Iron Status in Young Children with Autism Spectrum Disorder

A thesis presented in partial fulfilment of the requirements for the degree of

Masters in Science
in
Nutrition and Dietetics

at Massey University, Albany,
New Zealand.

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2015

Abstract

Background: Autism Spectrum Disorder (ASD) is diagnosed when a child shows unusual social behaviour, difficulty communicating both verbally and non-verbally, and performing repetitive tasks. Children with ASD often present with unusual feeding patterns and behaviours, and overseas research shows children with ASD are at greater risk of iron deficiency and iron deficiency anaemia. Maintaining adequate iron levels is very important, as during periods of growth and development iron has a role to play in both brain structure and function. Therefore it is important for children to eat foods rich in iron or foods that optimise iron bioavailability. In New Zealand, the prevalence of iron deficiency in children is relatively low, being 1.6%. However, there is no published data on iron status or dietary factors associated with iron status in New Zealand children with ASD.

Objective: To investigate factors influencing iron status in a cohort of children with Autism Spectrum Disorder living in New Zealand.

Methods: Sixty nine children with ASD between the ages of 2.5-8 years took part in this cross-sectional study. Participants were required to complete an estimated four-day food diary, a dietary questionnaire, and a Behavioural Paediatrics Feeding Assessment Scale (BPFAS) questionnaire. Serum ferritin, serum iron, total iron binding capacity, transferrin saturation and haemoglobin were measured to determine iron status. Statistical analysis was performed using independent t-tests, Mann-Whitney, Chi-square and Fishers' exact test.

Results: Iron depletion was present in 32.9% of the sample population (serum ferritin ≤ 20 µg/L), and iron deficiency was present in an additional 4.3% (serum ferritin ≤ 12 µg/L). No participants had iron deficiency anaemia (serum ferritin SF ≤ 12 µg/L plus Hb ≤ 110 g/L in 1-5 years; or Hb ≤ 115 g/L in 5-8 years). Participants in the iron replete group had significantly higher dietary protein intake ($P = 0.003$) and vitamin A intake ($P = 0.036$) compared to iron insufficient participants. The iron insufficient group had a significantly higher BPFAS frequency score than the iron replete group ($P = 0.022$), and significantly more participants in the iron insufficient group had a BPFAS score ≥84 ($P = 0.020$), indicating more feeding issues. No differences in patterns or factors affecting dietary intake were seen when comparing iron insufficient and iron replete groups.

Conclusion: Children with ASD in New Zealand appear to be at an increased risk of iron depletion and iron deficiency compared to neuro typical developing children. While no differences in dietary iron intake were seen between the iron replete and iron insufficient groups, iron replete participants had significantly higher intake of protein and vitamin A. More feeding behavioural problems were

identified in the iron insufficient group compared to the iron replete group. Further studies with a larger sample size should be undertaken to investigate other factors (dietary and non-dietary) to establish determinants of iron status in this population.

Acknowledgements

I would like to acknowledge a number of people who made this research possible. Firstly I would like to thank the participants and their families, for their time and effort dedicated to this study. The research would not have been able to be carried out without them. I would also like to thank my academic supervisory team. Dr Pam von Hurst for her guidance and support throughout this project as well as her knowledge. Dr Kathryn Beck for her support and extensive knowledge about iron and dietary assessment methods, as well as providing additional supervision at the end of this thesis. Dr Martin Dickens for his input, feedback and support throughout the thesis. I am grateful for each and every one of you, for your time and effort into providing feedback and making this project happen.

I would also like to thank Owen Mugridge, the research manager, who did a lot behind the scenes to ensure the project was completed in an efficient and timely manner. Also for Harjar Mazahery, the PhD candidate on the VIDOMA trial for her time in checking the food dairies and dietary questionnaires as well as collecting the anthropometric data. I would also like to thank Aimee Waring, another MSc student doing the thesis 'Dietary intakes, use of exclusion diets and supplements in children aged 2.5-8 years with ASD in New Zealand,' for her help in entering the food dairies into Foodworks as well as providing extra encouragement and support throughout the thesis process.

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List of Abbreviations

ABA	Applied Behaviour Analysis
AI	Adequate Intake
AVB	Applied Verbal Behaviour
ASD	Autism Spectrum Disorder
BPFAS	Behavioural Paediatrics Feeding Assessment Scale
CD4	Cluster of Differentiation 4 cells
CD8	Cluster of Differentiation 8 cells
CHO	Carbohydrate
CI	Confidence Interval
CRP	C-reactive Protein
Dcytb	Duodenal Cytochrome B Reductase
DF	Dietary Fibre
DHB	District Health Board
DMT1	Divalent Metal Ion Transporter 1
DNA	Deoxyribonucleic Acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, fifth edition
DSM-4	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
DTT	Discrete Trial Training
DVD	Digital Versatile Disc
EAR	Estimated Average Requirement
FAO	Food and Agriculture Organisation of the United States
Fe	Iron
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
FFQ	Food Frequency Questionnaire
FPN1	Ferroportin-1
GFCF	Gluten-Free Casein-Free Diet
GP	General Practitioner
Hb	Haemoglobin

HCP1	Haem Carrier Protein 1
HDEC	Health and Disabilities Ethics Committee
Hct	Haematocrit
Hep	Hepcidin
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
MCV	Mean Cell Volume
MFP	Meat Fish Poultry Factor
MSc	Masters in Science
NDNS	National Diet and Nutrition Survey (UK)
NHI	National Health Number
NRV	Nutrient Reference Values
NZ	New Zealand
NZEO	New Zealand European
PDD	Pervasive Developmental Disorder
PDD-NOS	Pervasive Developmental Disorders – Not Otherwise Specified
PRT	Pivotal Response Training
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RDI	Recommended Daily Intake
RNA	Ribonucleic Acid
SD	Standard Deviation
SF	Serum Ferritin
SFA	Saturated Fatty Acids
sTfR	Soluble Transferrin Receptor
sTfR:SF	Transferrin Receptor to Serum Ferritin Ratio
TEACCH	Treatment and Education of Autistic and Related Communication-Handicapped Children
TIBC	Total Iron Binding Capacity
TS	Transferrin Saturation
UL	Upper Level of Intake

UK	United Kingdom
USA	United States of America
VIDOMA	Vitamin D, Omega-3 in Autism Study
WDHB	Waitemata District Health Board
WHO	World Health Organisation
ZPP	Zinc Protoporphyrin

Chapter 1: Introduction

1.1. Background

Autism Spectrum Disorder (ASD) affects over 40,000 children and adults throughout New Zealand and is becoming a larger issue as the number of people diagnosed in the last 20 years has dramatically increased (Autism New Zealand Inc., 2014). ASD is a neurodevelopmental disorder in which clinical diagnosis is based upon a child showing signs of unusual social behaviour, uncharacteristic verbal and non-verbal communication, and repetitive behaviour or limited ability to perform tasks before the age of three years (American Psychiatric Association, 1994). Although all three symptoms need to be present for a diagnosis, the severity of the symptoms varies for every individual. The high functioning end of the spectrum is known as Asperger syndrome, where the individual has average or higher intelligence and fewer language problems. The spectrum ranges to those with severe cases of classic autism where difficulties socialising, understanding verbal and non-verbal cues and dislike of human contact are apparent (Howlin, 2006). Autism typically affects the cerebral cortex, basal ganglia, corpus callosum, cerebellum, brain stem, hippocampus and amygdala which are involved in higher mental functions, movement, behaviour, coordination, muscles for speaking and short term memory (Amaral, Schumann, & Nordahl, 2008). Common issues in children with ASD include hyperactivity, attention deficits, repetitive behaviours, self-injury, tics, strong interests, as well as mood and social problems (Ministry of Health and Education, 2008).

Children with ASD often have unusual feeding patterns and behaviours at meal times, with the prevalence of feeding issues in these children ranging from 13% to 80% (Coffey & Crawford, 1971; Jones, 1982; Perske, Clifton, McClean, & Stein, 1977). Children with ASD often have restricted food intake due to heightened sensitivity to the texture, appearance, taste, smell and temperature of food (Williams, Dalrymple, & Neal, 2000). One study found these children eat a smaller variety of food from within each food group (Williams et al., 2000). Several theories exist for the high prevalence of feeding difficulties in this group of children. One of these is that children with ASD are supertasters (Cermak, Curtin, & Bandini, 2010), which may lead to dislike of bitter tastes and preference for sweet foods (Bartoshuk, 2000). High food selectivity in children with ASD is of concern as it may lead to nutritional deficiencies as a results of dietary restriction (Cornish, 1998; Herndon, DiGuiseppi, Johnson, Leiferman, & Reynolds, 2009; Raiten & Massaro, 1986).

Research from several countries have confirmed that children with austism are at high risk of developing iron deficiency (ID) and iron deficiency anaemia (IDA). Turkish children with autism aged 3-16 years, were shown to have ID (24.1%) and IDA (15.5%) (Herguner, Kelesoglu, Tanidir, & Copur,

2012). Similarly, ID was seen in 52.2% of participants and IDA in 11.5% of Welsh children with autism (Latif, Heinz, & Cook, 2002). Australian children with autism had poorer iron status compared to the general population, with 3.0% having iron depletion, 7.5% having ID and 3.0% IDA (Sidrak, Yoong, & Woolfenden, 2014). However, the prevalence of IDA in American children with autism was not higher than the general population when using serum ferritin (SF) and transferrin saturation as markers for iron status (Reynolds et al., 2012). It is difficult to compare the prevalence of ID and IDA between studies as the cut off values are different for each study.

Iron is an essential nutrient, especially during periods of growth and development in children and adolescence (Ministry of Health, 2012). Iron is stored in different forms and has a variety of physiological roles. Iron is involved in oxygen transport; DNA and RNA synthesis; electron transport; cellular respiration, proliferation and differentiation (Lieu, Heiskala, Peterson, & Yang, 2001). It also has a major role in brain development by participating in the formation of myelin sheaths, dendrites and neurotransmitters (J.L Beard, Connor, & Jones, 1992).

Maintaining iron homeostasis is important as deficiency during periods of rapid growth can lead to impaired brain development (Oski, 1993); impaired cognition; decreased attention span and ability to learn; neuro motor dysfunction (Lozoff, Jimenez, & Wolf, 1991); immune dysfunction (Das et al., 2014); and potential growth impairment (J.L Beard, Haas, & Gomez, 1983). Although there is no evidence to suggest iron supplementation can reverse developmental issues (Logan, Martins, & Gilbert, 2001), iron supplementation is associated with improved behaviour such as better social interaction and attentiveness and reduced breath-holding in children with IDA (Daoud, Batieha, AlSheyyab, Abuekteish, & Hijazi, 1997; Lozoff et al., 1991). Although ID does not cause ASD, the negative effects associated with ID and IDA on brain development will certainly not help children with ASD to reach their full potential.

Both dietary and non dietary factors have the potential to impact on iron status. Dietary iron can be classified into haem iron found in animal foods such as red meat, poultry, fish and seafood. Non-haem iron is found in plant foods as well as meat products, with sources including green leafy vegetables, legumes, breads, cereals and nuts. An estimated 25% of haem iron is absorbed compared to 17% of non-haem iron (Ministy of Health, 2012). Meat also contains the Meat Fish Poulty (MFP) factor, which enhances non-haem iron absorption (Monsen et al., 1978). Non-haem ferric iron absorption is enhanced by vitamin C (Lynch & Cook, 1980) due to an increased acidity in the intestinal lumen (Monsen et al., 1978). This converts ferric iron to ferrous iron making it more bioavailable (Lieu, Heiskala, Peterson, & Yang, 2001). Polyphenols (found in tea, coffee, legumes and cereals), phytates and oxalates (found in wheat, cereals, nuts and seeds), calcium and some proteins (e.g. soy protein)

inhibit non-haem iron absorption (Ministry of Health, 2012). One study conducted on children with ASD found 35% did not consume red meat or meat products, and that iron fortified cereals contributed 25% towards daily iron intake (Cornish, 1998). Sidrak et al. (2014) propose that ID risk is heightened in children with ASD due to problems sucking, swallowing or chewing, poor eating behaviour and an inadequate dietary intake of meat, chicken, eggs or fish. It is typical of autistic children to have restrictive diets and be unwilling to try new foods due to dislike of new smells, texture and taste (Raiten & Massaro, 1986). This could increase the risk of ID if they are avoiding iron-rich foods as well as having an unbalanced intake of dietary modulators of iron absorption.

