a clean nappy so that it could be weighed and subtracted from the infant's weight. To ensure that the measurement was accurate, the weighing procedure was performed for a second time and an average of the two weights recorded on the data collection sheet (Appendix E). If the second result was not within 50 g of the initial weight, a third weight was taken and the average of the three measurements recorded.

3.4.2.2. Length

The infants' lengths were measured using an age appropriate length board. The infant was placed on its back with its heels on the edge of the length board and shoulders and buttocks flat against the measuring surface. The parents were asked to gently but firmly hold the infant's legs in place with a hand on the infant's knees to maintain full extension. The head piece was moved in so that it was firmly placed against the infant's head, ensuring that the vertical line from the infants' ear canal to the lower border of the eye socket was perpendicular to the horizontal board. The measurement was recorded to the nearest millimetre. A second length measurement was taken with an allowance of one centimetre variability between the two measurements and an average of the two measurements recorded on the data collection sheet (Appendix E). If the difference was greater than one centimetre then a third measurement was taken and the average of the three measurements recorded.

3.4.2.3. Head Circumference

The infants' head circumferences were measured using a flexible plastic measuring tape. The infant was held upright in the parent's arms allowing the researcher to wrap the tape measure around the infant's head. The tape was placed just above the eyebrows and ears, ensuring that it was positioned around the largest part of the back

of the head. Once in the correct position the tape was gently pulled tight and the circumference measured to the nearest millimetre. A second circumference was measured to ensure the accuracy and reliability of the measurement with an allowance of five millimetres variability between the two measurements and an average of the two measurements recorded on the data collection sheet (Appendix E). If the difference was greater than five millimetres then a third measurement was taken and the average of the three measurements recorded.

3.4.3. Development of Questionnaires

3.4.3.1. Demographics Questionnaire

The demographic questionnaire (Appendix I) was designed to ascertain what ethnicity the mother most associated with, her date of birth, whether she had any previous pregnancies and if so, how many. The mother was also asked what ethnicity their infant belonged to, what was their date of birth, and their gestational age at birth. The ethnic groups included in this questionnaire were taken from the 2002 New Zealand Children's Nutrition Survey (Ministry of Health, 2003).

3.4.3.2. Feeding Practices Questionnaire

The feeding practices questionnaire consisted of 22 questions designed to assess the overall nutrition and consumption of iron rich foods by preterm infants in this study. Parents were asked whether they breast fed or predominately formula fed (defined as at least 80% of the infant's oral intake coming from infant formula) their infant when first discharged and how they were currently feeding them at the time of the home visit. The questionnaire also covered duration of breastfeeding (if applicable), type of formula (if applicable) and whether solids and liquids had been introduced (Appendix J). The questionnaire was based on the Health and Social Care Information Centre 'Infant Feeding Survey' (2010) and the questionnaire developed by Golding (1991). The questionnaire was also designed to reflect the foods currently being consumed by infants and children in New Zealand (Ministry of Health, 2003) and the 'first foods' currently recommended for preterm infants (Cormack, 2004; Cormack, 2013).

3.4.3.3. Supplement Questionnaire

The supplement questionnaire consisted of 11 questions. It was designed to determine whether an infant was discharged from hospital on iron supplements or not, what dose of supplements they were discharged home on, whether this dose had changed since discharge, if the parents were still giving the infant the supplements, and what barriers there were to giving the supplements (Appendix K). As only infants born before 32 weeks gestation, with a birth weight of less than 1800 g or with clinical ID or IDA are routinely discharged from hospital on iron supplements, not all of these questions were applicable to all infants in this study. In addition, some infants may have been prescribed supplements after discharge, particularly if they were found to be at particular risk of ID or IDA, which was taken into account in this questionnaire.

3.5. Standard Operating Procedure for Booking

Once verbal consent has been obtained from a parent for their infant to be enrolled in the study, an eligibility screen (Appendix L) was completed over the telephone. The eligibility screen questionnaire asked parents whether their infant was born preterm, if they were currently living in a home environment, if they had been an inpatient at Auckland City Hospital, and whether it had been less than four months since their infant was discharged from hospital. Provided that the infant was eligible for the study, the parent was given an appointment as close as possible to four months after their infants discharge date. The demographic questionnaire (Appendix I) was also completed by the researcher at this time as part of the booking process.

3.6. Standard Operating Procedure for Appointment

At the beginning of each home visit the researchers introduced themselves to the family. The parents were also asked if they had read the information sheet and given the opportunity to ask any questions. Parents who had not read the information sheet were given a copy brought by the researchers. Following this an informed consent form was required to be signed for each infant (Appendix M). As the infants could not give informed consent, this was sought from their parent/guardian. Mothers who were currently breastfeeding at the time of the home visit, either exclusively or predominately, were also given the option to have their iron status analysed. If the

mother consented to having her blood taken for analysis a maternal consent form was also required to be signed (Appendix N).

To ascertain information about supplement usage and feeding practices parents of the participants were asked to complete an online questionnaire using the Survey Monkey programme prior to their home visit. These questionnaires were initially carried out during the home visit however many mothers reported that it would be more convenient for them to complete the questionnaires in their own time. Using the Survey Monkey software reduced the length of time of each appointment and also standardised the way in which the questionnaires were delivered, aiding to remove interviewer bias. The researchers had access to the questionnaire responses which allowed them to ensure that the questionnaires were accurately completed prior to each home visit. Any information that was unclear or missing could then be clarified with the parents at the home visit. Parents who either did not have access to the internet or could not complete the online questionnaire prior to the home visit by the research team completed interviewer-administered questionnaires during the appointment.

Following confirming questionnaires had been completed or conversely administering the questionnaires, the aforementioned anthropometric measures were taken. These were taken prior to blood sampling as the heel prick caused the infant to become fairly distressed, making anthropometric measurements difficult. Weight, length and head circumference were measured using the previously mentioned techniques and recorded on the data collection sheet (Appendix E). Finally, blood samples were collected as per the methods above. The home visit was concluded by thanking the parents for their time, informing them how they would receive the results and letting them know that another appointment would be arranged for approximately one year corrected age.

3.7. Standard Operating Procedure for Data Collection from Medical Notes

Medical information regarding the infant's initial hospital stay after birth and any admissions since was obtained from the Auckland City Hospital database and documented using a Medical Notes questionnaire (Appendix O). The following information was recorded from the medical notes: gestational age at birth, birth weight, birth length, head circumference at birth, type of delivery, whether it was a singleton or multiple birth, whether they had been admitted to the NICU, if they had received any erythrocyte transfusions whilst in the hospital, feeding mode whilst in the NICU and at discharge, whether they had received any supplements during their hospital admission and what was prescribed at discharge, iron biomarkers at birth and at discharge, and any details of readmissions since birth.

3.8. Standard Operating Procedure for Informing Participants of Results

Parents were contacted as soon as the results returned from the laboratory if they or their infant had any results outside of the accepted ranges (Table 3.1). They were made aware of the abnormal results and consent was obtained to contact their family doctor. A letter describing the findings along with a copy of the blood results was sent to the parents and to the family doctor (Appendix P). During the home visit, parents were made aware of this process and that if they did not hear from the researchers that the results were within the normal range. A summary of the results of the study was also provided to all participants at the end of the study.

3.9. Statistical Analysis

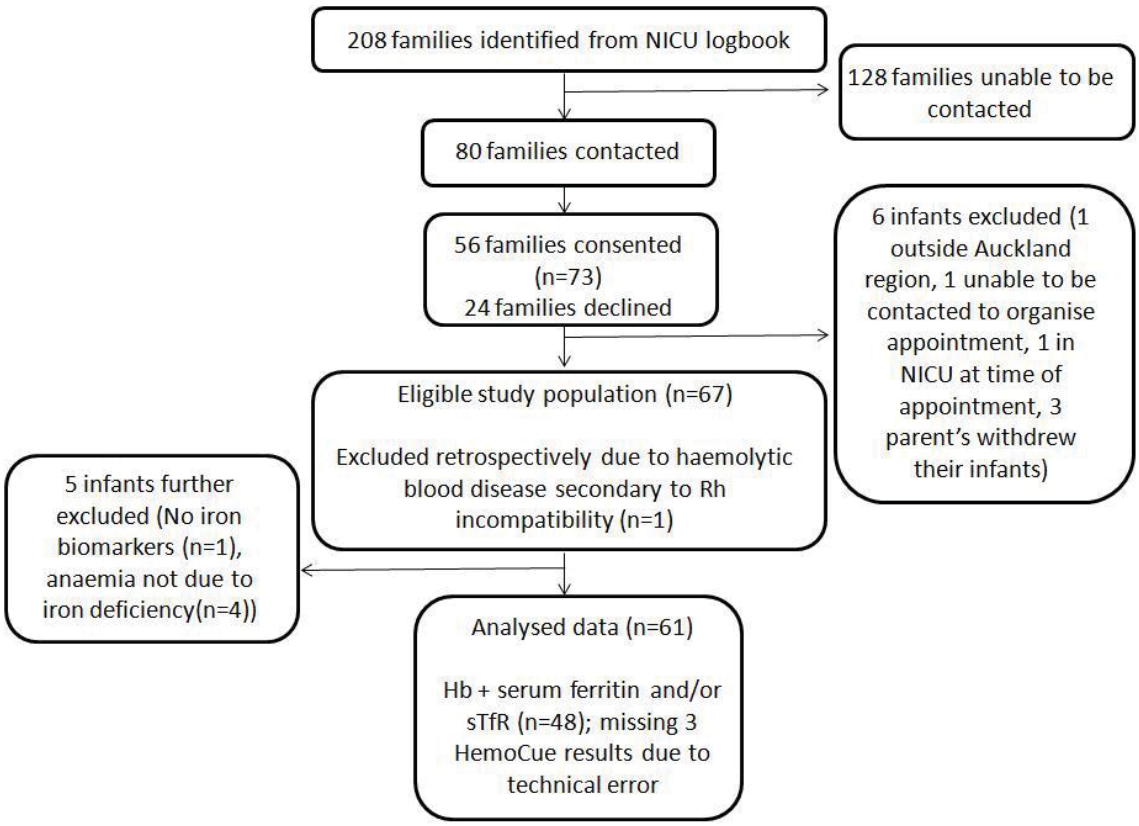
All data was coded and entered into SPSS (IBM SPSS Statistics Version 21) for statistical analysis. Descriptive statistics were carried out and the mean plus standard deviation (or geometric mean plus 95% confidence interval if the data was log transformed) or median plus 25^{th} and 75^{th} percentile documented. The primary outcome was iron status and this was compared between two groups, those receiving supplements post-discharge and those not receiving supplements post-discharge. Iron status was often categorised as optimal or suboptimal (by combining infants with ID and IDA) rather than optimal, ID and IDA to ensure an adequate sample size for statistical analysis. Comparisons between groups were performed using appropriate parametric and non-

parametric tests. Normality of distribution was tested using Kolmogorov-Smirnov tests, Shapiro-Wilk tests and normality plots. Prior to performing non-parametric tests, attempts to transform the data using log and square root transformations were undertaken and tests for normal distribution performed. When parametric data was collected, independent t-tests were performed, while Kruskal Wallis, Mann-Whitney tests or Chi Squared tests were performed in the case of non-parametric data being collected. Statistical analysis was also performed to determine the difference in iron status between infants who were breastfed and formula fed (defined as at least 80% of the infants oral intake coming from infant formula) at four months after discharge. Bivariate correlation tests were performed to identify whether maternal iron status of breastfeeding mothers had an effect on infant iron status. Growth parameters were expressed as z-scores and were calculated whilst controlling for gestational age (determined by the number of completed weeks), corrected age at home visit (determined by the number of completed weeks) and gender. Independent t-tests were performed to determine the effect that iron status and iron supplementation had on the changes in growth z-scores between birth and the home visit.

4. Results

4.1. Description of Participants

Figure 4.1 details the recruitment of preterm infants into this study through to the final number of participants for data analysis.



Hb= Haemoglobin; sTfR = Soluble Transferrin Receptor.

Figure 4.1: *Flow Diagram detailing Recruitment and Final Number of Participants for Data Analysis*

The characteristics of the participants at four months after hospital discharge are detailed in Tables 4.1. The cohort of infants had a median [25th, 75th percentile] gestational age of 34 weeks and 3 days [32+5, 35+6]. The cohort had a mean±SD birth weight of 2.07±0.65 kg, mean birth length of 44.4±4.6 cm, and median head circumference at birth of 31.5 [30, 33] cm. The majority of infants in this study were of European descent (55.7%), with the remaining infants coming from a range of different ethnicities (Table 4.1). The median time between discharge and home visit was 119 [112, 123] days. The shortest time between discharge and home visit was 93 days (3

months and 3 days) and longest was 163 days (5 months and 13 days). Three infants in total were seen later than five months after discharge.