Results from the 2002 National Children's Nutrition Survey (Ministy of Health, 2003) show a low prevalence of ID of 1.6% amongst New Zealand children, with a higher prevalance in children from Māori or Pacfic ethnicities. This survey also found the median dietary iron intake for males was 10mg, and for females 8.4mg for children aged 5-6 years. Despite concerns about dietary inadequacy and evidence that ID can affect attention, sleep, and cognitive development (J. Beard, 2003; Dosman et al., 2007; Osendarp & Eilander, 2011; Oski, 1993), to the best of our knowledge there are no studies in New Zealand which have examined iron status or the relationship between dietary intake, feeding behaviour and iron status in children with ASD.

1.2. Purpose of the Study

Research from other countries indicate the prevalence of ID and IDA in children with ASD is higher than neuro typical children, with possible reasons including highly selective diets and aversions to taste, texture, smell or appearance of the food. Very few of these studies have investigated dietary intake and practices which may affect iron status. At present, the iron status and factors affecting iron status in New Zealand children with ASD have not been researched. Therefore this cross-sectional study was conducted to describe the degree of iron deficiency in children with ASD in New Zealand as well as provide insight into dietary patterns, factors and behaviours that may influence iron status.

It is important for the current iron status of New Zealand children with ASD to be investigated as iron requirements increase during periods of rapid growth, and in addition, iron is an essential mineral needed in brain development. The findings from this study may help guide clinical practice. If this research shows New Zealand children with ASD are at risk of ID and IDA, easy and cost effective strategies could be put in place such as screening and supplementation. This has the potential to reduce the prevalence of ID and IDA in this population group and enable these children to reach their full potential.

1.3. Hypotheses

Children diagnosed with ASD are at higher risk of developing iron depletion, iron deficiency, and iron deficiency anaemia compared to neuro typical children. In addition, dietary iron and the intake of enhancers and inhibitors of iron absorption, and feeding behaviour is associated with iron status in children with autism.

1.4. Aim and Objectives

1.4.1. Aim

To investigate factors affecting iron status in a cohort of children with Autism Spectrum Disorder living in New Zealand.

1.4.2. Objectives

1. To describe the degree of iron depletion, iron deficiency and iron deficiency anaemia in children with autism
2. To explore dietary intake and practices associated with iron status in children with autism.
3. To investigate feeding behaviours associated with iron status in children with autism.

1.4. Structure of the Thesis

This thesis is divided into five chapters. The first chapter provides background information and the rationale for conducting this research. The second chapter reviews the existing literature on iron status in children with ASD as well as an overview of iron and factors influencing iron status. The third chapter outlines methods used to assess dietary intake and procedures to determine iron status in this population sample. The results of the study are presented in chapter four. Chapter five discusses the results, as well as providing a conclusion and recommendations for future research in this area.

1.5. Researcher's Contributions

Various people contributed in differing ways in order for this thesis to be conducted. Each researcher and their contribution is summarised in Table 1.1 below.

Table 1.1. The researchers involved in this study and their relevant contributions

Researcher	Contribution
Micaela Makker	Design of research questionnaires, food diary entry into FoodWorks, analysed data, performed statistical analyses, interpreted results, main author of thesis
Dr Pam von Hurst	Main academic supervisor, applied for ethics, research design, reviewed the thesis
Dr Kathryn Beck	Academic co-supervisor, research design, advice on dietary assessment methods and iron, assisted with interpretation of the results, reviewed the thesis
Dr Martin Dickens	Academic co-supervisor, reviewed the thesis
Dr Bobby Tsang	Participant recruitment, collection of serum samples
Owen Mugridge	Participant recruitment, conducted the research
Harjar Mazahery	Participant recruitment, conducted the research, checked food diaries
Sarah McDonald	Participant recruitment
Aimee Waring	Food diary entry into FoodWorks

Chapter 2: Literature Review

This chapter provides detail on the definition of Autism Spectrum Disorder (ASD), with possible co-morbidities and treatment options reviewed. Dietary patterns, eating behaviours, nutritional deficiencies and use of supplements in children with ASD are discussed. The role of iron is reviewed along with factors modulating iron absorption and determinants of iron status. Iron deficiency is defined and optimal methods to assess irons status and iron intake are discussed. The iron status, intake and requirements of children in New Zealand are also reviewed. This is followed by a review of the literature on iron status and determinants of iron status in children with ASD.

2.1. Autism Spectrum Disorder

2.1.1. Definition and Diagnosis

Autism Spectrum Disorder (ASD) is a life-long neurodevelopmental disorder in which clinical diagnosis is based upon criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) (American Psychiatric Association, 2013). The diagnostic criteria from DSM-5 are summarised below (American Psychiatric Association, 2013).

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested be the following, currently or by history:
    1. Deficits in social-emotional reciprocity.
    2. Deficits in nonverbal communication behaviours used for social interaction.
    3. Deficits in developing, maintaining, and understanding relationships.
B. Restricted, repetitive patterns of behaviour interests, or activities, as manifested by at least two of the following, currently or by history:
    1. Stereotyped or repetitive motor movements, use of objects, or speech.
    2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behaviour.
    3. Highly restricted, fixated interests that are abnormal in intensity or focus
    4. Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment.
C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life).
D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.
E. These disturbances are not better explained by intellectual disabilities or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

Diagnostic criteria taken from DSM-5 (American Psychiatric Association, 2013).

The severity of symptoms differs for each individual and can change over time (Bishop, 1989; Kanner, 1943). Due to the variance in severity, the term ASD is used to describe the whole range of disorders, also including Asperger's syndrome (Frith & Happe, 2005). People with Asperger's syndrome are at the higher functioning end of the spectrum and often have higher intelligence and better communication skills compared to people with classic autism (Sugden & Corbett, 2006). Also included within the autism spectrum are infantile autism, childhood autism, Kanner's autism, high-functioning autism, atypical autism, childhood disintegrative disorder, Pervasive Developmental Disorder (PDD), and Pervasive Developmental Disorders – Not Otherwise Specified (PDD-NOS) (American Psychiatric Association, 2013; Ministry of Health and Education, 2008).

Environmental risk factors for ASD include increasing parental age and low birth weight. A genetic component does exist in ASD with heritability approximately ranging from 37-90% (American Psychiatric Association, 2013). It has been estimated that ASD affects over 40,000 people in New Zealand (Ministry of Health and Education, 2008). ASD is becoming an increasingly larger issue as in the last 20 years the number of people diagnosed has dramatically increased. The rise in ASD prevalence may not be due to an increased incidence but an improved awareness and recognition (Wing & Potter, 2002), as well as the expansion of ASD criteria in DSM-4 (American Psychiatric Association, 2013). ASD is also diagnosed four times more often in males than in females (American Psychiatric Association, 2013).

Areas of the brain most commonly affected in ASD are the cerebral cortex, basal ganglia, corpus callosum, cerebellum, brain stem, hippocampus and amygdala. These areas are involved in higher mental functions, movement, behaviour, coordination, muscles for speaking and short term memory (Amaral et al., 2008). Behavioural and emotional issues are also characteristic of ASD, with common problems being hyperactivity, attention deficits, repetitive and ritualistic behaviours, self-injury, tics, strong interests, as well as mood and social problems (Ministry of Health and Education, 2008).

2.1.2. The Triad of Impairment

The triad of impairment affects every individual with ASD to varying degrees. These include impaired (i) social interaction, (ii) social communication and (iii) behaviour flexibility (Wing & Potter, 2002). Social interaction impairment indicates the inability to determine thoughts and feelings of others (A. Cashin, Sci, & Barker, 2009) which can cause frustration and anxiety for the child (A. J. Cashin, 2005). Impaired social interaction relates to the difficulty conveying and understanding both verbal and non-verbal communication cues. These include changes in facial expressions, gestures and tone of voice (A. Cashin et al., 2009). As any form of communication has to be taught, people with ASD often have

monotone speech, limited means of expressing themselves (A. J. Cashin, 2005), and do not understand the use of language to transfer information between people (A. Cashin et al., 2009). People with ASD also have reduced behaviour flexibility which is expressed through repetitive actions or limited interest in activities (American Psychiatric Association, 1994). Although some repetitive behaviours exist in typically developing children, research has shown children with ASD have significantly more repetitive behaviours than those with normal development (Bodfish, Symons, Parker, & Lewis, 2000; Hermelin & O'Connor, 1963; Prior & Macmillan, 1973). These repetitive behaviours can persist into adulthood if accompanied with intellectual impairment (A. Cashin et al., 2009). Repetitive behaviour has been linked to reduced imagination development (Happe, 1994) and resistance to unexpected changes to routine (A. Cashin et al., 2009).

In addition to the triad of impairment, people with ASD may be either hypo- or hypersensitive to certain external stimuli such as light, sounds, smell, touch or pain (Ayres, 1963). Children who are hypo- or hyper-sensitive may react negatively to simple routine tasks, such as getting up and ready in the morning, which are dependent on planning, switching and memory skills (Epstein, Saltzman-Benaiah, O'Hare, Goll, & Tuck, 2008). This may cause anxiety or panic upon anticipation of such an event (Leekam, Nieto, Libby, Wing, & Gould, 2007).

2.1.3. Conceptual Processing in ASD

Children with ASD have different cognitive processing styles compared to a neuro typical child (Gray & Attwood, 1999). The first difference is visual processing, meaning people with ASD process information visually rather than through language or verbally (A. Cashin et al., 2009).

The second difference is impaired abstraction (J. Scott, Clark, & Brody, 2000). In neuro typical individuals, abstraction allows information to be stored and ordered based on similar scenarios and concepts (A. J. Cashin, 2005), allowing a unified base of knowledge to be achieved. As individuals with ASD are unable to connect similar experiences and ideas, they store information based on visual codes which are ordered based on the sequence of events (J. Scott et al., 2000).

The third processing style is the theory of mind, also known as the basis of empathy (A. J. Cashin, 2005). In neuro typical individuals this theory in conjunction with abstraction allows adaptation to social changes occurring around them. For individuals with ASD who store information visually and in segments, they may struggle in unfamiliar situations or new environments as they have no past behaviour to revert to and do not know how to react. This can cause anxiety for the child, heightening the fight or flight response which may be expressed via repetitive behaviours to block out the threatening situation (A. J. Cashin, 2005).

2.1.4. Co-morbid Disorders

Children with ASD often have comorbidities, the most common being mental retardation, epilepsy, speech and language disorders, hearing and visual impairments (American Psychiatric Association, 2013). Motor disorders are more common in children with Asperger's Syndrome, these include developmental disorder, clumsiness and gait abnormalities (Gillberg & Billstedt, 2000). Behavioural and emotional issues such as sleep problems, sensory abnormalities, attention deficits and unusual activity levels are also prevalent in children with ASD (Gillberg & Billstedt, 2000). Additionally anxiety and mood disorders are also common in these children (Amaral et al., 2008; Ghaziuddin, Tsai, & Ghaziuddin, 1992). The complexity of cases with mental retardation makes the assessment and diagnosis of psychopathology very difficult (Ghaziuddin et al., 1992).

2.1.5. Possible Treatments

ASD is a life-long disability for which there is no medical cure (Wing, 1996). However, children who are diagnosed when they are young and who receive early interventions are proven to have better outcomes (Ministry of Health and Education, 2008). Interventions such as education and support aid the development of skills to equip children to reach their full potential (Ministry of Health and Education, 2008).

Behavioural interventions have been proven to be the most effective in treating children with Autism (Schriebman, 2000). Behavioural interventions such as Applied Behaviour Analysis (ABA) at age 3-4 years can significantly improve outcomes, with some children achieving normal functioning (M. Connor, 1998; Lovaas, 1987; Rogers, 1998). There are three different types of ABA. The first is Discrete Trial Training (DTT) in which a consequence is provided based on the ability of the child to complete a task which is broken down into simple steps (T. Smith, 2001). The second is Pivotal Response Training (PRT) which incorporates motivators of children's learning which are used as rewards (Koegel, Koegel, Harrower, & Carter, 1999). The third is Applied Verbal Behaviour (AVB) where the goal is to develop the child's communication skills by expressing their needs and providing the child with feedback to reinforce the behaviour (Sundberg & Michael, 2001).

Educational interventions can improve the development of children with Autism. One educational intervention is Treatment and Education of Autistic and Related Communication-Handicapped Children (TEACCH) which focusses on verbal and social skills, as well as daily activities using a structured approach (Schriebman, 2000). Other input such as speech and language therapy, occupational therapy, optometry and social skill interaction therapy to help organise sensory information are sometimes required (Winchester Hospital, 2015). Additionally pharmaceutical

medications are also used to treat symptoms and other issues such as anxiety (Winchester Hospital, 2015).

2.2. ASD and Dietary Patterns

2.2.1. Common Restrictive Diets

The implementation of restrictive or selective diets is very common in children with ASD. An internet survey completed by parents living in America found 27% of children with ASD were on a special diet (Green et al., 2006). The main reasons dietary interventions are used are to improve their child's rate of development and behaviour, and some even believe it will cure their child of the disorder (Levy & Hyman, 2005).

Children with ASD may have higher food sensitivities to allergens than typically developing children due to abnormal immune or digestive systems (Jyonouchi, Geng, Ruby, Reddy, & Zimmerman-Bier, 2005; Jyonouchi, Geng, Ruby, & Zimmerman-Bier, 2005; Jyonouchi, Sun, & Itokazu, 2002). Incomplete food breakdown may trigger the immune system and cause an immune reaction, immediate reaction, or a delayed reaction. The child's behaviour may change in response to pain or discomfort caused by the food allergen (Adams, 2013). Therefore avoidance of food allergens or food intolerances is often undertaken to prevent immune reactions, gastrointestinal disturbances and negative behaviours (Adams, 2013).

The most common exclusion diets in children with ASD are the gluten-free, casein-free (GFCF), yeast free, sugar free, egg free, specific carbohydrate diet, Feingold diet, low glycaemic index, and additive and preservative free diets. The most popular elimination diet in children with ASD is the GFCF diet which is largely supported by anecdotal evidence, with children having improved language, social interactions, or even being cured of their autism (Levy & Hyman, 2005) This diet involves the elimination of gluten (wheat, barley, rye) and casein (protein in milk and milk products) from the diet. The theory behind this popular diet is the incomplete breakdown of gluten and casein containing foods which cross a leaky gut membrane. These peptides cross the blood brain barrier to interfere with neurotransmitter breakdown, causing abnormalities in cognition and characteristic ASD behaviours (Panksepp, 1979). However, evidence for this hypothesis is limited (Adams, 2013). Only two small randomised control trials (RCT) (n= 20, n= 15) have been performed to investigate the effects of the GFCF diet on symptoms of autism (Elder et al., 2006; Knivsberg, Reichelt, Hoien, & Nodland, 2002). These studies showed significant improvements in autistic traits, social isolation and communication and interaction in children with ASD; however there were no significant improvements in these three outcomes when compared to the control group (Millward, Ferriter, Calver, & Connell-Jones, 2008). Evidence for the efficacy of the GFCF diet is poor, and larger RCTs need to be performed (Mari-Bauset,

Zazpe, Mari-Sanchis, Llopis-Gonzalez, & Morales-Suarez-Varela, 2014; Millward et al., 2008). Despite the poor clinical evidence for the efficacy and use of these diets they remain popular and are used as a type of treatment for ASD (Levy & Hyman, 2005).

2.2.2. Feeding and Eating Behaviours

Along with parental dietary restrictions, children with ASD often have unusual feeding patterns and behaviours surrounding food and meal times. The prevalence of feeding problems in children with developmental disabilities varies between 13% and 80% (Coffey & Crawford, 1971; Jones, 1982; Perske et al., 1977). Parents of children with ASD have reported their children to have more feeding issues than neuro typical developing children (K.A Schreck, Williams, & Smith, 2004), such as limited food acceptance which can result in the child eating as few as five different foods (Cermak et al., 2010). Although it is well known through anecdotal evidence and parental reports that children with ASD have feeding difficulties, it has only been confirmed through research in recent times (Cermak et al., 2010).

It has been found that children with ASD have restricted food intake due to limited food variety (Ahearn, Castine, Nault, & Green, 2001), dislike of textures (Ahearn et al., 2001; Archer, Rosenbaum, & Striener, 1991) and refusal of food (Archer et al., 1991). In a parental survey (n=100) of children with ASD, 67% reported their child was a picky eater, with factors having the greatest effect being texture (higher preference for pureed foods), appearance, taste, smell, and temperature of the food (Williams et al., 2000). In addition, eating behaviours most commonly reported were unwillingness to try novel foods, eating very few foods, mouthing objects and certain rituals surrounding eating (Williams et al., 2000). Similarly, K.A. Schreck and Williams (2006) found 72% of children with ASD (n=138) had restricted food variety which was associated with how the food was presented, the use of a particular utensil, different food items touching on the plate, as well as texture and oral motor problems. Additionally this study highlighted the ASD group ate a smaller variety of foods from each food group (fruits, dairy, vegetables, proteins, starches) than normally developing children. An important finding to note from this study is the severity of ASD did not determine the severity of feeding problems. The most significant factor affecting food behaviour in these children was family food preferences, with the children who had the most restrictive diets coming from families who also had restrictive eating practices (K.A Schreck et al., 2004). Other studies have also found children with ASD have high food selectivity (Klein & Nowak, 1999; Whiteley, Rogers, & Shattock, 2000), compared to neuro typical children (Raiten & Massaro, 1986; K.A. Schreck & Williams, 2006; K.A Schreck et al., 2004). Although the evidence appears convincing, it has been noted differing definitions of food selectivity and picky eating between studies makes comparisons and conclusions difficult.

Several hypotheses exist for high food selectivity and feeding difficulties among children with ASD. It has been suggested that children with ASD are supertasters, meaning they are more sensitive to bitter tastes (Cermak et al., 2010). This may lead to food refusal and aversions to bitter foods and the preference for sweeter foods (Bartoshuk, 2000). Another hypothesis is children with ASD have sensory overresponsiveness (Ayres, 1963), i.e. when a child is highly sensitive to touch it may cause negative behavioural responses to external stimuli. This may lead to picky eaters as overresponsiveness in the mouth may contribute to the child having issues with differing food textures, causing them to restrict their intake to textures they can tolerate (Twachtman-Reilly, Amaral, & Zebrowski, 2008; Williams et al., 2000). Feeding issues associated with sensory sensitivity cause increased stress which can affect family meal times and quality of life (Epstein et al., 2008; Groden et al., 2001). Other hypotheses for high food selectivity include (i) learned aversions to food secondary to gastrointestinal problems such as constipation, decreased appetite and gastro-oesophageal reflux disease (D. Field, Garland, & Williams, 2003), (ii) feeding difficulties being an unclassified characteristic of ASD, and (iii) need for repetition and uniformity in daily tasks (Ahearn et al., 2001; Williams et al., 2000).

High food selectivity in children with ASD is of concern as it may persist after early childhood (Tomchek & Dunn, 2007; Twachtman-Reilly et al., 2008). If this is the case, these children will be at risk of nutritional insufficiencies as a result of dietary restriction (Cornish, 1998; Herndon et al., 2009; Raiten & Massaro, 1986; Williams et al., 2000). It has been suggested the most beneficial way of helping ASD children with feeding difficulties is to have a multi-disciplinary approach between dietitians, occupational therapists and psychologists (Cermak et al., 2010). The collaboration of these professions will allow for appropriate foods to be identified, the modification of food texture, provision of specialised eating utensils, as well as altering the environment and behaviour (Cermak et al., 2010). These interventions will help to ensure the child obtains adequate nutrition, reduce stress at family meal times, and ensure optimal health of the child.

2.2.3. Identification of Feeding Issues

As there is a high prevalence of feeding issues in children with ASD, it is important to be able to identify those who have increased feeding problems. The Behavioural Paediatrics Feeding Assessment Scale (BPFAS) has been developed by Crist et al. (1994) to provide insight into the quantity and range of foods consumed, appetite as well as parental concern of their child's feeding. This has been validated for use in both clinical and normal populations between 7 months to 7 years (Crist & Napier-Phillips, 2001).

The BPFAS has also been used in children with ASD to determine behaviours associated with eating in this population group. This tool has been validated for use in young children with ASD to determine feeding problems (Allen et al., 2015). One study using the BPFAS (n=82) found children aged 2-12 years with ASD had slightly more feeding issues than neuro typical children, with ASD children less able to eat independently and less likely to accept new foods (Martins, Young, & Robson, 2008). Although the has been validated and used in ASD populations, it has not be used to determine the association between feeding issues and iron status in children with ASD.

2.2.4. Nutritional Deficiencies

Restricted intake of foods for a prolonged period can pose a potential health risk due to the development of nutritional deficiencies (Cermak et al., 2010). However, although there is an association between ASD and eating problems, studies looking at ASD and nutritional status have found mixed results (Cermak et al., 2010; Raiten & Massaro, 1986; Shearer, Larson, Neuschwander, & Gedney, 1982).

There have been various studies investigating the nutritional status of children with ASD based on the analysis of food records. One such study in China found children with ASD (n=111) had inadequate intakes of vitamins A, B6 and C, folic acid, calcium and zinc (Xia, Zhou, Sun, Wang, & Wu, 2010). Another study of children with ASD (n=17) based in England found one or more of these children had inadequate intakes of iron, vitamins D, C, niacin, riboflavin and zinc (Cornish, 1998). This same study also noted an inverse relationship between dietary variety and nutritional adequacy (Cornish, 1998). Other studies have used control groups of neuro typically developing children to compare dietary intake from food records of children with ASD. Some studies showed no difference between nutrient intakes between these two groups (Lockner, Crowe, & Skipper, 2008; Raiten & Massaro, 1986; Schmitt, Heiss, & Campbell, 2008). One study found children with ASD (n=77) had higher intakes of vitamins B6 and E, and lower intakes of calcium than neuro typical children. This same study also found both groups of children to have inadequate intakes of calcium, iron, vitamin D, vitamin E and fibre compared to the recommended intakes for their age (Herndon et al., 2009). These studies have produced somewhat conflicting results with nutrient intakes being the same, lower or higher in children with ASD compared to neuro typical children. Control groups allow for baseline comparisons in a population sample; therefore eliminating confounding factors and variables which may influence the results. However, not all of these studies had a control group, sample size was often very small, and like all studies investigating dietary intake, under-reporting of dietary intake is a possibility. Most studies didn't make a comparison with food selectivity, making it difficult to determine if having high food selectivity is associated with greater risk of nutritional deficiencies (Cermak et al., 2010).

A number of other studies have assessed nutritional status of children with ASD using plasma concentrations of biomarkers. A study in Arizona found children with ASD (n=55) aged 5-16 years had significantly lower levels of plasma biotin, lithium, calcium and magnesium compared to age matched controls (n=44) (Adams, Audhya, McDonough-Means, Rubin, Quig, Geis, Gehn, et al., 2011). A British study found children between 7-16 years old with ASD (n=12) had significantly lower levels of zinc and copper compared to neuro typical children (n=30) (Jackson & Garrod, 1978). Similarly, a Turkish study on children aged 4-12 years found lower zinc and red blood cell (RBC) concentration in the ASD group (n=45) compared to the control group (n=41) (Yorbik et al., 2004). Two studies found children with ASD to had higher levels of vitamin B6 compared with a control group, which the authors have concluded to be a vitamin B6 metabolic imbalance (Adams, George, & Audhya, 2006; Adams & Holloway, 2004). A study conducted in Egypt found children with ASD (n=70) had lower vitamin D levels when compared to neuro typical children of the same age (n=42) (N.A Meguid, Hashish, Anwar, & Sidhom, 2010), however this is contradictory to another study which found no difference in vitamin D levels between children with ASD (n=55) and a control group (n=44) (Adams, Audhya, McDonough-Means, Rubin, Quig, Geis, Gehn, et al., 2011). Additionally, four studies have found lower plasma omega-3 fatty acid concentrations in children with ASD when compared to a control group (Bell et al., 2004; Bell et al., 2010; Vancassel et al., 2001; Wiest, German, Harvey, Watkins, & Hertz-Picciotto, 2009). This is of clinical significance as omega-3 fatty acids are essential in brain development (Innis, 2007), so therefore deficiency in this population may prevent these children from reaching their full potential. Iron is also an important mineral in brain development, and various studies have found children with ASD to be more at risk of having inadequate iron status (Keen, 2008). This will be covered in further detail in Section 2.6.