Table 4.1: *Characteristics of Preterm Infants (<37 weeks gestation) in the Study at Four Months after Hospital Discharge*

Characteristics	**n= 61**
Gender n (%)	
Males	35 (57.4)
Females	26 (42.6)
Gestational Age (weeks + days)	
Median [25th, 75th percentile]	34+3 [32+5, 35+6]
≤32 weeks n (%)	14 (23.0)
>32 weeks n (%)	47 (77.0)
Birth Weight (kg)	
Mean±SD	2.07±0.65
≤1.8kg n (%)	23 (37.7)
>1.8kg n (%)	38 (62.3)
Length at Birth (cm)	
Mean±SD	44.4±4.6
Head Circumference at Birth (cm)	
Median [range]	31.5 [30, 33]
Infant Ethnicity n (%)	
European	34 (55.7)
Maori	8 (13.1)
Pacific Island	6 (9.8)
Asian	8 (13.1)
Indian	5 (8.2)
Singleton Birth n (%)	28 (45.9)
Twin n (%)	33 (54.1)
Chronological Age at Home Visit (days)	
Median [25th, 75th percentile]	138 [130, 157]
Corrected Age at Home Visit (days)	
Mean±SD	102±16.8
Time Since Discharge (days)	
Median [range]	119 [112, 123]

4.2. Preterm Infant Iron Status after Discharge

The mean haemoglobin concentration at four months after discharge was 118±10.3 g/L, the mean serum ferritin at four months after discharge was 51.5±27.2 µg/L and the median soluble transferrin receptor (sTfR) concentration was 1.7 [1.5, 2.0] mg/L (Table 4.2). One serum ferritin result was excluded from data analysis because the

infant had a C-Reactive Protein (CRP) of greater than 5 mg/L. Four infants were excluded from the data analysis as they were identified as being anaemic (haemoglobin less than 110 g/L) but had adequate iron stores (serum ferritin >12 µg/L and/or sTfR<2.4 mg/L), indicating that the anaemia was due to a reason other than iron deficiency (Figure 4.1). Infants with anaemia but no biomarkers of iron stores were included in this study as it could not be ruled out that the anaemia was in fact due to iron deficiency.

Table 4.2: *Iron Status of All Preterm Infants in Study at Four Months after Discharge*

	Haemoglobin[a] n= 58	**Serum Ferritin n=50**	**Soluble Transferrin Receptor n=46**
Mean/ Median	118±10.3 g/L	51.5±27.2 µg/L	1.7 [1.5, 2.0] mg/L
Range	82 – 136 g/L	10 – 126 g/L	1.2 – 3.2 mg/L

[a] *36 haemoglobin results from laboratory analysis, 22 haemoglobin results from the HemoCue*

The incidence of overt iron deficiency anaemia (IDA) in this cohort of preterm infants was 16.4% at four months after discharge (Table 4.3). An additional 6.6% of preterm infants in this study were also classified as iron deficient as they had a serum ferritin below 12 µg/L and/or a sTfR greater than 2.4 mg/L (Table 4.3).

Table 4.3*: Incidence of Iron Deficiency and Iron Deficiency Anaemia in Preterm Infants at Four Months after Discharge*

	Iron Deficiency Anaemia[a]	**Iron Deficiency[b]**	**Optimal Iron Status[c]**	**Iron Overload[d]**
n (%)	10 (16.4)	4 (6.6)	47 (77.0)	0 (0)

[a] *Defined as haemoglobin <110 g/L, serum ferritin <12 µg/L and/or sTfR>2.4 mg/L;*
[b] *Defined as haemoglobin >110 g/L, serum ferritin <12 µg/L and/or sTfR>2.4 mg/L;*
[c] *Defined as haemoglobin>110 g/L, serum ferritin >12 µg/L and sTfR<2.4 mg/L;*
[d] *Defined as serum ferritin >300 µg/L*

4.3. Iron Supplementation and Iron Status

4.3.1. Characteristics of Supplementation Group vs Non-Supplementation Group

Of the 61 infants included in this study, a total of 30 infants received iron supplements when discharged home (Table 4.4). All infants who were born before 32 weeks gestation and/or with a birth weight of 1800 g or less were prescribed iron

supplements after discharge. Preterm infants receiving supplements at discharge were born significantly more preterm (32+5 [30+0, 34+3] weeks vs 35+3 [34+3, 36+5] weeks; *U*= 138, *P*<0.001 (2-tailed), large sized effect r=0.60) and weighed significantly less at birth (1.58±0.48 kg vs 2.54±0.40 kg; *t*=-8.457, 59 df, *P*<0.001 (2-tailed), large sized effect r= 0.74) than those who did not receive supplements. There was also a significant difference in gender, birth length, head circumference and chronological age at home visit between infants who received iron supplements after discharge and those who did not (Table 4.4). There was, however, no significant difference in iron supplementation between singleton born preterm infants and those who were twins (*P*=0.53). There was also no statistically significant difference in the corrected age at home visit or time in days between discharge and home visit.

Table 4.4: *Characteristics of Preterm Infants Who Received Iron Supplements after Discharge Compared to Preterm Infants Who Did Not*

Characteristics	Infants who received supplements after discharge n= 30	Infants who did not receive supplements after discharge n= 31	*P* value
Gender n (%)			
Males	12 (40)	23 (74.2)	
Females	18 (60)	8 (25.8)	0.007*
Gestational Age (weeks+days)			
Median [25th, 75th percentile]	32+5 [30+0, 34+3]	35+3 [34+3, 36+5]	<0.001**
≤32 weeks n (%)	14 (46.7)	0 (0)	
>32 weeks n (%)	16 (53.3)	31 (100)	<0.001*
Birth Weight (kg)			
Mean±SD	1.58±0.48	2.54±0.40	<0.001**
≤1.8kg n (%)	23 (76.7)	0 (0)	
>1.8kg n (%)	7 (23.3)	31 (100)	<0.001*
Length at Birth (cm)			
Mean±SD	41.8±4.12	46.9±3.43	<0.001**
Head Circumference at Birth (cm)			
Median [25th, 75th percentile]	30 [28, 31]	33 [31.5, 34]	<0.001**
Infant Ethnicity n (%)			
European	17 (56.7)	17 (54.8)	
Maori	5 (16.7)	3 (9.68)	
Pacific Island	1 (3.33)	5 (16.1)	
Asian	3 (10.0)	5 (16.1)	
Indian	4 (13.3)	1 (3.23)	
Singleton Birth n (%)	15 (50)	13 (41.9)	
Twin n (%)	15 (50)	18 (58.1)	0.53
Chronological Age at Home Visit (days)			
Median [25th, 75th percentiles]	150 [137, 173]	131 [124,141]	0.001**
Corrected Age at Home Visit (days)			
Mean±SD	98.8±19.0	104±14.3	0.22
Time Since Discharge (days)			
Median [25th, 75th percentiles]	119 [113, 122]	120 [109, 125]	0.44

** Significant difference between groups P<0.05 (Chi Square test); ** Significant difference between groups P<0.05 (Independent t-tests and Mann-Whitney tests)*

4.3.2. Effect of Iron Supplementation on Iron Biomarkers at Four Months after Discharge

The iron status of infants receiving iron supplements after discharge differed significantly from the iron status of those infants who did not at four months after discharge (Table 4.5). Preterm infants who received iron supplements after discharge had significantly higher haemoglobin concentrations (123±8.3 g/L vs 113±9.9 g/L; *t*=3.979, 56 df, *P*=<0.001 (2-tailed), medium sized effect r=0.47), significantly higher serum ferritin concentrations (64.1±23.9 µg/L vs 39.5±24.9 µg/L; *t*=3.526, 47 df, *P*=0.001 (2-tailed), medium sized effect r=0.46) and significantly lower sTfR concentrations (1.5 [1.4, 1.8] mg/L vs 1.9 [1.55, 2.35] mg/L; *U*=145, *P*=0.005 (2-tailed), medium sized effect r=0.41) at four months after discharge than those infants not receiving iron supplements. There was also a significant difference in haematocrit, mean cell volume (MCV), and mean cell haemoglobin (MCH) at four months after discharge. There was, however, no significant difference in red blood cell count.

Table 4.5: *Iron Status of Preterm Term Infants Who Received Iron Supplements after Discharge and Those Who Did Not*

Biomarker	Infants who received supplements after discharge n=30	Infants who did not receive supplements after discharge n=29	*P* value
Haemoglobin (g/L)[a]	123±8.29	113±9.86	<0.001*
Red Blood Cell Count ($x10^6/\mu L$)	4.41±0.33[b]	4.35±0.39[c] n=22	0.60
Haematocrit/PCV	0.34 [0.33, 0.37][b]	0.32 [0.31, 0.33][c]	0.003*
MCV (fL)	78.1±1.93[b]	74.2±4.48[b]	0.001*
MCH (pg/cell)	28.0±0.92[b]	26.5±2.43[b]	0.016*
Serum Ferritin (µg/L)	64.1±23.9	40.2±24.4	0.001*
Soluble Transferrin Receptor (mg/L)	1.5 [1.4, 1.8]	1.9 [1.55, 2.35]	0.005*

*[a] 36 haemoglobin results from laboratory analysis, 22 haemoglobin results from the HemoCue; [b] n=17; [c] n=22; * Significant difference between groups P<0.05 (Independent t-tests, Mann-Whitney test); MCH= Mean Cell Haemoglobin; MCV= Mean Cell Volume; PCV = Packed Cell Volume*

4.3.3. Effect of Iron Supplementation on Optimal vs Suboptimal Iron Status

The incidence and characteristics of preterm infants with suboptimal iron status at four months after discharge was also explored (Table 4.6 and 4.7). Optimal iron status

was defined as a haemoglobin concentration of greater than 110 g/L, a serum ferritin greater than 12 µg/L and a sTfR less than 2.4 mg/L, while suboptimal iron status was classified as at least one of these biomarkers being outside the acceptable reference range. Analysis showed that 23% of the infants in this study had suboptimal iron status at four months after discharge (Table 4.6). Infants born after 32 weeks gestation were significantly more likely to have suboptimal iron status at four months after discharge compared to infants born more preterm (*P*=0.026). There was also a significant difference in iron status between infants who received iron supplements and those who did not, with those infants not receiving iron supplements after discharge 4.95 times more likely to have suboptimal iron status at four months after discharge than those receiving supplements, x^2 (df)=5.599, *P*=0.018. Birth weight, however, did not significantly affect iron status at four months after discharge (Table 4.6).

***Table 4.6**: Incidence and Characteristics of Preterm Infants with Suboptimal Iron Status at Four Months after Discharge*

Characteristics	Optimal Iron Status	Suboptimal Iron Status[a]	P value
Incidence n (%)	47 (77.0)	14 (23.0)	-
Gestational Age			
≤32 weeks n (%)	14 (100)	0 (0)	
>32 weeks n (%)	33 (70.2)	14 (29.8)	0.026*
Birth Weight			
≤1.8kg n (%)	20 (87)	3 (13)	
>1.8kg n (%)	27 (71.2)	11 (28.8)	0.15
Received Iron Supplements			
Yes n (%)	27 (90)	3 (10)	
No n (%)	20 (64.5)	11 (35.5)	0.018**

*[a] Suboptimal iron status defined as haemoglobin <110 g/L and/or serum ferritin <12 µg/L and/or sTfR >2.4 mg/L; * Significant difference between groups P<0.05 (Fisher exact test); ** Significant difference between groups P<0.05 (Chi Square test)*

Subgroup analysis of the iron status of infants born after 32 weeks gestation was also performed (Table 4.7). There was no significant difference between the iron status of moderate to late preterm infants who received supplements and those who did not. There was however a non-significant trend showing an increased risk of having suboptimal iron status if infants born after 32 weeks gestational age did not receive iron supplements after discharge (35.5% vs 18.7%).

Table 4.7: *Incidence of Suboptimal Iron Status at Four Months after Discharge in Preterm Infants Born After 32 Weeks Gestation Receiving Iron Supplements Compared to Those Not Receiving Supplements.*

Characteristics	Optimal Iron Status	Suboptimal Iron Status[a]	*P* value
Received Iron Supplements after Discharge			
Yes n (%)	13 (81.3)	3 (18.7)	
No n (%)	20 (64.5)	11 (35.5)	0.32

[a] *Suboptimal iron status defined as haemoglobin <110 g/L and/or serum ferritin <12 µg/L and/or sTfR >2.4 mg/L, * Significant difference between groups P<0.05 (Fisher's Exact test)*

4.3.4. Iron Supplement Compliance at Four Months after Discharge

Of the 30 infants who received iron supplements after discharge, only five infants had stopped taking them at the time of the home visit. Reasons given for stopping iron supplementation were that solids had already been introduced (n=3) and that the mother had not sought a second prescription after running out of supplements (n=2). Of the 25 infants who were still taking the iron supplements at four months after discharge, 11 infants were receiving supplements every day, 12 were receiving supplements most days per week (4-6 days/week) and 2 were occasionally receiving the supplements (1-3 times/week). Mothers reported that the barriers to giving iron supplements every day were that the iron supplement caused stains (n=1), constipation (n=3), worsened reflux (n=2), difficulty remembering (n=14) and that the infant disliked the taste (n=5).