2.2.5. Supplementation

Due to nutritional deficiencies in children with ASD parents often choose to supplement their child. An internet survey completed by parents living in America (n=552) of children with ASD found that 43% were using vitamin supplements (Green et al., 2006). This same study found the proportion of children with ASD using supplements were as follows: vitamin C (30.8%), Vitamin B6 (30.1%), essential fatty acids (28.7%), magnesium (26.5%), vitamin A (22.0%), multi-vitamin (15.8%) (Green et al., 2006). Another study also showed the proportion of participants using various vitamin and mineral supplements: omega-3 fatty acids (29.2%), vitamin C (18.8%), vitamin B12 (16.8%), and vitamin B6 and magnesium (16.8%) (Hall & Riccio, 2012). The use of iron supplementation was not investigated in this study.

Additionally, the effects of supplementation have been studied in terms of the effects of supplementation on behaviour, sleep, gastrointestinal function, communication and language. A

randomised, double-blind, placebo controlled study (n=141) found significant improvements in language, tantrums, hyperactivity and overall symptoms after children with ASD took a multi-vitamin/mineral supplement for three months (Adams, Audhya, McDonough-Means, Rubin, Quig, Geis, & Gehn, 2011). They also found the children with the lowest baseline levels of vitamin K and biotin improved the most with supplementation (Adams, Audhya, McDonough-Means, Rubin, Quig, Geis, & Gehn, 2011). Another study (n=44) found micronutrient supplementation was on par or more effective than standard medication when looking at benefits from measures such as the Childhood Autism Rating Scale, Childhood Psychiatric Rating Scale, Clinical Global Impressions, and Self-Injurious Behaviour, when compared to the control group (n=44) (Mehl-Madrona, Leung, Kennedy, Paul, & Kaplan, 2010). Another randomized, double-blind, placebo-controlled study (n=20) found a moderate dose multi-vitamin/mineral supplement for three months improved sleep and gastrointestinal function in children with ASD (Adams & Holloway, 2004). Several studies have shown children with ASD have vitamin B6 levels higher than the reference range before supplementation (Adams et al., 2006; Adams & Holloway, 2004). However, it has also been found that these children have low pyridoxal kinase activity, an enzyme responsible for converting vitamin B6 into its active form. Therefore a high dose of vitamin B6 has been found to be beneficial in aiding the conversion of B6 into its active form, which is required for many reactions such as the formation of neurotransmitters (Adams et al., 2006; Adams & Holloway, 2004). This has been associated with improved behaviour such as reduced physical aggression and improved social interactions (LeLord, Muh, Barthelemy, Martineau, & Garreau, 1981).

Additionally, there have been studies which have investigated the effects of essential fatty acid supplementation on children with ASD. Studies have found fish oil supplementation not only improves omega-3 fatty acid concentrations in these children (Bell et al., 2010; N. A. Meguid, Atta, Gouda, & Khalil, 2008) but can cause improvements in language and learning skills (Patrick & Salik, 2005) as well as communication and social interaction (Yui, Koshiba, Nakamura, & Kobayashi, 2010). However one open-label supplementation study in young adults with ASD (n=19) did not show any significant improvements in autistic symptoms or behaviour, although the sample size was small (Politi et al., 2008). Two other studies have found no significant improvements with hyperactivity after fish oil supplementation (Amminger et al., 2007; Bent, Bertoglio, Ashwood, Bostrom, & Hendren, 2011). It has been suggested that supplementation with fish oil may pose the most benefits for children who have poor fish intake, and a treatment may need to be longer than 12 months in order to experience maximum benefits (Adams, 2013). Studies have also looked at the effects of vitamin D supplementation on children with ASD, as it had been suggested vitamin D deficiency is a potential environmental risk factor for ASD (Kocovska, Fernell, Billstedt, Minnis, & Gillberg, 2012). A clinical

review of the literature concluded that although evidence is limited vitamin D may have a role in ASD pathogenesis through its roles in brain homeostasis and gene regulation (Kocovska et al., 2012). Further research in this field is required.

The behavioural effects of iron supplementation in children with ASD has only been investigated in one study (n=33) (Dosman et al., 2007). The study found iron supplementation for eight weeks resulted in 77% of participants having improved sleep as well as improved mean SF, mean corpuscular volume, and haemoglobin (Hb) concentrations.

2.3. Iron

2.3.1. Biochemistry

There are two forms of iron, the reduced form known as ferrous iron (Fe^{2+}), and the oxidised form, ferric iron (Fe^{3+}) (Geissler & Singh, 2011). Iron not bound to a protein is referred to as free iron and has the ability to form free radicals and cause cell damage (C. A. Smith, Mitchinson, Aruoma, & Halliwell, 1992; Sussman, 1992). Therefore it is imperative for iron regulation to be appropriately controlled at the absorption, transportation, delivery and storage stages to ensure correct bodily functioning and to minimise free radical production (Lieu et al., 2001).

2.3.2. Function

Iron is an essential nutrient, especially during periods of growth and development in children and adolescence (Ministry of Health, 2012). Iron is stored in different forms in the body and thus has a variety of roles. Iron is involved in oxygen transport; DNA and RNA synthesis; electron transport; cellular respiration, proliferation and differentiation; and the regulation of gene expression (Andrews, 1999; Boldt, 1999; Conrad, 1999; Gerlach, Ben-Shachar, Riederer, & Youdim, 1994; Wessling-Resnick, 1999). It also has a major role in brain development by participating in the formation of myelin sheaths, dendrites and neurotransmitters (J.L Beard et al., 1992). Therefore maintaining iron homeostatis is particually important for normal brain development, particually for learning and memory (Gerlach et al., 1994).

2.3.3. Iron Absorption

Iron absorption occurs primarily in the crypt cells of the duodenum and jejunum, allowing iron to enter into systemic circulation (Conrad, Parmley, & Osterloh, 1987; Wood & Han, 1998), Figure 2.1 shows the mechanisms of iron absorption. Ferric iron (Fe^{3+}) needs to be reduced in the small intestine via ferrireductase to ferrous iron (Fe^{2+}) (Ekmekcioglu, Feyertag, & Marktl, 1996; Riedel, Remus, Fitscher, & Stremmel, 1995) before absorption can occur (Conrad, 1999), as ferrous iron is more efficiently absorbed (Conrad, Weintraub, Sears, & Crosby, 1966).

Dietary iron is classified into two forms, haem iron and non-haem iron. Haem iron is derived from Hb and myoglobin in animal protein sources such as red meat, fish and poultry (Lynch & Cook, 1980). Non-haem iron is also found in meat products as well as plant foods such as green leafy vegetables, legumes, breads, cereals and nuts. Along with differing levels of solubility, the absorptive mechanisms of haem and non-haem iron also differ.

In the intestinal lumen, duodenal cytochrome B reductase (Dcytb) converts non-haem iron into a more soluble form. Ferrous iron is then specifically absorbed into the enterocyte via the divalent metal ion transporter 1 (DMT1), (Fleming et al., 1997; Gunshin & Mackenzie, 1997) which is located on the brush border of the cell with the highest concentration being in the duodenum (Canonne-Hergaux, Gruenheid, Ponka, & Gros, 1999; Gruenheid, Cellier, Vidal, & Gros, 1995; Gunshin & Mackenzie, 1997). Once inside the enterocyte, iron enters an iron pool where is can be used within the cell for mitochondrial haem synthesis or stored as ferritin, otherwise it is transported to the basal membrane where it enters systemic circulation via ferroportin-1 (FPN1) (Conrad, 1999). The destination of the iron is dependent on the body's requirements (Geissler & Singh, 2011).

The mechanism of haem iron absorption into the enterocyte is not as well understood as non-haem iron (Qiu et al., 2006). Haem enters the enterocyte via haem carrier protein -1 (HCP1). Some research indicates haem iron is digested by enzymes in the intestinal lumen before entering the enterocyte (Majuri & Grasbeck, 1987) where it is broken down by haem oxygenase to release ferric iron (Uzel & Conrad, 1998). This iron then enters the same iron pool within the enterocyte and has the same end points as non-haem iron depending on cellular need (Lieu et al., 2001).

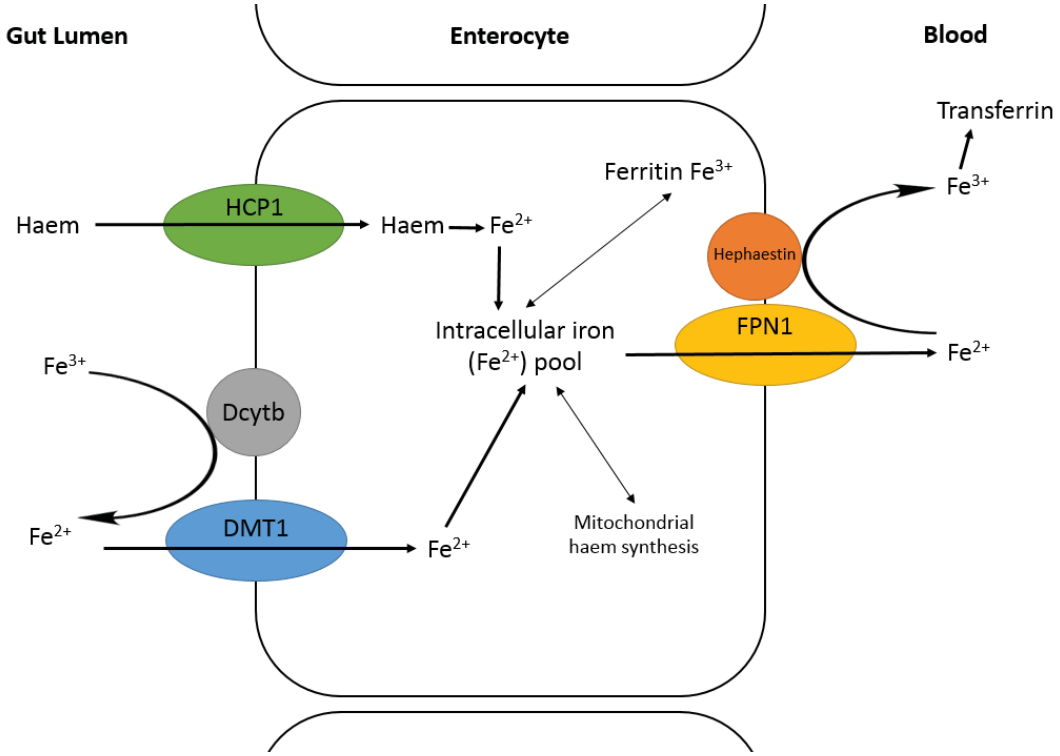

Figure 2.1: Mechanism of iron absorption from gut lumen, through the enterocyte and into the circulating blood. Dcytb = Duodenal Cytochrome B reductase; DMT1 = Divalent Metal Ion Transporter-1; Fe^{2+} = ferrous iron; Fe^{3+} = ferric iron; FPN1 = Ferroportin-1; HCP1 = Haem Carrier Protein-1.

2.3.4. Factors Affecting Iron Absorption

Dietary factors have a major influence on iron absorption. These factors include the quantity of food consumed which contains iron, type of dietary iron, as well as the presence of other dietary factors which may enhance or inhibit iron absorption.

Once consumed approximately 15-35% of haem iron is absorbed, however this may vary from 10% during periods of iron sufficiency to 40% if an individual is iron deficient (Hercberg, Preziosi, & Galan, 2007). Haem iron is less prone to the influence of gastric pH as well as dietary factors, however the presence of calcium inhibits haem iron absorption (Hallberg, Brune, Erlandsson, Sandberg, & Rossander-Hulten, 1991) while meat and soy protein have been shown to enhance haem iron absorption (Hallberg, Bjorn-Rasmussen, Howard, & Rossander, 1979).