4.4. Feeding Practices after Discharge and Iron Status

4.4.1. Effect of Breastfeeding vs Formula Feeding on Iron Status at Four Months after Discharge

At discharge from hospital, 43 (70.5%) infants were exclusively breastfed, 15 (24.6%) received a combination of formula and breast milk, and three (4.9%) received only formula. Breastfeeding rates, however, declined significantly by four months after discharge. At the time of the home visit only 27 (45%) infants were breastfed, 13 (21.7%) received a combination of formula and breast milk, and 20 (33.3%) were exclusively formula fed.

The effect of breastfeeding and formula feeding on infant iron status was also explored, whilst controlling for iron supplementations (Table 4.8). Two-way ANOVAs revealed that there was a significant main effect of iron supplementation after discharge on haemoglobin concentration (*P*=0.003) and serum ferritin (*P*=0.001) at four months after discharge. There was however no interaction between iron supplementation and mode of feeding after discharge in respect to haemoglobin (*P*=0.348) and serum ferritin concentrations (*P*=0.358) at four months after discharge. Further statistical analysis showed that there were no differences in haemoglobin, serum ferritin, or sTfR concentrations between infants who were breast fed and predominately formula fed in either the supplementation or the non-supplementation groups (Table 4.8). No infants were consuming post-discharge infant formula at four months after discharge which contains a higher concentration of iron than standard infant formula.

***Table 4.8**: Combined Effect of Iron Supplementation and Feeding Method on Preterm Infant Iron Status at Four Months after Discharge.*

Biomarker	**Infants who received supplements upon discharge**			**Infants who did not receive supplements upon discharge**		
	Breast Fed n=10	Predominately Formula Fed n=20	*P* value	Breast Fed n=17	Predominately Formula Fed n=13	*P* value
Haemoglobin (g/L)	125±7.65	122±8.53	0.31	112±11.7	115±6.58	0.33
Serum ferritin (µg/L)	73.6±32.5	59.5±17.0	0.16	43.3±27.1	36.4±20.8	0.52
Soluble Transferrin Receptor (mg/L)	1.5 [1.4, 1.6]	1.7 [1.4, 1.8]	0.19	1.85 [1.6, 2.6]	1.9 [1.6, 2.0]	0.49

** Significant difference between groups P<0.05 (Independent t-tests, Mann-Whitney Test)*

There was, however, a significant difference between the infants who received supplements and those who did not. The infants who were breastfed and received iron supplements after discharge had significantly higher haemoglobin concentrations (*P*=0.005, large sized effect r=0.54), higher serum ferritin concentrations (*P*=0.026, medium sized effect r=0.46), and lower sTfR concentrations (*P*=0.008, large sized effect r=0.58) than those who did not receive supplements. Likewise, infants who were

predominately formula fed and received iron supplements after discharge had significantly higher haemoglobin concentrations (*P*=0.046, medium sized effect r=0.36), higher serum ferritin concentrations (*P*=0.007, large sized effect r=0.52), and lower sTfR concentration (*P*=0.045, medium sized effect r=0.41) than those who did not receive supplements.

4.4.2. Introduction of Solids and Iron Status at Four Months after Discharge

The introduction of solids was also explored as part of this study. Twenty six percent of infants in this study had been introduced to solids by four months after discharge; however, none were firmly established on a solid diet. The mean corrected age that solids were introduced was 2 months and 19 days (2.69±1.20 months) and ranged from zero months to four months corrected age. The mean chronological age that solids were introduced was 3 months and 24 days (3.84±1.63 months). No infants in this study had been introduced to meat, poultry or seafood by four months after discharge. In addition no infants had been introduced to cow's milk as a drink by four months after discharge. All 16 infants who were consuming solids at four months after discharge were consuming iron-fortified cereals at least once daily. Statistical analysis of the data found that whether solids had been introduced or not had no effect on any of the iron biomarkers at four months after discharge (haemoglobin *P*=0.92, serum ferritin *P*=0.24, sTfR *P*=1.0).

4.5. Pre-Discharge Characteristics and Iron Status

Of the 53 infants who had their haemoglobin measured at birth, all were well above the cut-off for anaemia, with the mean haemoglobin at birth being 173±25.0 g/L and ranging from 125 g/L to 224 g/L. While the majority of infants had their haemoglobin measured at birth, only 33 had a repeated haemoglobin measurement taken before discharge from Auckland City Hospital. The mean haemoglobin concentration at discharge was 144±35.0 g/L and ranged from 90-227 g/L. Seven infants were discharged with anaemia (haemoglobin <110 g/L). Six of these infants received iron supplements whilst in the NICU or upon discharge. The infant not given iron supplements had a gestational age of 35+3 weeks and a birth weight of 2.11 kg.

However, despite being discharged home anaemic, his iron status was adequate at four months after discharge.

In addition, statistical analysis found that there was no relationship between being born with intrauterine growth restriction (IUGR) and haemoglobin (*P*=0.75), serum ferritin (*P*=0.90) or sTfR (*P*=0.60) concentrations at four months after discharge. There were also four infants in this study who received erythrocyte transfusions whilst in the Auckland City Hospital NICU; however, due to the small sample size, correlations were not performed.

4.6. Maternal Characteristics and Iron Status

4.6.1. Maternal Characteristics

A total of 47 mothers were included in this study. The women had a mean age of 34±5.06 years and approximately 63.8% of them most associated with being European, with a much smaller percentage most associating with the other ethnicities (Maori, Pacific Island, Asian and Indian). Just over half of the women in this study had no previous births (53.2%) and all women reported they had not smoked during their pregnancy. Approximately forty nine percent (48.9%) of the women reported that they had experienced no complications during their pregnancy, 36.2% reported they had experienced pre-eclampsia or hypertension, 6.4% said they had diabetes or gestational diabetes during their pregnancy and a further 8.5% reported they experienced some other maternal complication.

4.6.2. Effect of Maternal Iron Status on Infant Iron Status at Four Months after Discharge

Of the mothers who were breastfeeding, three were taking iron supplements, seven were taking Elevit, two taking Blackmores Pregnancy and Breastfeeding supplement, and four taking a women's multi-vitamin at the time of the home visit. In total 51.6% of the breastfeeding mothers in this study were taking a supplement containing iron at the time of the home visit .

Table 4.9 details the relationship between the iron status of breast feeding mothers and the iron status of preterm infants at four months after discharge. Of the 31 mothers who were breastfeeding at the time of the home visit, only one mother was diagnosed with ID and none had IDA. Although none of the 31 mothers reported that they had IDA at the time of their infant's birth, 13 did report that they had been diagnosed with anaemia just prior to or during their pregnancy. Bivariate correlation showed that there was no correlation between maternal iron status and infant iron status at four months after discharge.

Table 4.9: *Correlation Coefficients Showing the Relationship between Maternal Iron Status and the Iron Status of Preterm Infants at Four Months after Discharge.*

Characteristics	**Infant Haemoglobin**		**Infant Serum Ferritin**		**Infant Soluble Transferrin Receptor**	
	r	*P*	R	*P*	s	*P*
Maternal Haemoglobin	-0.312	0.06	-0.245	0.16	0.171[a]	0.35[a]

[a] *Non-Parametric; * Significant difference between groups P<0.05 (Bivariate correlations).*

4.7. Iron Status and Infant Growth

Analysis of the data found that there was no relationship between iron status at four months after discharge and any of the growth parameters (Table 4.10). There was also no effect of iron supplementation on growth between birth and home visit (Table 4.11).

Table 4.10: *Relationship between Iron Status at Four Months after Discharge and Changes in Growth Z-Scores between Birth and Home Visit.*

Growth Parameters	**Optimal Iron Status n=47**	**Suboptimal Iron Status[a] n=14**	***P* value**
Weight Z-Score Mean±SD	-0.12±1.47	0.40±0.88	0.22
Length Z-Score Mean±SD	-0.03±1.61	0.22±2.19	0.65
Head Circumference Z-Score Mean±SD	-0.28±1.00	-0.66±1.52	0.27

[a] *Suboptimal iron status defined as haemoglobin <110 g/L, and/or serum ferritin <12 µg/L and/or sTfR >2.4 mg/L*

***Table 4.11**: Relationship between Iron Supplementation after Discharge and Changes in Growth Z-Scores between Birth and Home Visit.*

Growth Parameters	**Infants who received supplements after discharge n= 30**	**Infants who did not receive supplements after discharge n= 31**	***P* value**
Weight Z-Score Mean±SD	-0.09±1.68	0.08±1.05	0.64
Length Z-Score Mean±SD	0.06±2.13	-0.00±1.29	0.89
Head Circumference Z-Score Mean±SD	0.47±1.12	-0.26±1.17	0.48

5. Discussion

The aim of this study was to investigate the iron status of preterm infants in Auckland, New Zealand at four months after discharge from hospital. As previously mentioned, four months post-discharge was chosen as an appropriate time point to assess infant iron status as red blood cells have a life span of approximately 120 days and any reductions in haemoglobin levels as a result of iron depletion could take up to three months to be detected (Ullrich et al., 2005). In addition, a second prescription for iron supplements must be sought at around three months post-discharge; with compliance in supplementation reported to decrease after this point. The decision to conduct home visits was based on the fact that it was more convenient for the parents and prevented the infants having to return to hospital where they may have been exposed to pathogens. To our knowledge this is one of only a few studies to explore the iron status of preterm infants living in New Zealand especially with regards to the relationship between iron status and current iron supplementation practices

5.1. Study Population Characteristics

The study population was recruited through Auckland City Hospital between February and August 2013. In total 61 preterm infants were included in this study, of which 28 were from singleton births and 33 from twin births. There were an uneven number of twin infants in this study as three were excluded from final data analysis. All infants in this study were born preterm, with gestational ages ranging from 24+2 to 36+6 weeks. The majority of the infants recruited into this study were born after 32 weeks gestation (77%). The mean±SD birth weight of this study population was 2.07±0.65 kg which was significantly lower than the recorded median [25^{th}, 75^{th} percentile] birth weight of infants born in Auckland, New Zealand (3.42 [3.13-3.7] kg) (Pot et al., 2012). This reflects part of the clinical picture of these preterm infants and the effect of their shortened gestational length on in utero growth.

5.2. Iron Status of Preterm Infants at Four Months after Discharge

5.2.1. Iron Status at Four Months after Discharge

Fourteen (23%) preterm infants in this study had suboptimal iron status at four months after discharge, of which ten (16.4%) were diagnosed with iron deficiency anaemia

(IDA). Interestingly, no infants had iron overload (defined as a serum ferritin greater than 300 µg/L) at four months after discharge despite four infants receiving erythrocyte transfusions whilst in the Neonatal Intensive Care Unit (NICU) (between one and seven erythrocyte transfusions) as well as iron supplements after discharge.

5.2.2. Biomarkers of Iron Status

While the optimal method of assessing iron status is unclear; complicated by the existence of multiple biomarkers; current evidence suggests that a number of different iron indices should be measured to predict iron status (Clark, 2009). A combination of three biomarkers were used in this study to determine iron status at four months after discharge; haemoglobin, serum ferritin and soluble transferrin receptor (sTfR).

5.2.2.1. Biomarker of Anaemia

Haemoglobin was used as the key marker of anaemia in this study. Two methods were used in this study to determine haemoglobin concentration at four months after discharge. The primary method for determining infant haemoglobin concentrations was laboratory analysis of capillary blood samples. While this was the more accurate method of determining infant haemoglobin concentrations, and was always reported in preference when results from both methods were collected, it was not always possible to collect a haemoglobin sample for laboratory analysis. This is due to the practical issues associated with paediatric capillary blood collection such as infant distress and poor blood flow.

To ensure that a haemoglobin concentration was available for each infant, the HemoCue Hb 201+ Analyzer was also utilised to measure infant haemoglobin during the home visit. Recent studies have validated the use of the HemoCue in infant populations as an accurate and convenient alternative to traditional laboratory analysis. A study published by Rechner, Twigg, Davies, and Imong, (2002) showed that in a group of 82 infants there was little difference in the mean haemoglobin concentrations measured by the HemoCue (150.3 g/L) and that measured by traditional laboratory analysis (152.8 g/L). In addition, Nkrumah et al. (2011) found that in children aged 1-4 years there was near perfect correlation in haemoglobin

readings between the HemoCue and traditional laboratory analysis using the Sysmex KX21N (r=0.994). Schapkaitz et al. (2012) also found that in a study of 44 patients aged six months or less that the mean haemoglobin concentration from the HemoCue (118 g/L; range 48-187 g/L) was comparable with the mean from the automated haematology analyser (118 g/L; range 52-192 g/L) with a Bland-Altman difference plot revealing only a small amount of bias between the two methods (0.2%). The small amount of bias of the HemoCue may however cause some infants to be misclassified; therefore the haemoglobin values from the laboratory should always be reported in preference to the HemoCue result if both are available.

A cut-off of <110 g/L was used to define infant IDA in this study. This cut-off has been used in multiple studies exploring the iron status of preterm infants (Friel et al., 2003; Heath et al., 2002, Thom et al., 2003) and is recommended by both the World Health Organisation (WHO) (2011a) and Baker and Greer (2010) as the optimum value to use as a cut-off to diagnose IDA in infants. Other studies have used a slightly lower cut-off of 105 g/L, but employing this cut-off for this study may have resulted in an underestimation of the true prevalence of IDA, especially in such a small sample size (Berglund et al., 2010; Georgieff & Innis, 2005). In addition, while severe IDA is an obvious concern for preterm infants, evidence shows that milder IDA (defined as a haemoglobin of 100-109 g/L) also results in more subtle but still potentially adverse effects with regards to neurodevelopment, especially if IDA occurs during sensitive periods of development (McCann & Ames, 2007; WHO, 2011a).

5.2.2.2. Biomarkers of Iron Deficiency

Two biomarkers were used in this study to determine infant iron deficiency (ID): serum ferritin and sTfR. Although serum ferritin is routinely used in research to diagnose ID, it is affected by infection and inflammation, making it less reliable in preterm infants for which infection and inflammation are common (Amin, Scholer, & Srivastava, 2012; Baker & Greer, 2010; Berglund et al., 2010; Grant et al., 2007a; Heath et al., 2002; Long et al., 2012; Lynch, 2010; Thom et al., 2003; WHO, 2011b). To combat these issues, this study reported C-reactive protien in conjunction with serum ferritin in the aim to eliminate the problems caused by inflammation and infection. A cut-off of 12 µg/L was

used in this study to indicate ID, in line with the recommendations from WHO (WHO, 2011b); although studies in infants have also used 10 µg/L as a cut-off (Grant et al., 2007a; Thom et al., 2003). Like the haemoglobin cut-off, employing the lower cut-off value may have underestimated the true prevalence of ID in this group of infants. Soluble transferrin receptor is a novel biomarker of iron stores which unlike serum ferritin, is not affected by infection and inflammation, possibly making it a more accurate biomarker in preterm infants (Lynch, 2010). It is however not yet validated in preterm infants and standardised cut-offs are not well defined in paediatric populations (Baker & Greer, 2010).

Whilst the two biomarkers were both used to diagnose ID, there was a drastic difference in this study between the rates of suboptimal iron status determined by serum ferritin and sTfR. Almost twice as many infants were classified as having suboptimal iron stores using sTfR concentration compared to serum ferritin, despite serum ferritin results being available for 50 infants and sTfR results only being available for 46. Similar findings were reported by Engle-Stone et al. (2013) who found that serum ferritin and sTfR concentrations resulted in different estimates of the national prevalence of ID and IDA among women and children in Cameroon. This is likely due to the fact that the two biomarkers measure very different aspects of iron metabolism. Serum ferritin provides an indication of the actual amount of stored iron in the infant's liver while sTfR gives an indication of tissue iron depletion (Cheng & Juul, 2011; Olivares et al., 2000). Interestingly, Olivares et al. (2000) found that sTfR appeared to be a better indicator of ID than serum ferritin as it correlated better with other biomarkers of iron status. They did however concede that sTfR may not be an adequate iron biomarker on its own due to low sensitivity and high cost. However due to its high specificity, it is satisfactory for confirming the presence of ID alongside other biomarkers (Olivares et al., 2000). Therefore both serum ferritin and sTfR were used to diagnose ID at four months after discharge, which is also in line with the recommendations by Clark (2009) and Grant et al. (2007b).

It should be noted that the optimal method for diagnosing ID and IDA in preterm infants is yet to be defined. The World Health Organisation currently

recommend that haemoglobin, serum ferritin and sTfR should be used to define ID and IDA in paediatric populations, however this is based on term infants and may not translate to preterm infants (Lynch, 2010; WHO, 2010). In addition both Olivares et al. (2000) and Lorenz, Peter, Poets and Franz (2013) reported that all of the iron biomarkers have specific limitations (such as poor sensitivity, poor specificity and that they are affected by infection/ inflammation), which is of particular importance in preterm populations. To compound this issue, cut-off values are not yet well defined for preterm infant populations for haemoglobin, serum ferritin or sTfR, making it difficult to determine the best method of diagnosing ID and IDA (Baker & Greer, 2010; Berglund et al., 2010; Georgieff & Innis, 2005; Grant et al., 2007a; Thom et al., 2003). In addition, as blood collection is often difficult in infants after discharge it is unlikely that more than three biomarkers could be measured to assess iron status.

5.2.3. Preterm Infant Iron Status in the Literature

The prevalence of IDA in this study was similar to the prevalence of IDA reported by Heath et al. (2002) who found that 11% of term infants living in Dunedin, New Zealand had IDA at 9 months of age. The study by Heath et al. (2002) was not however powered to determine the iron status of preterm infants who are thought to have lower haemoglobin concentrations than their term counterparts due to a shortened accretion period, increased utilisation for rapid growth and excessive losses through phlebotomy (Amin, Scholer & Srivastava, 2012; Georgieff & Innis, 2005; Rao & Georgieff, 2007). Studies focusing on the prevalence of IDA in low birth weight (LBW) infants, of which preterm infants make up a significant proportion, found that between 23% and 26.5% of infants had IDA in their first year of life, which is greater than the prevalence reported in this study (Ferri, Procianoy, & Silveira, 2013; Thom et al., 2003). These studies both defined IDA as a haemoglobin <110 g/L in conjuction with low iron stores and thus rates can be compared with the results of this study. The lower prevalence of IDA in this population may reflect the small sample size or perhaps the difference in iron supplementation practices between District Health Boards and countries, as a standardised recommendation is yet to be adopted.

The prevalence of suboptimal iron stores reported in this study was also lower than the prevalence detailed by other studies conducted in New Zealand. Grant et al. (2007a) reported that of 21 preterm infants living in the Auckland region aged six to 23 months, 30% had ID. An even higher prevalence of ID was reported in a cohort of LBW infants living in Dunedin at nine months of age, where 47% were diagnosed with suboptimal iron stores (Thom et al., 2003). Similar rates of ID were reported in international studies exploring the iron status of LBW preterm infants (40 and 48%) (Ferri et al., 2013; Franz et al., 2000). It should also be noted that the studies by Ferri et al. (2013), Grant et al. (2007a), and Thom et al. (2003) all defined ID as a serum ferritin of <10 μg/L, meaning that rates of ID are likely to have been even higher in these studies if the cut-off of 12 μg/L was used.

It was encouraging that no infants in this study had iron overload at four months after discharge despite previous studies reporting that approximately 19% of preterm infants have iron overload prior to discharge from the NICU (Amin, Scholer & Srivastava, 2012). This suggests that the current dose of iron is not high enough to result in iron overload even in formula fed infants. It also shows that, with adequate monitoring after discharge, routine iron supplementation of all preterm infants after discharge from hospital has the potential to be a safe and effective way to improve iron status at four months after discharge

5.3. Iron Supplementation and Preterm Infant Iron Status at Four Months after Discharge

5.3.1. Characteristics of Supplementation Group vs Non-Supplementation Group

In total, 30 infants received iron supplements after they were discharged home. As expected, preterm infants receiving supplements after discharge were born significantly smaller and more preterm than those who did not receive supplements. This is in line with the current supplementation protocol at Auckland City Hospital NICU whereby only those infants born less than 32 weeks gestation or less than 1800 g routinely receive iron supplements after discharge, unless clinically indicated (Cormack, 2012). It is encouraging that all infants born at less than 32 weeks gestation and/or with a birth weight less than 1800 g were prescribed iron supplements after

discharge, showing that current supplementation protocols at Auckland City Hospital NICU are being adhered to. It is also pleasing that all infants prescribed iron supplements at discharge from Auckland City Hospital were given them for at least the first three months after discharge.

5.3.2. Effect of Iron Supplementation on Iron Biomarkers at Four Months after Discharge

Preterm infants who received iron supplements after discharge had significantly higher haemoglobin concentrations at four months after discharge than those infants not receiving iron supplements. This is supported by current research which has shown a consistent link between iron supplementation and improved haemoglobin concentrations in preterm and LBW infants. A Cochrane review of 26 trials comparing enteral iron supplementation with minimal supplementation or placebo concluded that iron supplementation significantly increased infant haemoglobin concentrations at three to four months of age (Mills and Davies, 2012). A study not included in this meta-analysis but that supports these results is the double blinded, randomised control trial conducted by Friel et al. (2003) which found that supplementation with 7.5 mg of iron per day significantly increased haemoglobin levels at three and a half and six months. Aggarwal et al. (2005) also reported that LBW infants who received 3 mg/kg/day supplemental iron had higher haemoglobin concentrations from four weeks chronological age than those in the control group.

Infants in this study who received iron supplements after discharge also had significantly higher serum ferritin concentrations and lower sTfR concentrations at four months after discharge than those not receiving supplements. Unlike haemoglobin however, fewer studies have been able to link iron supplementation with increased serum ferritin levels. The Cochrane review by Mills and Davies (2012) found that there was a small benefit in terms of serum ferritin concentrations in favour of the non-supplement group at three to four months of age. In addition Aggarwal et al. (2005) found no benefit of iron supplementation on serum ferritin levels in preterm infants. Conversely, the systematic review by Long et al. (2012), which had less stringent inclusion criteria than the Cochrane review by Mills and Davies, had similar findings to

the results of this study, with the majority of studies included in their review showing a statistically significant increase in serum ferritin concentrations in the supplement groups compared to placebo. In addition, Berglund et al. (2010) found that supplementation of marginally LBW infants with 1-2 mg/kg/day of supplemental iron increased serum ferritin concentrations and reduced sTfR concentrations in a dose response manner, which further supports the findings of this study.

5.3.3. Effect of Iron Supplementation on Optimal vs Suboptimal Iron Status

Data analysis showed that 23% of the infants in this study had suboptimal iron status at four months after discharge; defined as at least one of the three biomarkers being outside the acceptable reference range. Infants born after 32 weeks gestation were significantly more likely to have a suboptimal iron status at four months after discharge compared to more preterm infants. There was also a significant difference in iron status between preterm infants who received iron supplements and those who did not, with those infants not receiving iron supplements after discharge almost five times more likely to have ID or IDA at four months after discharge. Similar results have been reported by Berglund et al. (2010), Long et al. (2012), and Mills and Davies (2012) who all found that iron supplementation was associated with a lower prevalence of ID and IDA in preterm and LBW infants.

While it is encouraging that no infants born less than 32 weeks had ID or IDA at four months after discharge, the iron status of infants not receiving iron supplements highlights that current post-discharge supplementation practices at Auckland City Hospital may be insufficient to meet the needs of moderate to late preterm infants. The current protocols at Auckland City Hospital NICU are based on those produced by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) which recommend that preterm infants born with a birth weight less than 1.8 kg should be routinely supplemented with elemental iron (Agostoni et al., 2009). However the ESPGHAN chose the 1.8 kg cut-off as they believed there was insufficient literature to provide recommendations for infants born with a heavier birth weight (Agostoni et al., 2009). The results of this study suggest that it may be more prudent to adopt the American Academy of Pediatrics (AAP) supplement guidelines who

recommend that all preterm infants (regardless of their gestational age and birth weight) should receive supplemental iron starting by one month and extending through to 12 months of age, especially if they are breastfed after discharge (Baker & Greer, 2010; Kleinman, 2009). The only group of preterm infants for which routine iron supplementation is not encouraged is preterm infants who receive multiple erythrocyte transfusions in the Neonatal Intensive Care Unit (NICU) (Baker & Greer, 2010; Kleinman, 2009).

Subgroup analysis of the results revealed that 16 infants born after 32 weeks gestation received iron supplements after discharge. While these infants are not routinely discharged home from Auckland City Hospital with iron supplements, moderate to late preterm infants may be prescribed iron supplements after discharge if they are found to have ID or IDA whilst in the hospital or if their family doctor considers them at risk of ID after discharge. Data analysis of this subgroup of infants revealed that there was no significant difference in iron status between infants who received iron supplements after discharge and those who did not. It is likely this study was not adequately powered to determine a difference in iron status in this subgroup. The results, however, showed that there was a non-significant trend towards having improved iron status after discharge for infants born after 32 weeks gestation who received iron supplements (18.7% vs 35.5%).

In addition, it is concerning that 18.7% of infants born after 32 weeks gestation had suboptimal iron status at four months after discharge despite receiving iron supplements. This highlights the fact that there needs to be routine monitoring of iron status after discharge from hospital for all preterm infants regardless of their gestational age and whether they have received iron supplements post-discharge. This could easily be achieved by ensuring that all preterm infants are screened for IDA at their three to four month Plunket visit using the HemoCue. Infants found to be at risk of IDA could then be referred to their family doctor for further investigation. Using the HemoCue would be an easy and cost effective way of screening for IDA and would have the added benefits of being less invasive, more acceptable to parents and would not require a paediatric phlebotomist. The HemoCue does however have its

limitations, primarily that it would not be able to screen for ID. In addition, the HemoCue does have the potential to misclassify infants according to their haemoglobin concentration, and thus a validation study would need to be conducted prior to implementing routine screening of all preterm infants after discharge. However, despite the limitations of the HemoCue and the risk of misclassifying infants, any screening would be an improvement compared to what is currently being offered.