Non haem iron makes a large contribution to the body's iron pool as it is a component in a wide range of foods (Monsen, 1988). However, non-haem iron has poorer bioavalibility with 2-20% being absorbed and it is significantly influenced by other dietary factors (Monsen, 1988). Non-haem ferric iron absorption is enhanced by ascorbic acid (J.D Cook & Monsen, 1977; Lynch & Cook, 1980) by increasing the intestinal lumen acidity. Meat also contains the meat, fish, poulty (MFP) factor, which enhances non-haem iron absorption although the mechanism is unclear (Monsen et al., 1978). These

factors increase absorption via converting ferric iron to ferrous iron or by maintaining the iron in a soluble form once released from food (Lieu et al., 2001). In addition, some evidence suggests vitamin A also enhances the absorption of non-haem iron by counteracting the inhibitory effects of tannins and phytates (Garcia-Casal, Layrisse, & Solano, 1997). However, this is controversial as another study found vitamin A did not enhance iron absorption (Walczyk, Davidsson, Rossander-Hulthen, Hallberg, & Hurrell, 2003).

Non-haem iron bioavailability is also influeced by dietary factors which may inhibit absorption. These include polyphenols (found in tea, coffee, legumes and cereals) (Hurrell, Reddy, & Cook, 1999), phytates (found in wheat, cereals, nuts and seeds) (Hallberg, Brune, & Rossander, 1989) calcium (Hallberg et al., 1991) and soy protein (J.D Cook, Morck, & Lynch, 1981). These inhibit absorption by binding iron to form an insoluble complex which cannot enter the enterocyte (Disler et al., 1975). It has also been discovered that high doses of minerals such as zinc, copper, cadmium, cobalt and maganese can prevent absorption of non-haem iron through competing with the DMT1 for transport from the intestinal lumen into the enterocyte (Monsen, 1988; Rossander-Hulten, Brune, Sandstrom, Lonnerdal, & Hallberg, 1991; Solomons, 1986).

2.3.5. Iron Storage

The liver is the main iron storage site in the body, with two thirds of iron attached to the storage protein ferritin (Geissler & Singh, 2011). The highest concentration of ferritin is in the liver, with the spleen and bone marrow also having high concentrations (Geissler & Singh, 2011). Synthesis of ferritin is increased during iron sufficiency and decreased during ID and IDA (Gdaniec, Sierzputowska-Gracz, & Theil, 1998; Zahringer, Baliga, & Munro, 1976). SF can be used as a biomarker to determine iron stores as there is a positive relationship between the amount of iron stored and the concentration of SF (Walters, Miller, & Worwood, 1973). The role of ferritin to bind iron is important as it prevents free iron forming reactive oxygen species and cell damage (Theil, 1998).

2.3.6. Iron Homeostasis

The regulation of iron concentration in the body is vital to prevent deficiency as well as toxicity. This is occurs by ensuring the absorption of iron offsets losses through desquamated skin cells, sweat, urine, gastrointestinal secretions, hair, and blood losses such as menstruation in premenopausal women (Geissler & Singh, 2011). If stores become too low due to inadequate absorption, tissue iron stores become exhausted (Geissler & Singh, 2011). This in turn leads to reduced iron delivery, causing iron dependant functions to become impaired. Erythropoiesis without iron leads to reduced Hb production and IDA (Geissler & Singh, 2011). On the contrary, excessive iron intake can lead to an increase in free

radical production and cause cellular damage, iron imbalance, neurodegenerative disorders and possibly some cancers (C. A. Smith et al., 1992; Sussman, 1992).

Iron absorption is regulated in a variety of ways, but the main regulatory mechanism is systemic need (Finch, 1994). Hepcidin, a hormone synthesised in the liver controls iron absorption (Ganz, 2004) by down regulating iron absorption in the small intestine as well as inhibiting the release of iron from macrophages and hepatocytes (Nicolas et al., 2002). Thus, in times of iron sufficiency and inflammation hepcidin production is increased (reducing iron absorption), and during ID and IDA hepcidin synthesis is suppressed (enhancing iron absorption) (Geissler & Singh, 2011). The second regulatory mechanism is the mucosal block (Andrews, 1999). This refers to the inhibition of iron absorption after an initial exposure to dietary iron due to the inability of intestinal enterocytes to absorb iron. Maturing enterocytes with iron absorbing capacity need to move up the villi before absorption can take place (Geissler & Singh, 2011). This may result in a delay of one to two days before intestinal changes allow the absorption and transport of iron to match systemic need (Andrews, 1999). Therefore the mucosal block may also occur in an iron deficient state (Frazer et al., 2003). The third iron absorption regulatory mechanism is the erythropoietic factor, this impacts iron absorption depending on need for the production of RBC (Finch, 1994).

2.3.7. Stages of Iron Deficiency

A continuum of ID exists from iron stores being depleted, to impaired functional capacity of Hb due to reduced production of RBCs. The stages of ID are outlined in Table 2.1 below. The first stage of ID is termed iron depletion where iron stores decline, indicated by decreased concentration of serum SF to reflect low stores in the liver, spleen, and bone marrow (P. R. Dallman, 1986). During iron depletion, Hb concentration remains within the normal reference range. The second stage of ID is classified by the exhaustion of iron stores, reduced serum iron and iron transportation (P. R. Dallman, 1986) to levels which are insufficient to meet requirements for normal cellular function. However the rate of erythropoiesis is still adequate (DeMaeyer et al., 1989). In this second stage functional and physiological changes effect to some organs despite I concentrations being within the normal range (J. L. Beard & Connor, 2003). The third stage is IDA which occurs when the physiological function of Hb is impaired due to a reduction in erythropoiesis (Dallman, 1986). IDA is officially identified when the Hb concentration is more than two standard deviations below the mean Hb concentration in an age, gender and altitude matched population (World Health Organisation, 2001).

Table 2.1. Stages of iron deficiency (Coad & Conlon, 2011; R.S Gibson, 2005; Lee & Nieman, 2010)

	Normal	**Iron Depletion**	**Iron Deficiency without Anaemia**	**Iron Deficiency with Anaemia**
Total iron binding capacity (µg/dL)	330±30	360	390	410
Serum ferritin (µg/L)	100±60	20	10	<10
Serum iron (µg/dL)	115±50	115	<60	<40
Transferrin saturation (%)	35±15	30	<15	<15
Soluble transferrin receptors	Normal	Normal	High	High
Haemoglobin (g/L)	120-160	120-160	120-160	<120

2.3.8. Iron Biomarkers

The various biomarkers used to determine the iron status of an individual, each have their own advantages and disadvantages which are shown in Table 2.2. Due to various limitations and benefits of each biomarker it is recommended that several biomarkers be used to determine iron status as opposed to just one (Burke, Leon, & Suchdev, 2014).

Table 2.2: Advantages and disadvantages of biomarkers used to assess iron status

Biomarker	Uses/Measures	Advantages	Disadvantages
Serum Ferritin (SF)	Iron storage	- Sensitive indicator of ID - Responds well to iron interventions	- An acute phase protein so increases during inflammation, possibly masking ID
Haemoglobin (Hb)	Assess anaemia	- Easy and cheap - Good screening tool for severe ID	- Not specific or sensitive to iron status - Measures function rather than status
Haematocrit (Hct)	Percentage of RBCs in blood	- Easy to measure	- Provides no extra information above Hb
Mean Cell Volume (MCV)	Indicates IDA when MCV is low and red cell distribution is high	- Useful in clinical setting	- Levels decrease late in ID - Doesn't represent iron status
Serum Iron	Circulating iron	- Differentiates between ID and anaemia associated with chronic disease, inflammation or neoplastic disease	- Contaminated by iron from other sources - Affected by time of day and after eating - Doesn't detect iron bound to Hb
Total Iron Binding Capacity (TIBC)	Iron binding sites on transferrin	- More stable than other measures	- Only changes with depletion of iron stores
Transferrin saturation	Circulating iron	- Differentiates between ID and anaemia associated with chronic disease, inflammation or neoplastic disease	- Decreased concentration during inflammation
Soluble Transferrin Receptor (sTfR)	Upregulated in state of ID	- Good accuracy in paediatric population with prolonged infection - Less sensitive to inflammation than SF	- Levels change late in ID - Affected by other causes of low RBC production - Expensive
Transferrin receptor to serum ferritin ratio (TfR:SF)	Proportional to stored iron or iron deficit	- High sensitivity in response to iron supplementation	- Not validated in paediatric population - SF responses to inflammation - Assay dependant
Reticulocyte Haemoglobin concentration	Measures iron in RBC indicating iron availability to cells	- Accurate when compared to other biomarkers - Recommended for paediatric populations - Not affected by inflammation - Gold standard for ID diagnosis	- Assay not widely available

Zinc Protoporphyrin (ZPP)	Reflects iron shortage in the last step of Hb formation	- Sensitive indicator of severe ID, but not of moderate ID - Little blood volume is needed	- Not specific to iron, affected by lead poisoning and inflammation - Ranges not well defined for infants
Hepcidin (Hep)	Liver hormone involved in iron homeostasis. Biomarker of iron status and function.	- Increased levels during iron sufficiency and decreased during ID - Blood or urine can be used	- Normal range not well defined, limiting its use - Influenced by inflammation

ID = Iron Deficiency; IDA = Iron Deficiency Anaemia; RBC = Red Blood Cell

2.3.9. Clinical Presentation of Iron Deficiency

IDA can be characterised by fatigue, light headedness, weakness, pallor, faintness, lack of concentration, and lack of immunity. These symptoms are not specific to IDA, so to confirm diagnosis biochemical lab tests are required (James D. Cook, 2005), with fatigue being the main reason Hb levels are checked (Killip, Bennett, & Chambers, 2007). The biochemical measurements to diagnose ID and IDA are discussed in greater depth in Section 2.3.8.

2.3.10. Determinants of Iron Status

ID has become an important public health issue (Andrews, 2008) as it is the most common micronutrient deficiency throughout the world (DeMaeyer & Adiels-Tegman, 1985). Both dietary and non-dietary factors can influence the iron status of children. It is important to discuss these factors so they can be targeted in the treatment and prevention of ID and IDA.

The physiological demand for iron is the most important factor in iron status (J.D Cook, 1990). Approximately 12-14 µg of iron per kg is lost from the body daily from the gastrointestinal tract, urinary tract, and the skin (J.D Cook, 1990). Additionally iron requirements increase during periods of rapid growth and development, such as during childhood and adolescence. This is due to the growth of body tissues as well as the expansion of the red cell mass (J.D Cook, 1990). The growth demand declines during late childhood, but increases again during adolescence, additionally adolescent females are at increased ID risk due to blood losses through menstruation (D. E. Scott & Pritchard, 1967).

Dietary supply of iron incorporates both the amount of dietary iron consumed as well as the bioavailability of the iron. Dietary factors associated with ID and IDA include the consumption of foods high in haem and non-haem iron, as well as the intake of other dietary components which may enhance or inhibit dietary iron absorption.

A limited amount of studies have looked at the impact dietary factors have on iron status in children. One longitudinal Swedish study (n=127) investigated dietary factors affecting Hb and SF concentrations in children aged 6-12 months, with a follow up at four years of age. No associations were found between mean daily iron, meat, ascorbic acid, calcium or dairy product consumption and Hb concentrations. A significant correlation was found only in boys between meat consumption and SF concentrations (Ohlund, Lind, Hornell, & Hernell, 2008). One cross-sectional Icelandic study (n=130) in children aged two years found children who consumed more than 500g of cow's milk per day had increased risk of having a poor iron status (Gunnarsson, Thorsdottir, & Palsson, 2004). Another cross-sectional study in Iceland (n=188) in children aged 6 years found iron status was positively associated with meat, fish, juice, and multi-vitamin supplementation, and negatively associated with dairy products (Gunnarsson, Thorsdottir, & Palsson, 2007). A large British study (n=1859) in pre-school

children found children consuming >400mg per day of milk or cream were less likely to consume foods from other food groups. Children who had high dairy product consumption in addition to eating small amounts of meat, fish, fruit and nuts had the highest risk of ID. However this became non-significant when consumption of foods which enhance iron status increased (Thane, Walmsley, Bates, Prentice, & Cole, 2000). Another cross-sectional UK study (n=904) found pre-school children who had a high cereal consumption had significantly higher iron intakes than low cereal consumers. However, those with high cereal intakes did not have higher SF levels and ate less vitamin C and meat. Therefore dietary advice should be to increase the amount of iron-rich foods eaten, as well as to modify the diet to improve absorption (Gibson, 1999).