5.3.4. Iron Supplement Compliance at Four Months after Discharge

Parental compliance with administering iron supplements to preterm infants is an understudied area. Auckland City Hospital currently recommend that iron supplements should be continued preferably until 12 months of age or until the infant is well established on a balanced diet of solids (Cormack, 2012). Results of this study showed that of the 30 infants who received iron supplements after discharge, only five infants (16.7%) had stopped taking them at the time of the home visit; none of whom were well-established on solids. While there is a paucity of evidence in this area, it appears that the compliance in this cohort of infants and mothers was fairly high. A study conducted through Waitemata DHB in Auckland found that of 88 preterm infants discharged from Waitemata Hospital between June 2009 and May 2010, only 52.9% (95%CI 44.08 to 61.72) had their iron supplement prescriptions filled after discharge (Mohamed & van den Boom, n.d.).

This study also provided useful insight into the barriers which parents face with regards to giving their infants iron supplements every day. This study found that the most common barrier to regularly giving their infant iron supplements was that the mothers had difficulty remembering. Other barriers were that the supplement caused stains, constipation, worsened reflux and that the infants disliked the taste.

5.4. Infant Feeding Practices after Discharge and Iron Status at Four Months after Discharge

5.4.1. Effect of Breastfeeding vs Formula Feeding on Iron Status at Four Months after Discharge

At discharge from hospital, 70.5% of infants in this study were breastfed. Interestingly, breastfeeding rates at discharge were much lower than those reported in the "Growing Up in New Zealand" study, who reported than 96.3% of infants in the study were exclusively breastfed at hospital discharge (Morton et al., 2012). This may reflect the fact that preterm infants often have difficulty establishing breastfeeding before discharge due to physiological immaturity causing a less coordinated suck, problems staying alert during feeds, and difficulty expressing clear hunger and satiety cues (Buckley & Charles, 2006).

At four months after discharge, when the infants were between 2 months and 14 days and 5 months and 6 days corrected age, the rate of breastfeeding dropped to 44.3%. Similar rates of breastfeeding at four months of age were reported in the "Growing Up in New Zealand" study (47.3%) (Morton et al., 2012). While the reduction in breastfeeding rates after discharge in this study is also reflected in the general infant population, it may also reflect the fact that early discharge of moderate to late preterm infants can reduce the likelihood of continued exclusive breastfeeding as breastfeeding may not have been fully established at time of discharge (Tomashek et al., 2006).

As expected, infants who received supplements after discharge had an improved iron status at four months after discharge; however this was not affected by mode of feeding. The results of this study showed that there was no difference in haemoglobin, serum ferritin, or sTfR concentrations between infants who were breast fed and those who were predominately formula fed in either the supplementation or the non-supplementation groups. It should be noted however that the study may however have been under-powered to determine a significant difference in the iron status between these groups even if it truly existed. These findings are supported by results from the Cochrane Review by Mills and Davies (2012) who concluded that there

was no statistically significant difference in mean haemoglobin concentration between formula-fed (WMD 2.4 g/L; 95% CI -0.42 to 5.2) and breastfed infants (WMD 2.7 g/L; 95% CI -2.7 to 8.1).

Exclusive breastfeeding of preterm infants is a hotly debated topic, with concern that this may predispose infants to ID and IDA within their first year of life (Hall et al., 1993; Olivares et al., 1992; Rao & Georgieff, 2009; Roze et al., 2012). Human breast milk contains approximately 0.5 mg/L of elemental iron, and while it is highly bioavailable, it may not be sufficient to meet the additional iron requirements of preterm infants (Mills & Davies, 2012; Rao & Georgieff, 2007; Rao & Georgieff, 2009). A recent study however by Finkelstein, O'Brien, Abrams and Zavaleta (2013) found that term infants are able to up-regulate iron absorption from breast milk when iron stores are depleted at two and five months of age. This may account for the fact that there was no difference between the iron status of breast fed and predominately formula fed infants at four months after discharge in this study, although the same mechanism is yet to be studied in preterm infants.

Of interest, no infants were discharged home with post-discharge infant formula. Post-discharge infant formula contains additional nutrients for growth compared to regular infant formula, including 12 g/L of iron (Cormack, 2013). Two infants in this study were born at less than 33 weeks gestation and were fully formula fed at time of discharge, thus eligible to receive post-discharge formula if they had poor growth. This is of interest because studies have found that post-discharge formula improves growth parameters in very low birth weight and extremely low birth weight preterm infants (Carver et al., 2001; Lucas, Bishop, King, & Cole, 1992; Lucas et al., 2001).

5.4.2. Introduction of Solids and Iron Status at Four Months after Discharge

Sixteen (26%) infants in this study had been introduced to solids by four months after discharge however none were firmly established on a solid diet. The mean corrected age that solids were introduced was 2 months and 19 days and the mean chronological age was 3 months and 24 days. This is slightly younger than the recommendations by

Palmers and Makrides (2012) who state that solids should be introduced at approximately three months corrected age. Six infants in this study were introduced to solids before three months corrected age, with one infant being introduced to solids at zero months corrected age. Early introduction of solids is generally discouraged as the infant may not be developmentally ready (Morgan et al., 2004; Palmer & Makrides, 2012). In addition, of the 49 infants who were at least three months corrected age at the time of the home visit, 34 had not yet been introduced to solids. Of more concern, two of these infants had not been introduced to solids before seven months of age. Late introduction of solids predisposes an infant to ID and IDA as after about six months of age it becomes increasingly difficult to meet iron requirements from breast milk alone (Kuo, Inkelas, Slusser, Maidenberg & Halfon, 2011; Ministry of Health, 2008). In this study however, whether solids had been introduced or not had no effect on any of the iron biomarkers at four months after discharge. This is possibly because the number of infants having started solids was small and the amount of solids that the infants were consuming on a daily basis was insufficient to significantly affect their iron status, with most infants in this study consuming less than two tablespoons per meal.

Of interest, no infants in this study had been introduced to meat, poultry or seafood by four months after discharge. Studies have shown that intake of meat at approximately 13 weeks chronological age has been associated with improved iron status (Marriott et al., 2003). Current guidelines on feeding the preterm infant suggest that iron rich foods such as pureed or finely minced meat should be one of the first foods introduced to infants (Ministry of Health, 2008).

5.5. Pre-Discharge Characteristics and Iron Status

5.5.1. Iron Status at Birth

All infants who had their iron status measured at birth had adequate haemoglobin concentrations, ranging from 125 g/L to 224 g/L. The results of this study show that even infants born extremely preterm (before 28 weeks gestation) had adequate haemoglobin concentrations at birth (125-155 g/L), although they were lower than the haemoglobin concentrations of less preterm infants. However as serum ferritin or sTfR are not routinely measured in Auckland City Hospital NICU, the iron stores of these

infants could not be assessed at birth and discharge. Current literature states that preterm infants have poorer iron status at birth than term infants as a result of missing out on the critical accretion period during the third trimester (Amin, Scholer & Srivastava, 2012; Long et al., 2012; Rao & Georgieff, 2007). It is possible that these infants had compromised iron stores at birth which were undetected (in line with the literature), however it is promising that none had overt IDA at birth.

5.5.2. Effect of Pre-Discharge Characteristics on Iron Status at Four Months after Discharge

Multiple studies have shown that delayed cord clamping improves infant iron status at birth and in the first year of life by allowing extra transfer of foetal blood from the placenta to the infant (Andersson et al., 2011; Chaparro & Lutter, 2009; Collard, 2009; McDonald, Middleton, Dowswell, & Morris, 2013; Venancio et al., 2008). Unfortunately, the time of cord clamping is not routinely recorded at Auckland City Hospital therefore the effect of cord clamping on infant iron status prior to discharge and at four months after discharge could not be explored.

As only four infants in this study received erythrocyte transfusions within the NICU, correlations unfortunately could not be performed. Current iron supplementation guidelines recommend that infants who receive multiple erythrocyte transfusions should not be routinely supplemented as each transfusion typically supplies the infant with 8 mg/kg of iron, leaving the infant with adequate iron stores for up to six months (Agostoni et al., 2009; Griffin & Cooke, 2010; Kleinman, 2009; Rao & Georgieff, 2009). Arad, Konijn, Linder, Goldstein and Kaufmann (1988) for example found that preterm infants who received more than 100 mL of packed red blood cells whilst in the NICU had significantly higher serum ferritin concentrations at four to five months of age compared to those infants who were transfused with smaller amounts.

The results of this study showed that there was no relationship between intrauterine growth restriction (IUGR) and infant iron status at four months after discharge. This is contrary to research which has shown that being born with IUGR is associated with an increased risk of developing ID and IDA (Siddappa et al., 2007). If

the cause of IUGR is extrinsic to the foetus (maternal or uteroplacental), transfer of oxygen and nutrients (especially iron) to the foetus is decreased (Siddappa et al., 2007). Foetal erythropoiesis is subsequently up-regulated so that more oxygen binding haemoglobin can be synthesised which is at the expense of stored iron (Siddappa et al., 2007). A recent study revealed that approximately 50% of infants born with IUGR have ID at birth (Patidar, Shrivastava, Agrawal, & Dwivedi, 2013). Again, as only nine infants in this study were born with IUGR, it is likely that the study was inadequately powered to determine a difference in iron status at four months after discharge in relation to IUGR, even if a difference truly existed.

5.6. Maternal Characteristics and Iron Status

5.6.1. Maternal Characteristics

Of interest, 36.2% of women in this study reported they had experienced pre-eclampsia or hypertension, 6.4% said they had diabetes or gestational diabetes during their pregnancy and a further 8.5% reported they experienced some other maternal complication during their pregnancy. The high rate of maternal complications is not surprising considering pre-eclampsia and maternal hypertension in particular have been linked to the aetiology of preterm birth (Siddappa et al., 2007). The sample size however was too small to detect whether there was a correlation between these maternal complications and infant iron status at four months after discharge.

5.6.2. Effect of Maternal Iron Status on Infant Iron Status at Four Months after Discharge

As expected, there was no correlation between the iron status of breastfeeding mothers and their infants iron status at four months after discharge. This is due to the fact that no mothers had IDA at the time of the home visit. Iron concentration in breast milk is not affected by ID or mild IDA as regulatory mechanisms in the mammary gland conserve the iron content of breast milk at the expense of maternal stores (Domellof et al., 2004). Once maternal iron stores however are exhausted, breast milk iron concentration begins to decrease. This is reflected in the study by Meinzen-Derr et al. (2006) who found that maternal IDA was independently associated with a three-fold increased risk of infant IDA at six months of life.

5.7. Iron Status and Infant Growth

As expected there was no relationship between iron status and any of the growth parameters. Whether an infant had optimal or suboptimal iron status at four months after discharge had no effect on the changes in weight (*P*=0.22), length (*P*=0.65) or head circumference (*P*=0.27) between birth and home visit. In addition, iron supplementation after discharge had no effect on any of the growth parameters. This is in line with current literature which has consistently shown no link between iron supplementation and any growth related parameters including growth rate, length, head circumference and weight (Aggarwal et al., 2005; Berglund et al., 2010; Friel et al., 2003; Mills & Davies, 2012; Sichieri et al., 2006). It is interesting that there was no relationship between growth in head circumference and iron status or iron supplementation in this study as ID and IDA are associated with poor neurodevelopmental outcomes, of which head circumference can be a surrogate marker (Georgieff & Innis, 2005; Lozoff et al., 2006; Mascheki, Ellenriederi, Hecher, & Bartmann, 2009; Tamura et al., 2002).

6. Conclusions

6.1. Summary of the Study

This study was designed to provide a situational analysis of the iron status of preterm infants in Auckland, New Zealand at four months after discharge from hospital. Sixty one preterm infants born between 1 October 2012 and 30 April 2013 at Auckland City Hospital were included in this study. At four months after discharge, haemoglobin, serum ferritin and soluble transferrin receptor (sTfR) were analysed for each infant to determine their iron status. Information on iron supplementation practices and mode of feeding was also collected using an online questionnaire to determine their effect on infant iron status at four months after discharge. The iron status of breast feeding mothers was also assessed at this time point. Growth between birth and appointment was also determined and compared with iron status and supplement use at four months after discharge. Statistical analysis using independent t-tests, Mann-Whitney tests, Chi Square tests, and bivariate correlations were performed. A *P* value of <0.05 was considered statistically significant.

The primary objective of this study was to describe the iron status of preterm infants in Auckland, New Zealand at four months after discharge. Results of this study suggest that while the rates of iron deficiency (ID) and iron deficiency anaemia (IDA) are lower compared to those in previous studies of preterm infants, a substantial proportion of infants in this study had suboptimal iron status (23%) at four months after discharge. No infant in this study had iron overload despite four infants receiving erythrocyte transfusions in the Neonatal Intensive Care Unit (NICU) and iron supplements after discharge. The alternative hypothesis (H_1) that preterm infants in Auckland, New Zealand have a lower than recommended iron status was therefore accepted.

The second objective of this study was to compare iron status at four months after discharge between preterm infants who received iron supplements and those who did not. Infants who received iron supplements after discharge had significantly higher haemoglobin and serum ferritin concentrations along with lower sTfR concentrations at four months after discharge. The risk of ID or IDA at four months

after discharge was also significantly increased if an infant did not receive iron supplements after discharge (*P*=0.018). As only infants who are born at less than 32 weeks gestation and/or less than 1800 g routinely receive iron supplements after discharge, this meant infants born after 32 weeks gestation were at a higher risk of developing ID and IDA (0% vs 29.8%). Some moderate to late preterm infants received iron supplements after discharge which appeared to a certain degree to protect against ID and IDA (18.7% vs 35.5%), although it was not statistically significant. The alternative hypothesis that iron supplementation improves infant iron status at four months after discharge was subsequently accepted.

The third objective of this study was to compare the iron status between preterm infants who had been breast fed until at least four months after discharge and preterm infants who had been predominately formula fed. The study found that there was no difference in iron status between infants who were breastfed and predominately formula fed after controlling for supplement usage. The null hypothesis was therefore accepted.

The fourth objective of this study was to assess the effect of pre- and post-discharge factors and feeding practices on iron status in preterm infants at four months after discharge. The study concluded that there was no relationship between intrauterine growth restriction (IUGR) and iron status at four months after discharge. There was also no relationship between maternal haemoglobin of breastfeeding mothers and infant iron status. The introduction of solids also had no effect on infant iron status at four months after discharge, possibly because iron rich meats had not been introduced and because the infants were only consuming small amounts of solids at the time of this study. Unfortunately the sample size was inadequately powered to determine the effect of erythrocyte transfusions on infant iron status at four months after discharge. The null hypothesis again accepted.

The final objective was to assess the relationship between iron status and growth in preterm infants at four months after discharge. There was no relationship between iron status at four months after discharge or iron supplementation after

discharge and any growth parameters. Growth was determined by subtracting weight z-scores, length z-scores and head circumference z-scores at birth by the corresponding z-scores at the home visit. Z-scores were calculated whilst controlling for gestational age, corrected age at the home visit, and gender. The null hypothesis was therefore again accepted.

6.2. Conclusion

Results of this study suggest that changes to Auckland City Hospital NICU iron supplementation protocols should be made. Routine supplementation of all preterm infants after discharge with 2-3 mg/kg/day supplemental iron should be considered, with the exception of infants who have received multiple erythrocyte transfusions whilst in the NICU. This is in line with current recommendations from the American Academy of Pediatrics (Kleinman, 2009).

In addition to routine supplementation of all preterm infants after discharge, there should be routine screening of iron status at three to four months corrected age. This study showed that even infants who received iron supplements after discharge were at risk of suboptimal iron status at four months after discharge. As red blood cells have a lifespan of approximately 120 days, IDA is unlikely to be detected until at least three months post-discharge, making three to four months post-discharge an appropriate time point for screening (Ullrich et al., 2005). In addition, this would tie in with the current support offered by Plunket who provide free home and clinic visits for all infants at three to four months of age. Utilising the HemoCue would be an inexpensive and easy way of screening preterm infants for IDA after discharge. Infants who had suboptimal haemoglobin concentrations according to the HemoCue could then be referred to their family doctor for further investigations.

This study also highlighted that many parents of preterm infants are waiting too long to introduce solids, in particular, iron rich meats. At the three to four month Plunket visit, parents should be educated about the appropriate age to introduce solids (at approximately three months corrected age and before seven months

chronological age), with greater emphasis placed on the introduction of iron rich solids (Palmers & Makrides, 2012).

6.3. Strengths

At present there is a paucity of evidence on the iron status of moderate to late preterm infants (Berglund et al., 2010; Tomashek et al., 2006). This study provides valuable insight into the iron status of this population with 77% of this cohort of infants being born after 32 weeks gestation. As these infants do not routinely receive iron supplements after discharge and are the least likely to receive erythrocyte transfusions whilst in the NICU, they are the ones most at risk of developing ID and IDA. Although this study was inadequately powered to perform comparisons between the iron status of infants born with differing degrees of prematurity, having a larger proportion of infants born after 32 weeks gestation allowed for good subgroup analysis. In addition, as infants born after 32 weeks gestation make up the majority of preterm infants born in New Zealand each year, the results of this study can be extrapolated to other hospitals around New Zealand (National Maternity Monitoring Group, 2013). Many hospitals in New Zealand only treat infants born after 32 weeks gestation as they do not offer tertiary level care; making the results of this study particularly pertinent to them.

It is well known that non-compliance with oral iron medication is common amongst paediatric populations, especially as the infants do not like the taste (Grant et al., 2007b). Grant et al. (2007b) reported that adherence to daily medication for several months is difficult, however did not report rates of compliance. To our knowledge, this is the only study to report the rates of non-compliance after discharge in New Zealand preterm infants as well as reasons that parents stop administering the supplement to their infants. This gives health professionals insights into barriers which parents may face and misconceptions they may have with regards to iron supplementation, and allow them to tailor their advice accordingly.

A further strength of the study was the use of the online questionnaire programme. To ascertain information about supplement usage and feeding practices,

parents of the participants were asked to complete an online questionnaire prior to their home visit. This standardised the way in which the questionnaires were delivered, aiding to remove potential interviewer bias.

A fourth strength of this study was that a point-of-care device was used to help determine the haemoglobin concentration of the infants in this study. Despite the previously mentioned limitations, using the HemoCue ensured that a haemoglobin result could be obtained for almost every infant. Blood collection can be difficult in infants due to distress and poor blood flow, which is highlighted by the fact that only 36 haemoglobin concentrations could be obtained from laboratory analysis. The HemoCue is advantageous in paediatric populations as it requires only 10 µL of blood compared to at least 250 µL required for traditional assessment of haemoglobin (Nkrumah et al., 2011; Schapkaitz, Mahlangu, Letsoalo, 2012).

Another strength of this study was that a trained paediatric phlebotomist was employed to collect the infant blood samples. As blood collection from infants can be difficult, it was important that a trained phlebotomist was available to take the bloods. The phlebotomist also trained with the phlebotomists at Auckland City Hospital to ensure that his technique mimicked that used in the NICU and to optimize blood collection.

The final strength of this study was in its design. Most previous studies have looked at the iron status of preterm infants at a particular time point based on their corrected or chronological age (Aggarwal et al., 2005; Berglund et al., 2010; Franz et al., 2000; Friel et al., 2003; Sankar et al., 2009). This study however opted to assess infant iron status at four months after discharge. Using time since discharge as the time point for assessment has more clinical significance than corrected or chronological age when determining the effect of post-discharge factors on iron status. This is because not all infants are discharged at 40 weeks gestation; some may be discharged before this while more unwell preterm infants may have much longer stays in the NICU.

6.4. Limitations

For the study to be adequately powered to determine iron status based on a difference in mean haemoglobin of at least 5 g/L, 76 preterm infants needed to be recruited. As only 61 infants were included in the final data analysis, the study may lack power to accurately determine a difference in iron status between preterm infants living in Auckland at four months after discharge. There is little available information on the nutritional status of preterm infants after discharge, and determination of the sample size was difficult. The power calculation was based on just one study investigating the iron status of low birth weight infants in New Zealand, and therefore may not be completely accurate (Thom et al., 2003). Our study does however provide valuable insight into the post-discharge iron status and feeding and supplementation practices of preterm infants living in New Zealand and is a basis for larger studies to be conducted.

Another limitation of this study was that medical records from other hospitals could not be accessed. Seventeen infants were transferred to hospitals other than Auckland City Hospital for continued care prior to discharge home. In addition, infants may have been readmitted to hospitals other than Auckland City Hospital after initial discharge. Information about erythrocyte transfusions, iron supplementation and other procedures which may have affected infant iron status at these other hospitals could not be collected. Having access to medical records from Counties Manukau District Health Board and Waitemata District Health Board would have been beneficial and allowed for more accurate determination of factors which may affect iron status at four months after discharge. Conducting a multi-centre study could also provide a solution to this problem.

While it was a strength of this study that the cohort consisted mostly of moderate to late preterm infants, as only 23% of the infants were born at less than 32 weeks gestation, the study was inadequately powered to perform comparisons between the iron status of infants born with differing degrees of prematurity. As more moderate to late preterm infants are born each year compared to very and extremely preterm infants (592 compared to 228) it is not surprising fewer infants born before 32

weeks gestation were recruited (Pot et al., 2012). As there was only a short period of time for recruitment, it made it difficult to recruit a large enough sample size of infants born before 32 weeks gestation. Running the study over a longer period of time, focusing on a longer recruitment period, would have meant that more infants born very and extremely preterm could have been recruited into this study, ensuring a larger overall sample size and enabling sub-group comparisons to be made.

Although the aim of this study was to present a situational analysis of preterm infant iron status at four months after discharge, infants who were discharged more than five months prior to appointment were also included in statistical analysis (n=3). This highlights the difficulties associated with conducting home visits as part of scientific research as appointment dates needed to be convenient for the families involved in the study.

To determine the iron status of each infant, capillary blood samples were taken via a heel prick. This method of collection was chosen in preference to venous blood collection due to limitations in resources and to avoid subjecting the infants to unnecessary invasive procedures. The literature however shows that the concentration of iron biomarkers determined using capillary samples is less precise than the corresponding concentrations from venous blood samples due to the fact that interstitial fluid may dilute capillary samples (Gibson, 2005). The results of this study may therefore not accurately reflect the real prevalence of ID and IDA in preterm infants; although the effect of biomarker dilution is likely to be small.

Finally, there were limitations with regards to determining iron status at four months after discharge. In order for the iron status of an individual to be assessed, at least two biomarkers must be reported; one reflecting iron stores and one reflecting red blood cell indices (Lynch, 2010). Despite using different methods to determine iron status, including a point-of-care device for detecting anaemia, a full set of results were not available for all infants in this study. Iron status could only be described for 48 infants in this study, with three infants only having biomarkers reflecting their iron stores and ten infants having only haemoglobin concentrations. This means that there

may have been infants who were classified as having ID whereas in fact they actually had IDA. In addition, it is possible that the infants who only had haemoglobin concentrations may have had ID which could not have been detected, therefore underestimating the rate of ID in this group.

Despite the limitations of this study it is clear that current post-discharge iron supplementation protocols should be addressed. This study has shown that preterm infants who are discharged home from hospital without iron supplements have an increased risk of developing ID and IDA within the first four months after discharge. This is of concern as a suboptimal iron status early in life has been linked with gastrointestinal disturbances, immune and thyroid dysfunction, and temperature instability (Collard, 2009; Rao & Georgieff, 2009). The most devastating effects of ID and IDA in preterm infants are on neurodevelopment and cognition, with ID and IDA associated with poorer recognition memory, altered motor movements and learning difficulties later in life (Georgieff & Innis, 2005; Siddappa et al., 2004; Tamura et al., 2002). Routine iron supplementation for all preterm infants regardless of their gestational age or mode of feeding (but with the exception of those receiving multiple erythrocyte transfusions) appears to be a safe and effective way to reduce the risk of ID and IDA at four months after discharge.

6.5. Recommendations for Future Studies

1. To determine the iron status of preterm infants at four months after discharge in the whole of New Zealand by conducting a multi-centred study. This would ensure that a larger sample size could be recruited and allow for comparisons to be made between the iron statuses of preterm infants born in different District Health Boards, who may have different supplementation protocols.
2. Conduct a randomised control trial to determine the effect of iron supplementation on the iron status of infants born after 32 weeks gestation.
3. Investigate the optimal dose and duration of iron supplementation in preterm infants.

4. Future research should also examine common practices with regards to the introduction of solids to preterm infants, focusing in particular on the introduction of iron rich meat.

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Appendix A

Study Poster for Auckland City Hospital Neonatal Intensive Care Unit Staff

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after discharge

AIM OF THE STUDY

The aim of this study is to investigate the iron and vitamin D status of preterm babies living in Auckland at 4 months post hospital discharge and at 1 year corrected age.

WHY IS THIS STUDY IMPORTANT?

- Preterm birth is associated with nutrient deficiencies and poor growth.
- Preterm babies are at a high risk of developing deficiencies in fat soluble vitamins (A, D and E) and iron.
- Not having enough iron and vitamins during the babies first year of life can cause poor physical growth, gastrointestinal disturbances, reduced immunity, and short and long term neurodevelopmental impairments and cognitive abnormalities.
- Following discharge from Auckland City Hospital, the iron and vitamin D status of these babies is not normally assessed.
- It is not known what percentage of these babies are deficient in one or both of these nutrients.
- Therefore it is not currently known whether feeding and supplementation practices meet the nutrient requirements of these infants.

WHY PARENTS SHOULD ENROL THEIR BABIES IN THIS STUDY

- Parents would find out about their babies iron and vitamin D status at 4 months after leaving hospital and at one year corrected age.
- Knowing this could prevent deficiencies going untreated and long lasting negative health effects occurring.
- This study will also help in the future treatment of preterm babies, if it is found that a large number of these babies are deficient in these nutrients, routine

screening and/or supplementation of all preterm babies after discharge can be implemented.