There are several limitations to these studies, most have relatively small sample sizes, therefore may not have identified small associations between dietary factors and iron biomarkers. Although observational data takes into account adaptive responses and looks at the diet as a whole, correlations can be misleading if dietary data is incorrect and measures of iron status are not sensitive. Additionally most studies only analyse a small range of exposures and do not take into account non-dietary factors which may influence iron status, such as inflammation.

Duration of breastfeeding and the type of first foods given to a child have major influence on iron status during infancy and early childhood. Although both breast milk and cows' milk contain the same amount of iron (0.5-1mg/L), the bioavailability of the iron is different, with approximately 50% of iron in breast milk absorbed compared to about 10% in whole cows' milk (Oski, 1993). The current recommendation is for infants to be exclusively breastfed for their first six months of life, at which point complementary foods high in iron should be introduced (Kramer & Kakuma, 2001). The rationale behind this is that at six months of age, iron stores acquired during gestation decline due to rapid growth and RBC expansion, therefore dietary iron intake requirements exceed that received from breastmilk (Zimmermann & Hurrell, 2007). The prevalence of ID in infants and young children has declined in the USA following the introduction of iron fortified complementary foods in the 1970s (Yip, Binkin, Fleshood, & Trowbridge, 1987). It is also recommended children do not drink cows' milk in their first year of life as the consumption of cows' milk is negatively associated with iron status at age 12 months (Freeman, Mulder, Van't Hof, Hoey, & Gibney, 1998).

A number of non-dietary factors may also influence iron status. Studies have shown IDA is more prevalent in those of lower socioeconomic status as well as in developing countries (Muller & Krawinkel, 2005). This may be due in part to the use and availability of non-iron rich complementary foods (World Health Organisation, 2001). In addition ID is also common in late infancy and early childhood in children who belong to social and ethnic minority groups (Fernandez-Ballart, Domenech-

Massons, Salas, Arija, & Marti-Henneberg, 1992; Mekki, Galan, Rossignol, Farnier, & Hercberg, 1988; Warrington & Storey, 1989). Children who are born prematurely are at increased risk of ID as 80% of iron accretion occurs in the last trimester of pregnancy (Baker & Greer, 2010). Another risk factor for ID is high body weight during childhood. One study (n=9698) found children who were overweight or who were at risk of being overweight were twice as likely to have ID compared to those who were not overweight (Nead, Halterman, Kaczorowski, Auinger, & Weitzman, 2004). The health implications of ID and IDA during childhood are discussed in further detail in Section 2.4.

2.3.11. Dietary Methods to Assess Iron Related Nutrition

As discussed in Section 2.3.4, iron status is influenced by dietary iron intake as well as dietary factors which may enhance or inhibit the absorption of iron. To determine the association between dietary intake and iron status it is important to investigate the diet a whole (K.L Beck & Health, 2013).

To assess dietary intake, five main methods or tools are used (R. S. Gibson, 2005): (i) weighed food records, when the participant records and weighs each food item and beverage at the time they are consumed; (ii) estimated food records involves the participant recording food items and beverages at the time they are eaten based on estimations using household measures, food packaging and photographs; (iii) a food frequency questionnaire (FFQ) requires the participant to record the frequency of foods from a list consumed over a defined period of time; (iv) a 24 hour recall requires recollection of food and drink items consumed the day prior or over a 24 hour period; and (v) a diet history involves the recall of usual daily food intake and an FFQ to determine consumption of key food items (K.L Beck & Health, 2013). The type of dietary analysis method used is determined by a number of factors including the literacy and numeracy skill of participants, financial resources, if the dietary data are needed for groups or individuals, and if the goal of the research is to determine nutrients, food or dietary patterns (K.L Beck & Health, 2013). Each dietary method has advantages and disadvantages which are summarised in Table 2.3 (Ambrosini et al., 2012; Arab, Estrin, Kim, Burke, & Goldman, 2011; Baranowski, Sprague, Baranowski, & Harrison, 1991; Kesse-Guyot et al., 2012; Kim, Jo, & Joung, 2012; Meyer et al., 2011; Ribas-Barba, Serra-Majem, Roman-Vinas, Ngo, & Garcia-Alvarez, 2009; Stumbo, 2013).

Table 2.3: Advantages and disadvantages of methods used to assess dietary intake

Method	Advantages	Disadvantages
Weighed food record	- Used in groups or individuals - Determines actual or usual intake if enough days are collected - Risk of inadequate intake can be calculated if enough days are collected	- High participant and researcher burden - High literacy and numeracy skills - High cost - Inaccuracies in recording when child is away from home
Estimated food record	- Quantities based on household measures - Valid and reliable - Used in groups or individuals - Determines actual or usual intake if enough days are collected - Less time consuming and flexible weighed food records - Risk of inadequate intake can be calculated if enough days are collected	- High literacy and numeracy skills - High cost - Inaccuracies in recording when child is away from home
FFQ	- Identifies usual intake - Low numeracy and literacy skills needed - Low participant burden - Low cost	- Can only be used in groups - Doesn't identify actual intake - Needs to be validated in population group before using - Doesn't calculate risk of adequate intake
Single 24-hour recall	- Appropriate for use in groups - Identifies actual and usual intake - Low numeracy and literacy skills needed - Risk of inadequate intake can be calculated	- Not appropriate for individuals - Moderate participant burden - Moderate cost
Multiple 24-hour recall	- Used in groups or individuals - Identifies actual and usual intake if enough days are collected - Low numeracy and literacy skills needed - Risk of inadequate intake can be calculated if enough days are collected	- High participant burden - High cost
Diet history	- Appropriate for use in individuals - Estimates usual intake - Low numeracy and literacy skills needed	- Not appropriate for use in groups - Unable to estimate actual intake - Moderate participant burden - Moderate cost - Doesn't calculate risk of inadequate intake

FFQ = food frequency questionnaire

2.4. Iron Importance during Childhood

2.4.1. Brain Development and Cognitive Performance

Many studies have investigated the importance of iron for brain development and function (J.L Beard, Connor, & Jones, 1993; Lozoff et al., 1987; Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998). The accumulation of iron in the brain varies depending on the brain region (J. L. Beard & Connor, 2003). The nucleus accumbens, substantia nigra, deep cerebellar nuclei, red nucleus and parts of the

hippocampus have the highest iron concentration (Yehuda, 1990). The concentration of cerebral iron is tightly controlled through homeostatic mechanisms which are also influenced by dietary iron intake (Pinero, Li, Connor, & Beard, 2000). The majority of brain iron is found in the microglia and oligodendrocytes (J.L Beard et al., 1993; J. R. Connor et al., 1987; Erikson, Byron, & Beard, 2000) and is therefore essential for monoamine neurotransmitter synthesis and also for the enzymes involved in the myelinisation process (Lozoff, 2007; McCann & Ames, 2007). The development of the central nervous system is dependent on enzymes and proteins which contain iron (Lozoff, 2007). Animal studies have found ID alters brain development by affecting the formation of myelin sheaths thus impairing neural speed transmission (J. R. Connor & Menzies, 1996). ID also alters brain cell structure and function by impairing neuronal metabolism, growth of dendrites and synapse formation in animal studies (P.R Dallman & Spirito, 1977; Jorgenson, Wobken, & Georgieff, 2003; Pokorny & Yamamoto, 1981; R. Rao, Tkac, Townsend, Gruetter, & Georgieff, 2003; Rihn & Claiborne, 1990).

Neural developmental impairments due to ID are associated with altered learning, attention, memory and psychomotor functions (Lozoff, 2007). One study tested cognitive development in children at both three and five years of age (n=55). Those children who had IDA had significantly lower cognitive scores than children of the same age who had normal iron status (Palti, Meijer, & Adler, 1985). Another study showed children who had been iron deficient as infants (n=48) had poorer arithmetic, writing, reading, school progress, motor function and also experienced more anxiety, depression and social problems than children who had normal iron status in infancy (n=114) (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000). ID in children three years and younger poses the most detrimental effects to psychomotor and neurologic development (J. R. Connor & Menzies, 1996; Lozoff, 2007; Roncagliolo et al., 1998; Wang, Zhan, Gong, & Lee, 2013).

The use of iron supplementation to improve or reverse the negative effects of ID or IDA in children has been relatively inconclusive. If ID occurs during infancy, the neural developmental implications are less likely to be reversed with iron supplementation. A systematic review of randomised control trials (RCTs) and preventative trials in ID infants, concluded there were no improvements in cognitive development when supplemented with iron (Grantham-McGregor & Ani, 2001). However, the authors suggested more research was necessary to investigate if the irreversible developmental impairments in infants is due to ID or due to poor social backgrounds (Grantham-McGregor & Ani, 2001). Conversely, if ID occurs in children older than two years of age, results from RCTs show iron therapy in these children can reverse developmental impairments (Pollitt, Leibel, & Greenfield, 1983; Pollitt, Saco-Pollitt, Leibel, & Viteri, 1986; Soewondo, Husaini, & Pollitt, 1989; Stoltzfus et al., 2001).

2.4.2. Behaviour

As iron is a mineral involved in brain development, some studies have identified ID and IDA to be associated with increased behavioural problem incidents (Idjradinata & Pollitt, 1993; Lozoff et al., 1987; Lozoff et al., 2000; Lozoff, Wolf, & Jimenez, 1996), fewer social interactions and impaired learning in children (Grant, Wall, Brewster, et al., 2007; Grantham-McGregor & Ani, 2001; Lozoff, 2007). Another study (n=33) showed that restless sleep in children with ASD and ID significantly improved after 8 weeks of iron supplementation, suggesting a link between ID and sleep disturbance (Dosman et al., 2007). However, other studies done in children with ASD indicate there is no association of ID with behaviour (Bilgic et al., 2010; Dosman et al., 2007).

2.4.3. Growth

During periods of growth, the demand for iron increases due to red cell mass expanding as well as increased energy requirements (Yip, Johnson, & Dallman, 1984). It has been assumed ID will impair growth as iron is an important factor in immune function, appetite, thermogenesis and thyroid hormone metabolism (J.L Beard et al., 1983; Lawless, Latham, Kinoti, Pertet, & Stephenson, 1994). Studies have found children with IDA weighed less and were shorter than typical children (Judisch, Naiman, & Oski, 1966; Lozoff, Brittenham, Viteri, & Urrutia, 1982), and observational studies have reported impaired physical growth (Owen, Lubin, & Garry, 1971; K. V. Rao, Radhaiah, & Raju, 1980). However findings from observational studies may be limited due to confounding factors such as: (i) parasites causing ID and impairing growth, (ii) speed of growth impacting iron status, for example children with slow growth may appear iron replete and those with rapid growth may appear iron deficient, (iii) micronutrient deficiencies e.g. zinc, may also impair growth (Sachdev, Gera, & Nestel, 2007). A review of RCTs found supplementation with iron did not improve physical growth in children (Ramakrishnan, Aburto, McCabe, & Martorell, 2004; Sachdev et al., 2007).

2.4.4. Immune Function

Iron is an essential mineral needed for cellular proliferation, therefore it is also necessary for normal maturation and development of the immune system to elicit a specific response to infection (J. Beard, 2001; Kumar & Choudhry, 2010). Iron is an important component of enzymes involved in the immune response as well as the production and function of cytokines (J. Beard, 2001). Therefore maintaining a normal iron status is crucial to prevent susceptibility towards infection (Das et al., 2014).

The effect of iron depletion on immunity has been studied extensively with evidence suggesting a greater effect on cell mediated immunity than humoral immunity (Kumar & Choudhry, 2010). Studies investigating the effect of ID on humoral immunity show conflicting results with some studies showing no effect on immune function (Ekiz, Agaoglu, Karakas, Gurel, & Yalcin, 2005; Feng, Yang, & Shen, 1994; Sadeghian et al., 2010) and others indicating reduced serum immunoglobulin

concentrations (Guzikowska et al., 1988). Differences in these studies may be due to differences in sample size or selection criteria (Das et al., 2014).