HOW YOU CAN HELP US

You can help us by talking to parents about this study, at a time that you feel is appropriate, and by discussing with them the benefits they will experience if they enrol their baby in this study. If you have any further questions, please do not hesitate contacting one of the key researchers on charlottefmoor@gmail.com or briaremmett@yahoo.com.

Appendix B

Contact Letter

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Dear Parent or Caregiver,

We are writing to you because your baby was born preterm (before 37 completed weeks of gestation).

Preterm babies are at risk of having iron and fat soluble vitamin deficiencies. Preterm babies are born too soon, which can mean they have not had enough time to develop adequate nutrient stores. These nutrients all have important roles within the body and are needed for the normal growth, health and well being of your baby. Preterm babies are particularly at risk of having iron and vitamin D deficiencies. We would therefore like to find out whether the current feeding and supplementation practices are enough to stop deficiencies developing in these babies born preterm. From this study you will find out if your baby is deficient in any of these nutrients.

We are interested in investigating nutrient stores in any baby born preterm, including those with varying degrees of immaturity (from those babies born extremely preterm to those who are only just preterm). The study is being run through Massey University in collaboration with Auckland District Health Board and specialist health professionals from the Neonatal Intensive Care Unit. If your baby was born before 37 weeks gestation and you live in the Auckland area you may qualify to take part in this study. The aim of the study is to look at the nutrient status and feeding practices of preterm babies after they have been discharged from hospital.

If you think you might be interested in this study, please complete the sheet below and leave at reception OR contact Cath Conlon (PhD) on:

c.conlon@massey.ac.nz

OR 09 414 0800 extension 41206

OR TXT 0211730428

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 13/06. If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 84459, email humanethicsoutha@massey.ac.nz.

Appendix C

Information Sheet

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

INFORMATION SHEET

You have been sent or given the information sheet about this research study because your baby was born preterm (before 37 weeks' gestation) and we would like to invite you to take part in a study looking at the nutrition of preterm babies after hospital discharge. Thank you for taking the time to think about enrolling your baby in this study.

About the researchers

We are a group of researchers from Massey University in Albany and the Neonatal Intensive Care Unit (NICU) at Auckland Hospital. Our research team includes Cath Conlon (PhD) and Pamela von Hurst (PhD) (Massey University), Professor Frank Bloomfield (Professor of Neonatology and Specialist neonatologist, National Women's Health, Auckland City Hospital), Barbara Cormack (Neonatal Dietitian, Auckland City Hospital), Briar Emmett and Charlotte Moor who are both doing their MSc in Nutrition and Dietetics at Massey University.

Project Description and Invitation

Feeding preterm babies is often hard. We are recruiting **all** babies born before 37 weeks' gestation in the Auckland area, including extremely preterm babies, moderately preterm babies and late preterm babies (who may not have experienced any problems due to being born preterm) in order to look at their nutrition after they have been discharged from hospital. Because preterm babies are born too soon, they often have not had enough time to develop sufficient nutrient stores. Iron and vitamins D, A and E are some of these nutrients which are often low in preterm babies. These nutrients all have important roles within in the body. They are needed for the normal growth, brain development and the health and well-being of your baby. Therefore, we would like to find out whether the current feeding and supplementation practices are enough to prevent deficiencies in these babies. Results of this study will hopefully guide future feeding and supplementation practices of preterm babies.

We are asking you to consider including your baby in this study as she/he was born preterm (before 37 weeks' gestation). Preterm babies are unlike any other babies and it is therefore not possible to conduct this research in any other group.

Project Procedures

This study will involve 2 home visits over your baby's first year of life to find out about feeding, nutrition and how she/he has grown. We would like to visit you in your home or you can attend our research facilities at Massey University in Albany, Auckland at a time which is convenient to you. Our first visit would be 4 months after your baby was discharged home from hospital and our second visit would be when your baby is 1 year old (corrected age).

At each visit we would like to take a small blood sample from your baby so that we can look at his/her iron and vitamin status. This may cause your baby some discomfort. If you are breastfeeding, we would also like to take a blood sample from you. This will be to determine whether you are deficient in iron or vitamin D which could affect your baby's nutritional status.

If you or your baby are found to be deficient in iron or vitamin D, you may experience some distress. As part of this study however, bloods will be processed and analysed on the same day. This will allow us access to results within a few days of being collected. This information will then be forwarded promptly on to you and your GP and he/she will advise you on how these deficiencies can be corrected. A 25(OH)D (vitamin D) level below 27.5 nmol/L is consistent with vitamin D deficiency in babies. A serum ferritin of below 10 µg/L indicates iron deficiency and a serum ferritin over 400 µg/L indicates iron overload. A low serum ferritin and a haemoglobin below 105 g/L is consistent with iron deficiency anaemia.

Obtaining information on how preterm babies grow after they have been discharged is one of the key outcomes of the research, so we will measure your baby's weight, length and head circumference at each visit.

We would also like to collect some additional information using 4 simple questionnaires. These questionnaires will be used to collect information about you and your baby, on feeding practices, nutritional supplementation and sun exposure. At the second visit when your baby is 1 year corrected age we will also ask about starting solid foods.

Taking part in the study will take about an hour on each visit. The questionnaires will take between 20 to 30 minutes. Other measures which include weight, length and head circumference and a blood test will take between 10 to 15 minutes. We also allow time for you to ask questions. All blood samples will be taken by a trained phlebotomist or neonatal research nurse who has experience with taking bloods from babies.

We will also collect some health information about your baby from baby's medical notes. This is to record how your baby was fed after birth, his/her birth weight, head circumference and length, and whether any assessment of nutritional status was made during the hospital admission or after birth.

Benefits

By taking part in this study you will receive your baby's individual blood results on their iron and vitamin D status. Both of these nutrients are essential for optimal development and growth of your baby. If we find that your baby is low in these nutrients then we will refer you to your GP, often the solution is as easy as providing a supplement but its important that this is decided by your medical practitioner. You will also find out your babies' length, weight and head circumference, these will be taken by trained researchers. If you are breastfeeding and consent to giving a blood sample, you will also find out whether you are sufficient in iron and vitamin D. If these values are outside of the normal ranges you will be notified and these will be forwarded on to your GP. Your GP will be able to provide you with best advice and if any treatment is needed.

By taking part in this study you and your baby are helping us find out whether feeding and supplementation practices currently followed are appropriate to prevent iron and vitamin D deficiencies in babies born preterm. With your help we can find out whether feeding and supplementation practices are currently sufficient or whether these need changing or if babies need to be routinely monitored.

Data Management

Any information collected from you and your baby will be used only for the purposes of this study. This information will be stored in a secure manner at Massey University. Once collected, data will be entered into a database and analysed in a way that does not identify you or your baby.

We will not be sharing information about you or your baby outside of the research team. The information that we collect from this research project will be kept confidential. All questionnaires with information about you and your baby will be given a number and will not display any names.

A summary of your baby's results will be given to you. In addition, the overall findings from the study will be shared with all parents; however, individual results will be kept private. We will also publish the results of this study so that we can make sure that future feeding and supplementation practices are in the best interests of preterm babies.

If we find that any of the results from the blood sampling are outside of a normal range we will, with your permission, contact your baby's General Practitioner directly and give them a copy of the results.

Participant's 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;*
- *decline to have the blood sample taken from your baby or yourself (if breast feeding)*
- *withdraw from the study within the timeframe of data collection;*
- *ask any questions about the study at any time during participation;*
- *provide information on the understanding that your name or your baby's 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.*

Project Contacts

If you have any questions, you may ask them now or later. If you wish to ask questions later, you may contact any of the following

Researcher and Supervisor:

Cath Conlon (PhD)
c.conlon@massey.ac.nz
09 414 0800 extension 41206

TXT 0211730428
Massey University Oteha Rohe
Albany Highway,
Albany 0632
New Zealand

Researcher
Charlotte Moor
Charlottefmoor@gmail.com

Researcher
Briar Emmett
Briaremmett@yahoo.com

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 13/06. If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 84459, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

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.

Appendix D

Contact Details Slip

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Contact Details

Your Name:_____

Phone Number:_____

Mobile Number:_____

Email Address: _____

Appendix E

Data Collection Sheet

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Data collection sheets for Visit 1

Date: ___/____/___

Participant ID:_____

Participant DOB___/____/___

Date of discharge from hospital___/____/___

Age in days_____

Health Screening Questionnaire completed ☐

Criteria for inclusion in the study met ☐

Informed Consent completed ☐

Demographic Questionnaire completed ☐

Anthropometric Measurements taken ☐

Baby's weight at 4 months after discharge: _____kg

Baby's length at 4 months after discharge: _____ cm
: _____ cm
: _____ cm
Mean : _____ cm

Baby's head circumference at 4 months after discharge: _____cm
: _____ cm
: _____ cm
Mean : _____cm

Infant's Blood Tests:

Completed: ☐ Arranged: ☐

Date:_____

Needs to be arranged: ☐

Blood sampling was by: venipuncture/micro collection (*delete as appropriate*)

HemoCue ☐

Fitzpatrick Score ☐

Detail of any issues about blood collection

_____

_____

_____

Mother's Blood Tests:

Currently breast feeding or expressing breast milk YES/NO

If no, blood sample from mother not required. If yes:

Informed consent for blood sample ☐

Consent for blood sample declined ☐

Completed: ☐ Arranged: ☐

Date:_____

Needs to be arranged: ☐

HemoCue ☐

Fitzpatrick Score ☐

Feeding Questionnaire completed ☐

Sun exposure questionnaire completed ☐

Second Visit

Date arranged _____ ☐

To be arranged_____ ☐

Declined_____ ☐

Explained to parent how they will receive their results ☐

Thank parent for their time and participation in the study

Appendix F

Serum Ferritin Data Sheet

Appendix G

Serum Soluble Transferrin Receptor Data Sheet

Appendix H

C-Reactive Protein Data Sheet

Appendix I

Demographic Questionnaire

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Mother and Baby Demographics Questionnaire

Date: ___/____/___
Participant ID: _____

The following questionnaire has been designed to obtain information on demographic characteristics about the mother and baby.

Section 1
What we need to know about the mother

1. How old are you/the baby's mother?

_____

2. Which ethnic group do you/the baby's mother belong to? (Please circle the one that most applies to you)

- New Zealand/European
- Other European
- New Zealand Maori
- Cook Island Maori
- Fijian
- Niuean
- Samoan
- Tongan
- Tokelauan
- Other Pacific Island
- Chinese
- Other Asian
- Indian
- South East Asian
- Other

3. Was this your first baby? (Please circle)
Yes
No

If no, how many other children do you (the mother) have and what are their ages? (Please state)

_____
_____
_____

Section 2

What we need to know about your baby

1. What is your baby's date of birth?

_____

2. What is the gender of your baby? (Please circle)

Male

Female

3. Which ethnic group does your baby belong to? (Please circle the one that most applies to your baby)

- New Zealand/European
- Other European
- New Zealand Maori
- Cook Island Maori
- Fijian
- Niuean
- Samoan
- Tongan
- Tokelauan
- Other Pacific Island
- Chinese
- Other Asian
- Indian
- South East Asian
- Other

4. What gestational age was your baby born at?

_____Weeks_____Days

5. Which hospital was your baby discharged from?

_____

6. What date was your baby discharged home?

_____

Appendix J

Feeding Questionnaire

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Interviewer Administered Feeding Questionnaire

Date___/___/___
Participant ID:_____
Participant DOB___/___/___

Instructions that will be given to parents or prompts for interviewer are shown in italics. Substitute 'your baby' for the baby's name if the interview is taking place with the legal caregiver and not the parent

When your baby was discharged

These questions relate back to when your baby was discharged from the hospital.

1. On the day you were discharged from hospital how was your baby fed? (please circle the one which most applies)

   Breast fed
   Bottle fed
   Tube fed (*tube through their nose or mouth*)
   Combination of breast/bottle /tube
   Please specify for example breast fed and topped up with a tube feed
   _____
   _____
   _____
   _____
   _____

2. What was your baby fed when first discharged? (You can chose more than one option if relevant)
   Breast milk (Go to question 3)
   Breast milk and formula (Go to question 3)
   Formula (Go to question 4)
   Cow's milk (go to question 4)

Other: _____

3. If you were breastfeeding or giving expressed breast milk, how long did you continue this after discharge? (or document if still breast feeding/providing EBM)

(*Find out to the nearest week i.e. less than 1 week, 1 week, 2 weeks etc, if mother can't remember try to find out approximately, ask about the baby's age when breast feeding or expressing breast milk stopped but* clarify *whether this is their chronological age (age since their birth) or their corrected age).*

_____
_____
_____
_____
_____

3a. If you are currently breast feeding have you/the mother been diagnosed with iron deficiency? (*Please circle*)

Yes

No

Don't know (*any blood testing, previous anaemia, please provide details and if the mother is on any supplements/treatment*)

_____
_____
_____
_____
_____

3b. Are you currently vitamin D deficient? (*Please circle*)

Yes

No

Don't know (*any blood testing, please provide details and if the mother is on any supplements/treatment*)

_____
_____
_____
_____
_____

4. After you were discharged from hospital were you adding anything to your baby's feeds or giving any supplements after feeds?
   Yes _____(specify)
   No

5. If formula fed, what formula was your baby fed after discharge? (*Please circle*)
   Post-discharge preterm formula (S-26 Gold Pregro)
   De- Lact
   Heparon Junior
   Karicare Gold Plus 1 from Birth
   Karicare Gold Plus 2 from 6 months
   Karicare AR All Ages
   Karicare Follow On 2 From 6 months
   Karicare Goat 1 From Birth
   Karicare Goat 2 From 6 months
   Karicare Gold 1 From Birth
   Karicare Gold 2 From 6 months
   Karicare HA AR All ages
   Karicare HA Gold Plus All ages
   Karicare Infant 1 From Birth
   Karicare Soy All ages
   Kindergen
   Locasol
   MCT Peptide
   Monogen
   NAN HA 3
   NAN HA GOLD 1
   NAN HA GOLD 2
   Neocate Advance (unflavoured)
   Neocate Advance (vanilla)
   Neocate LCP
   Novalac AC
   Novalac AR
   Novalac IT
   Novalac SD
   Novalac Stage 1 and 2
   Nurture Follow-on Formula 2
   Nurture Gold Follow-on Formula 2
   Nurture Gold Infant Formula 1
   Nurture Plus Gold Infant Formula All Ages

Nurture Infant Formula 1
Pepti-Junior Gold
S-26 Gold AR
S-26 Gold Lactose Free
S-26 Gold Newborn
S-26 Gold Progress
S-26 Original Newborn
S-26 Original Progress
S26 Soy
SMA
Other _____(specify)
Don't remember

If mother is currently breast feeding or expressing breast milk go to question 6 otherwise skip to section on starting solids

6. If you are currently breast feeding or expressing breast milk (fully or partially) are you willing to have a blood test to check your iron and vitamin D status? *(please tick)*

Not applicable	□ (Go to section on starting solids)
Yes	□ (*arrange for mother to sign consent form and have a blood sample taken*)
No	□

7. Are you taking any supplements during this time? If yes, which ones (*collect brand name of any supplements the mother is taking*) *(please tick)*

Elevit	□
Blackmores Pregnancy and Breastfeeding Gold	□
Other multivitamin	□ *state which*_____
Iron	□
Calcium	□
Vitamin D	□
Iodine	□
Other	□*state which*_____

8. Are you a vegetarian/vegan? *(please tick)*
   Yes ☐
   No ☐

Baby's First Foods

1. Is your baby currently eating any solids? *(please circle)*
   Yes
   No (Go to question 10)

2. Who suggested that you started solids? *(please tick)*
   Plunket nurse
   General practitioner
   Neonatologist/paediatrician
   Family member
   Myself
   Other (please state)_____

   Explore the reason for starting solids (Find out if it was due to advice or because the baby seemed hungry)

   _____
   _____
   _____
   _____
   _____

3. When did your baby start eating solids? _____(Date)
   or if not known – age

   Weeks_____
   Months_____
   (ask whether this is their chronological age (age since their birth) or their corrected age).
   Don't remember

4. What was your baby's first food? *(please circle)*
   Ready-made baby food
   Homemade foods
   Rusk
   Baby rice
   Fruit

Vegetables
Yoghurt
Breakfast cereal
Meat
Other _____

5. When did you introduce red meat?
   Date:_____
   Or if you are unsure of date, how long after introducing the first food did your baby eat red meat?
   Days:_____
   Weeks: _____
   Haven't introduced red meat yet (*please circle if this applies*)

6. When did you introduce other meat, for example chicken, pork or fish?
   Date:_____
   Or if you are unsure of date, how long after introducing the first food did your baby eat other meat?
   Days:_____
   Weeks: _____
   Haven't introduced other meat yet (*please circle if this applies*)

7. How many times a day does your baby eat solid foods?
   _____

8. Approximately how much does your baby eat at each time? (*hint: teaspoons, tablespoons etc*)
   _____
   _____

9. How often do you usually give your baby these types of solid foods?

	More than once per day	Once per day	3 or more times per week	Once or twice per week	Less than once per week	Never
Fresh fruits						
Fresh Vegetables						
Ready made foods (such as jars of baby food)						
Breakfast cereals						
Rice or Pasta						
Breads						
Potatoes						
Potato products e.g. chips, crisps						
Butter or margarine						
Beef						
Lamb						
Pork including ham						
Chicken & other poultry						
Fish						
Eggs						
Beans, lentils, chickpeas						
Tofu						
Nuts						
Cheese or yoghurt						
Puddings or desserts						
Biscuits, sweets or cake						

10. Has your baby ever had any liquids other than breast milk or infant formula? *(please circle)*
    Yes
    No (Finish interview)

11. How old was your new baby the first time he or she drank liquids other than breast milk or formula?
    Weeks_____
    Months_____
    Don't remember

12. What was your baby's first liquid other than breast milk or formula? *(please circle)*
    Cow's Milk
    Soya Milk
    Goats Milk
    Juice
    Tea
    Water
    Other:_____

Appendix K

Supplement Questionnaire

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Interviewer Administered Supplement Questionnaire

Date___/___/___
Participant ID:_____
Participant DOB___/___/___

Instructions that will be given to parents or prompts for interviewer are shown in italics. Substitute 'your baby' for the baby's name if the interview is taking place with the legal caregiver and not the parent

We would like to find out whether your baby has received supplements such as iron or Vitadol C (*if mother has not heard of this just talk about iron or any other supplements*). Please be as honest as you can. All the data is confidential and only identified by a unique code. The purpose of the research is because we don't know whether every baby needs these supplements and by being honest you will help us to determine this.

1. Was your baby discharged home on any supplements? *(please tick)*
   Yes ☐
   No ☐ *(Finish questionnaire)*
   Not sure ☐ *(List supplements: Vitadol C, Ferro-Liquid (or Ferrous Sulphate) to jog the mother's memory)*

2. Which supplements was your baby discharged home on? *(please tick)*
   Vitadol C ☐
   Ferro-Liquid/ Ferrous Sulphate ☐
   Other:_____

3. What dose was your baby discharged from hospital on?
   (*Write N/A if vitamin/mineral not prescribed or don't know if mother doesn't know*)
   Vitadol C: _____
   Ferro-Liquid/Ferrous Sulphate: _____

Other: _____

4. Has the dose of this/these supplements ever been changed since your baby was discharged*? (please circle)*
   Yes
   No
   Don't Know

5. What was the dose changed to?
   (*Write N/A if vitamin/mineral not prescribed or don't know if mother doesn't know*)
   Vitadol C: _____
   Ferrod-Liquid/Ferrous Sulphate: _____
   Other: _____

6. After discharge, did you give these to your baby daily? *(please circle)*
   Yes, every day (*continue to question 8*)
   Yes, most days
   Yes, some times
   No, never

7. If not every day, how often did you give them to your baby?

_____
_____
_____
_____
_____
_____

8. What were some barriers/ issues you experienced with giving your baby supplements every day?

_____
_____
_____
_____
_____
_____

9. Are you still giving your baby these supplements? *(please circle)*
   Yes
   No
10. If no, when did you stop giving your baby these supplements?

_____
_____
_____

11. If no, why did you stop giving your baby these supplements?

_____
_____
_____
_____
_____

Appendix L

Eligibility Screening Questionnaire

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Eligibility Screening Questionnaire

Date: ___/____/___

Participant ID:_____

Please circle the correct response (to be completed by a researcher)

Was the baby born prior to 37 weeks gestation?	**Yes**	**No**
Is the baby currently living in a home environment	**Yes**	**No**
Was the baby born at Auckland City Hospital or as an inpatient of the Neonatal Intensive Care Unit?	**Yes**	**No**
Was the baby discharged from Auckland City Hospital or the NICU 4 months ago or less	**Yes**	**No**

If the answers to all of the above questions is 'yes' then the baby meets the criteria for taking part in the research study.

Appendix M

Infant Informed Consent Form

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

We would also like to ask your permission to have access to your babies medical records, these will only be used for the purpose of this study, and all data obtained will be kept confidential.

As the parent/legal caregiver to my baby

Baby's Name

Please print .....

I agree to have myself and my baby participate in this study under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Full Name of

Parent/legal caregiver

Please print .....

Appendix N

Maternal Informed Consent Form

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

PARTICIPANT CONSENT FORM

Maternal blood testing

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I am currently breast feeding my baby and I agree to have a blood test taken, under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Full Name - printed .....

Appendix O

Medical Notes Questionnaire

Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Data Collection from Medical Notes

Date___/___/___
Participant ID:_____
Date of discharge from hospital___/___/___

About the Birth

1. **Patient's DOB: _____**

2. **Patient's Gestational Age at birth:_____**

3. **Birth weight:_____**

4. **Birth length:_____**

5. **Head circumference at birth: _____**

6. **Is the infant a** Single birth
   Twins
   Triplets
   Other (Please state)_____

7. **Time of Cord clamping: _____**

8. **Did the infant suffer from IUGR?**
   (please circle)
   Yes
   No

Inpatient relevant data

1. **After the infant was born, was he or she put in an intensive care unit?** (Please circle)

Yes
No

2. Did the infant receive any blood/erythrocyte transfusions when they were in the NICU after birth?

Yes
No

If yes, how many?_____

3. Did the infant have an erythropoietin administration during admission?

Yes
No

If yes, how many?_____

<u>Feeding and Supplement History</u>

1. Did the infant receive parenteral nutrition?

Yes, how long? (Days)_____
No

2. Whilst in hospital did the infant receive enteral nutrition (EXPRESSED BREAST MILK and/or infant formula)?

Yes
No

If yes, what were they fed?

EBM only
FEBM only
NIF only
PIF only
EBM + NIF
EBM + PIF
FEBM + NIF
FEBM + PIF

3. If infant was fed formula, which formula were they fed?

NIF
PIF

4. **Whilst in hospital was the infant breast fed?**
   Yes
   No

5. **How was the infant fed when discharged?**
   Nasogastric or orogastric □
   Breast feeding □
   Bottle feeding □

6. **What and how was the infant being fed upon discharge?**
   _____
   _____
   _____

7. **If infant was fed formula, which formula were they fed?**
_____
_____
_____

8. **What supplements did the infant receive whilst in hospital?** (please tick all that apply)
   Vitadol C □
   Ferro-Liquid/ Ferrous Sulphate □
   Other:_____
   None: _____

9. **When were supplements started?** (*Date*)
   Vitadol C: _____
   Ferro-Liquid/ Ferrous Sulphate: _____
   Was this 4 weeks after birth Yes/No
   Other: _____

10. **What dose were supplements initially prescribed at?**
    Vitadol C: _____
    Ferro-Liquid/ Ferrous Sulphate: _____
    Other: _____

11. **What supplements were the infant discharged on?** (please tick all that apply)
    Vitadol C □
    Ferro-Liquid/ Ferrous Sulphate □
    Other:_____

None: _____

12. What dose of supplements was the infant discharged on?

Vitadol C: _____

Ferro-Liquid/ Ferrous Sulphate: _____

Other: _____

13. Infant Biochemistry (please note that many of these parameters are not routinely measured at birth or prior to discharge however the researchers should check the medical notes and record if measured, please also record date of measurement)***

Blood Sample Analysis of Preterm Infants at birth*		**Date**
Serum Ferritin		
Haemoglobin		
RBC		
Haematocrit		
MCV		
Mean Cell Hb		
Iron		
Iron binding capacity		
Iron saturation		
C-Reactive Protein		
25 hydroxyvitamin D (25(OH)D)		

Blood Sample Analysis of Preterm Infants at discharge or prior to discharge*		**Date**
Serum Ferritin		
Haemoglobin		
RBC		
Haematocrit		
MCV		
Mean Cell Hb		
Iron		
Iron binding capacity		
Iron saturation		
C-Reactive Protein (mg/L)		
25 hydroxyvitamin D (25(OH)D) (nmol/L)		

<u>Readmissions</u>

1. **Has the infant been readmitted to Auckland City Hospital since their birth?**
   Yes
   No

2. **Did the infant receive any blood/erythrocyte transfusions when they were in the NICU after birth?**
   Yes
   No

If yes, how many?_____

3. **Did the infant have an erythropoietin administration during admission?**
   Yes
   No

If yes, how many?_____

4. **The infant receive supplements during their admission?**
   Yes
   No

   If yes, what dose were the prescribed?
   Vitadol C:_____
   Ferrous Sulphate: _____

Appendix P

Letter to Family Doctor

(Insert date)
(Insert GP name and Practice address)

Dear Doctor,

_____(name) has been enrolled in the research study '**Post-discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge**' study run through Massey University Albany.

Recent blood tests taken as part of this research study to assess iron and vitamin D status have indicated that one or both of these results for _____(name) are outside of normal ranges. These results have been attached.
The parents have been informed of these results but have not been given any clinical advice. The parents have consented to these results being sent to their GP.
Please contact Cath Conlon (PhD) with any further questions.

Kind regards,

Cath Conlon (PhD)
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0632
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