It has been shown that children with ID have suppressed cell mediated immunity due to lower levels of T-lymphocytes, CD4+ cells and reduced CD4:CD8 ratio when compared to a control group (Das et al., 2014). Additionally, ID can decrease macrophage bacterial action (Hallquist, McNeil, Lockwood, & Sherman, 1992) as well as neutrophil activity (Spear & Sherman, 1992). With iron supplementation, the reduced cell mediated response can mostly be reversed (Das et al., 2014; Kuvibidila, Kitchens, & Baliga, 1999; Spear & Sherman, 1992).

Therefore ID can increase the susceptibility towards infection. However these studies should be interpreted carefully as ID often occurs alongside poverty, malnutrition and other nutritional deficiencies (J. Beard, 2001) which are also associated with higher infection rates.

2.5. Iron Status, Intakes and Requirements of Children Living in New Zealand

2.5.1. Prevalence of Iron Deficiency and Iron Deficiency Anaemia

Studies in the 1960s found IDA had high prevalence in children and infants in New Zealand, with more severe cases in Māori infants and children (Akel et al., 1963; Neave, Prior, & Toms, 1963; Reeves, 1968). Rates of IDA at six months of age ranged from 58% to 75% in Māori infants compared to 29% in European infants (Akel et al., 1963; Neave et al., 1963).

Results from the 2002 National Children's Nutrition Survey (Ministry of Health, 2003) identified the prevalence of ID without anaemia in New Zealanders aged 5-6 years to be 0.1% for males and 0.0% for females. The prevalence slightly increased for children between the ages of 7-10 years to 0.7% for both male and females. The National Children's Nutrition Survey found IDA in 0.0% of children between the ages of 5-6 years, however, the prevalence increased to 0.2% for males and 0.1% for females between the ages of 7-10 years (Ministry of Health, 2003). This survey didn't include the iron status of children aged 2-4 years, however regional studies indicate this population may be at risk of ID (two of the following: SF < 10µg/L, Fe saturation <10%, or MCV < 73fl) as it is present in 4-14% of infants and toddlers (Grant, Wall, Brunt, Crengle, & Scragg, 2007; Soh, Ferguson, McKenzie, Homs, & Gibson, 2004).

It has also been found that in New Zealand ethnicity is a bigger risk factor than socioeconomic status for ID in infants and toddlers, with lower prevalence in New Zealand Europeans and higher rates in Māori, Pacific, South-East Asian, Chinese and Indian (Grant, Wall, Brunt, et al., 2007).

Epidemiology studies from other countries indicate ID and IDA continues to be a major public health concern for young children. IDA rates in children under the age of six years range between 47.7-

61.3% in Guatemala, Haiti and Bolivia and between 20.1-37.3% in Nicaragua, Brazil, Mexico, Ecuador, El Salvador, Cuba, Colombia, the Dominican Republic, Peru, Panama and Honduras (Mujica-Coopman et al., 2015). The prevalence of IDA in children in developed countries is much lower, with 1.1% in Australia (Karr et al., 1996), one in 12 children in the United Kingdom (Gregory, Collins, Davies, Hughes, & Clarke, 1995), and less than 1% in American children (Looker, Dallman, Carroll, Gunter, & Johnson, 1997).

2.5.2. Iron Intakes and Requirements for Children

The National Children's Nutrition Survey (n=3275) provides information on the daily iron intake of children living in New Zealand (Ministry of Health, 2003). It found for children aged 5-6 years the median intake was 10mg and 8.4mg for males and females respectively. The median iron intake was higher in Māori females (10.1mg) compared to Pacific (9.3mg) and New Zealand European and other (NZEO) females (9.4mg). Pacific males had the lowest iron intake (10.6mg), followed by NZEO (12.4mg) and Māori males had the highest intake (13.3mg).

The survey also determined the proportion of children to have inadequate iron intake (Ministry of Health, 2003). They found 6.6% of children had inadequate dietary iron intake compared to the recommended intake, with more females having inadequate intake compared to males. It also found children aged 5-6 years were more likely to have an adequate iron intake when compared to children aged 7-10 years, males, and females aged 11-14 years who were not menstruating. Bread, beef and veal, potatoes, kumara, taro, beverages, vegetables, fruit and biscuits provided the largest source of iron in New Zealand children's diets.

The dietary iron requirements for children vary between ages, with differences in daily recommendations between countries. The dietary iron requirements for age and country are summarised in Table 2.4 below. The Australia New Zealand Nutrient Reference Values (NRV) are based on 14% iron absorption for 1-3 year olds and 18% for all other ages (Ministry of Health, 2006). The United Kingdom reference values are based on 15% iron absorption (Scientific Advisory Committee on Nutrition, 2010). For the United States and Canada reference values, the bioavailability of iron was estimated to be 18% for individuals with low iron stores, and 10% for vegetarians (Trumbo, Yates, Schlicker, & Poos, 2001). The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) provide two requirement values; one based on 10% iron absorption, and the other on 15% absorption (FAO and WHO, 2002). European reference ranges are based on 15% absorption (Reports of the Scientific Committee for Food, 1993).

Based on New Zealand recommendations, a child aged 1-3 years will have to eat two weetbix (3.01mg), two slices of bread (0.97mg), 50g chicken breast (0.95mg), 100g beef mince (3.07mg), one

quarter cup of spinach (0.67mg), half a medium potato (0.28mg) and one quarter cup of broccoli (0.23mg) throughout the day to reach their recommended daily intake (RDI) of 9mg.

Table 2.4: Recommended iron intake values according to differing Western world standards

Country	Age range (years)	EAR (mg/day)	RDI (mg/day)	UL (mg/day)
NZ/Australia*	1 – 3	4	9	20
	4 – 8	4	10	40
UK†	1 – 3	-	6.9	-
	4 – 6	-	6.1	-
	7 – 10	-	8.7	-
USA and Canada‡	1 – 3	-	7	40
	4 – 8	-	10	40
FAO/WHO¥	1 – 3	-	3.9^{ψ} or 5.8$^{¤}$	-
	4 – 6	-	4.2^{ψ} or 6.3$^{¤}$	-
	7 – 10	-	5.9^{ψ} or 8.9$^{¤}$	-
Europe~	1 – 3	-	3.9	-
	4 – 6	-	4.2	-
	7 – 10	-	5.9	-

*Values obtained from (Ministry of Health, 2006), †(Department of Health, 1991), ‡(Trumbo et al., 2001), ¥(FAO and WHO, 2002), ~(Reports of the Scientific Committee for Food, 1993), ^{ψ}Based on 15% absorption, ¤Based on 10% absorption

EAR = Estimated Average Requirement: a daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender

UL = Upper Level of Intake: highest intake that will cause no health effects, above this level of intake the potential risk of adverse effects increases

RDI = Recommended Daily Intake: daily average amount of nutrient required to meet 97-98% of the requirements of healthy individuals in a particular life stage and gender

NZ = New Zealand, UK = United Kingdom, USA = United States of America, FAO = Food and Agriculture Organisation of the United Nations, WHO = World Health Organisation

2.6. Iron Status and Determinants of Iron Status in Children with ASD

2.6.1. Iron status and Dietary Patterns in Children with ASD

Table 2.5 outlines studies that have investigated the prevalence of ID and IDA in children with ASD. It is important to note the differing criteria used in each study to determine ID and IDA as this limits the ability to compare studies.

Research from several countries have highlighted children with austism are at risk of developing ID and IDA. In Turkish children aged 3-16 years with autism (n=116), 24.1% were shown to have ID and 15.5% had IDA (Herguner et al., 2012). Similarly, ID was seen in 52.2% and IDA in 11.5% of Welsh children with autism (n=52) (Latif et al., 2002). Australian children with autism (n=144) had poorer iron status compared to the general population, with 3.0% having iron depletion, 7.5% having ID and 3.0% IDA (Sidrak et al., 2014). However, the prevalence of IDA in American children (n=368) with autism was not significantly higher than the general population when using SF and transferrin saturation as markers for iron status (Reynolds et al., 2012). However, this study did not measure

inflammatory biomarkers, which may mask ID when using SF to determine iron status (Worwood, 1997).

Very few studies have researched dietary practices and risk factors that contribute to children with ASD being more at risk of ID and IDA than the general population. Cornish (1998) assessed dietary iron intake of children with ASD through a retrospective study design. They found children with ASD had repetitive food patterns and that 35% did not consume red meat or meat products. Iron fortified cereals were found to contribute the greatest source of dietary iron, providing 25% of the daily iron intake. Another study found the biggest risk factors for ID and IDA in children with ASD were problems in sucking, swallowing or chewing; poor eating behaviour; and consuming inadequate amounts of meat, chicken, eggs or fish (Sidrak et al., 2014). It is typical of children with ASD to have restrictive diets and an unwillingness to try new foods (Raiten & Massaro, 1986), therefore if they restrict foods high in iron the risk of ID will increase. Additionally, one study indicates ASD children with more severe communication difficulties may be at greater risk of developing ID. The authors suggest these children may be more likely to have highly restrictive diets, or that communication difficulties are exacerbated by ID (Dosman et al., 2006).

As discussed in Section 2.2.2, it has been reported children with ASD often have difficult behaviours around food and mealtimes. No studies have investigated the association between feeding behaviours and iron status in children with ASD. Some research suggests preschool children with ASD may be particularly vulnerable to inadequate intake of iron, due to limited food acceptance being more typical in this age group (Cornish, 1998). This is of concern due to the associated effects of ID and IDA early in life, as discussed in Section 2.4.

2.6.2. Screening for ID in New Zealand ASD Children

Children with ASD in New Zealand do not come to the attention of the health care system unless they have co-morbidities or faltering growth. Therefore nutritional deficiencies, such as ID are likely to go unrecognised, and may exacerbate symptoms. It is of importance to note that the Centres for Disease Control Prevention recommends all population groups at risk of ID be screened (Centres For Disease Control Prevention, 2002).

Table 2.5: Summary of characteristics and results of studies exploring the prevalence of iron deficiency and iron deficiency anaemia in children with ASD

Reference	Study Population	Study Design	Iron Depletion, ID and IDA Criteria	Results
Sidrak et al., 2014	N=122 Age: 1-12 years Location: Australia	Retrospective review	Fe depletion: Plasma ferritin <10µg/L ID: Two or more of the following (i) SF <10µg/L; (ii) MCV <73-75fL; (iii) Fe saturation <10-12% IDA: Criteria for ID plus Hb <110g/L	- Fe depletion 3.0%, ID 7.5%, IDA 3.0% - Prevalence of ID in children with ASD greater than neuro typical developing children.
Herguner et al., 2012	N=116 Age: 3-16 years Location: Turkey	Cross-sectional observational design	ID: SF <10µg/mL for children <5 years, and <12µg/mL for school-aged children IDA: Hb <11.0g/dL for <5 years, and <12g/dL for school-aged children	- ID 24.1% and IDA 15.5% - Significant positive correlation between age and ferritin and haematological measures
Reynolds et al., 2012	N=368 Age: 2-11 years Location: United States	Cross-sectional observational design	Fe depletion: SF <12µg/L ID: SF <12µg/L + TS > 10% IDA: Hb <11g/dL for children <5, and <12g/dL for 6-11 years + SF <12µg/L	- 8% Fe depletion - 1% ID and 1 participant (0.3%) IDA - <2% had Fe intake below the EAR - Major Fe sources: enriched or fortified foods e.g. breakfast cereals.
Adams et al., 2011	N=55 Location: Arizona Age 5-16 years	Cross-sectional observational study design using age and gender matched controls	N/A	- Significantly higher RBC Fe in ASD group. Clinical significance unclear, but did relate to ASD severity. - Non significant difference in serum Fe and SF between two groups. - IDA an issue for those <5 years.
Bilgic et al., 2010	N=31 Location: Turkey Age: 18-60 months	Cross-sectional observational study	ID: SF <10µg/L IDA: SF <10µg/L + Hb <110g/L	- 32.2% had ID and 6.5% had IDA. - No association between ASD symptom severity, developmental delay and SF level.
Dosman et al., 2006	N=96 Location: Toronto, Canada	Retrospective chart review	ID: SF <10µg/L for ages 1-5 years and <12µg/L for >6 years MCV <80fL	- 7% had ID in 1-2 years (similar to neuro typical children). - 3-5 years, more than double the 5% prevalence.

	Age: 2-10 years		IDA: Hb <110g/L in 2-4 years and <120g/L in 5-10 years	- 6-10 years, 5x greater than the normal prevalence. - Low SF associated with severely impaired communication - reflecting restricted diets, or exacerbation of behavioural difficulties.
Latif et al., 2002	*Autism group: n=52* Age: 19months - 5 years *Asperger's Syndrome group: n=44* Age: 3-13 years Location: England	Retrospective study	ID: SF <12µg/L IDA: Hb <110g/L for <6years and <120g/L for 6-12 years + SF <12µg/L	*Autism group:* - ID 12% and IDA 11.5% *Asperger's group:* - ID 13.6% and IDA 4.5% - SF only available for 22 children

ASD = Autism Spectrum Disorder; CRP = C-reactive protein; EAR = estimated average requirement; Fe = iron; Hb = Haemoglobin; ID = Iron Deficiency; IDA = Iron Deficiency Anaemia; MCV = Mean corpuscular volume; RBC = Red blood cell; SF = Serum Ferritin; TS = transferrin saturation

2.7. Conclusion

ASD is a neurodevelopmental disorder which is characterised by the child showing unusual behaviour, difficulty communicating and performing repetitive tasks. Children with ASD often have feeding and behavioural problems, and in conjunction with the high use of exclusion diets in this population group, may increase their risk of nutritional deficiencies. Iron is an essential mineral in brain development, and as ASD is a neurological disorder, achieving optimal iron status is important in this population group. The prevalence of ID and IDA in New Zealand children is low, however other countries have shown children with ASD are at increased risk of ID and IDA. Although the prevalence of ID and IDA in children with ASD have been determined in other countries, there have been no studies in New Zealand to date looking at this issue. No studies have investigated the association between feeding behaviours and iron status in children with ASD. Additionally, very few studies have investigated the dietary practices which may lead this population to be at increased risk of ID. By investigating these, the adverse effects of ID and IDA in ASD children may be reduced.

Chapter 3: Study Methodology

3.1. Study Design

The 'Iron status in young children with Autism Spectrum Disorder (ASD)' study is a cross-sectional observational study, investigating data on the iron status and dietary iron intake of New Zealand children with ASD, along with dietary practices which may influence iron status. This thesis presents baseline data which was collected as part of the VIDOMA RCT, investigating the benefits of omega-3 and/or vitamin D supplementation in children with ASD. The methodology presented in this section relates the 'Iron status in young children with ASD' study.

3.2. Ethical Approval

Ethical approval was sought from the Health and Disabilities Ethics Committee (HDEC) and the RCT was registered on the Australian New Zealand Clinical Trial database (ACTRN12615000144516). Consent was also obtained from the Waitemata District Health Board (WDHB) for recruitment of children through the WDHB developmental co-ordinators and for data collection to be performed at North Shore and Waitakere Hospitals.

Written informed consent was acquired from each parent or caregiver prior to their child's involvement in the study. Each potential participant the research team made contact with was given a unique study code. This ensured the participant's identity remained anonymous throughout the study and also allowed the research team to keep track of participants that did not meet the inclusion and exclusion criteria.

3.3. Study Population

3.3.1. Setting

The study was conducted in Auckland, New Zealand. The majority of participants were located in Auckland, therefore data collection was either conducted at the Human Nutrition Research Unit at Massey University, Albany or at the paediatric outpatient department at North Shore or Waitakere Hospitals. All participants located outside of Auckland, travelled to Auckland for data collection.

3.3.2. Inclusion Criteria

All children lived in New Zealand, were between the ages of 2.5 to 8 years, and had a medical diagnosis of ASD by a paediatrician. The participants were diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders, fourth or fifth edition (DSM-4 or -5) (American Psychiatric Association, 2013) with any diagnoses along the autism spectrum being eligible for inclusion in this study. Participants were required to have onset of ASD symptoms at the age of 18 months or later to eliminate participants who had developmental delay from birth. The age group range was chosen

based on requirements for some of the assessment tools used in the VIDOMA RCT. Additionally, the family had to be proficient in the English language due to the nature of the RCT assessment tools.

3.3.4 Sample Size

The calculation used to determine the sample size needed to achieve statistical significance for the 'Iron status in young children with ASD' study came from Fox, Hunn, and Mathers (1998). Using available data on the iron status of children with ASD from other countries (Reynolds et al., 2012), it was calculated that 114 children were needed to be able to determine the prevalence of iron deficiency to within 5% based on an expected prevalence of 8%, with level of significance at $P < 0.05$.

3.3.5. Recruitment

Recruitment of participants occurred between November 2014 and September 2015. Initially recruitment of children with ASD was done through the WDHB developmental co-ordinators. They provided potential participants with a flier, answered any initial questions, and directed them to the VIDOMA study homepage on the Massey University website. The website contained extra information about the study and enabled participants to register their interest, allowing the researchers to contact those participants who were interested in taking part. Once means from the ASD co-ordinators were exhausted, the developmental paediatrician at WDHB involved in the study provided a list of potential participants who met the inclusion criteria. These participants were sent a letter via mail which outlined information about the study and encouraged them to contact the research team directly if interested. Other means of recruitment included advertising on the Children's Autism Foundation social media page, as well as through the general media (radio interview). The process of participant recruitment is illustrated in Figure 3.1.

3.3.6. Study Procedure

Figure 3.1 outlines the sequence of procedures involved in this study. Participants who expressed interest in the initial recruitment phase were contacted by the research team. The initial contact pack was completed over the telephone (Appendix A). This pack contained a contact details sheet enabling details to be collected on name, date of birth, National Health Identification (NHI) number, phone numbers, address, and general practitioner (GP) contact information for each participant. The pack also contained a family/medical history questionnaire with questions related to chronic conditions, medication use, ASD therapies, previous ID or use of iron supplements, presence of infection or illnesses, ethnicity, household income and mode of feeding as an infant (breast or formula).

Once the initial contact pack was complete, an information pack was sent to the participant which contained an information sheet, consent form, social stories, four day food diary (with instructions on how to complete), a dietary questionnaire, blood request form, directions to the

hospital, and a parking permit. The information sheet outlined the procedures associated with the study and provided detail on any potential risks or discomforts that may arise (Appendix B). A written consent form was completed by the parent or guardian, which confirmed the participants' understanding of the procedures, benefits, risks and participant rights associated with the study (Appendix C). Three social stories were included in this pack, which provided pictures on where the child was going, who they were going to meet and what procedures to expect (Appendix D). The aim of these were to reduce the element of surprise for the children and to aid compliance with necessary procedures. Participants were required to submit the consent form along with the food diary and dietary questionnaires (Appendix E) to the research team via an envelope provided.

Participants were checked for eligibility for the RCT by assessing compliance with the inclusion and exclusion criteria. Participants who had not returned their consent form to the research team were required to bring it to their appointment at North Shore or Waitakere Hospitals where a serum sample was collected. On knowledge of the participant's blood test results, the research team confirmed with the parents or caregivers if their child met the criteria for the RCT. Participants who did not meet the RCT inclusion criteria were invited to participate in the 'Iron status in young children with ASD' study.

Following confirmation of enrolment in the study, participants attended an appointment at Massey University, Albany research centre. Here the food diary and dietary questionnaire were checked for completeness and for collection of anthropometric data.

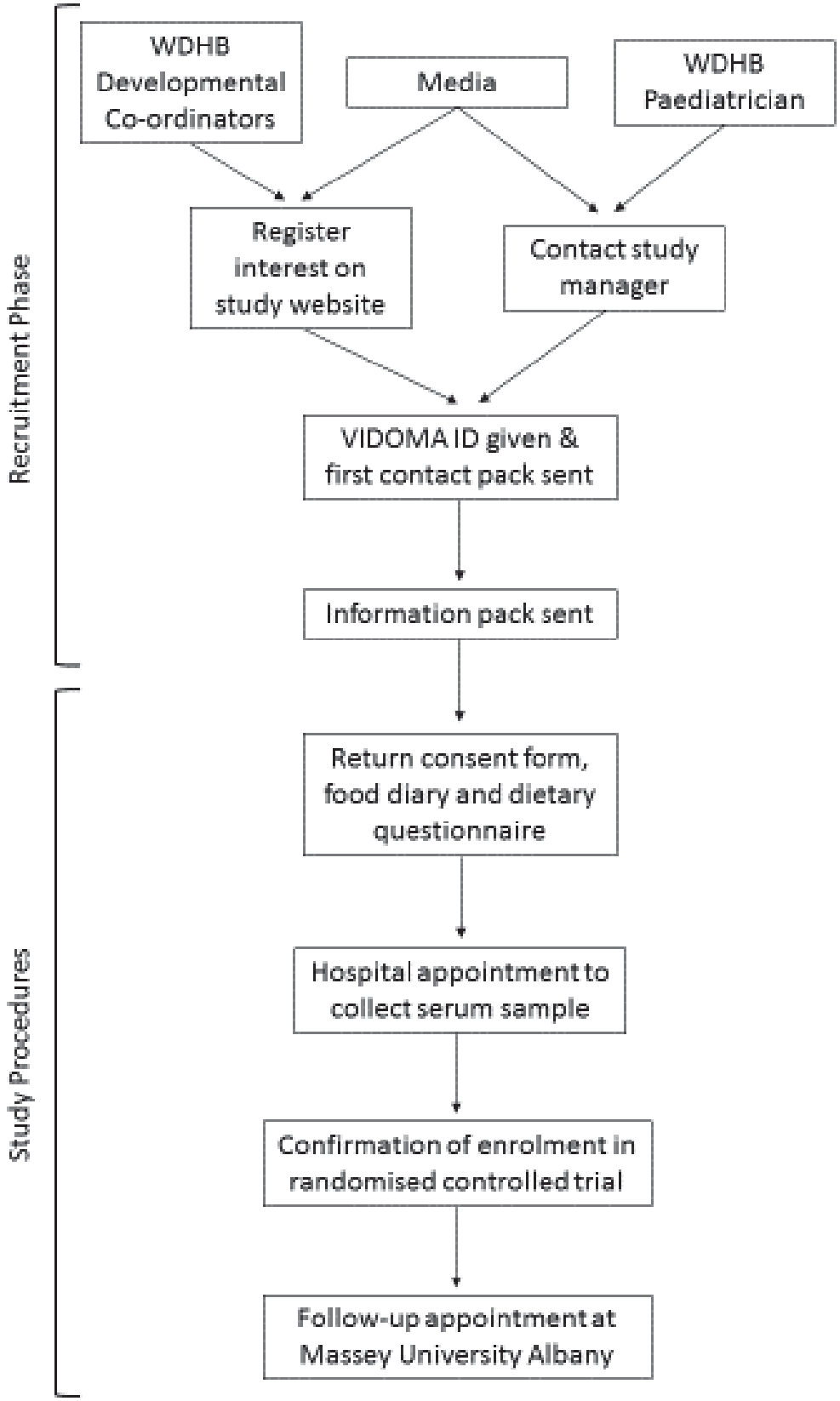

Figure 3.1: Flow diagram of recruitment and procedures involved in this study.

3.4. Measures to Assess Iron Status

3.4.1. Iron Biomarkers

To determine the iron status of the participants, blood was collected and the following biomarkers were measured: serum Fe, SF, TIBC, transferrin saturation and Hb. SF was measured as it represents the amount of storage iron and is a sensitive indicator of ID (Hallberg & Hulthén, 2002). Hb is commonly used to determine anaemia, and when done in conjunction with SF is a sensitive measure of IDA (World Health Organisation, 2001). Serum Fe and transferrin saturation were measured as they both reflect the amount of circulating iron and can differentiate between anaemia caused by ID, chronic disease or inflammation. However serum Fe does not detect iron bound to Hb and also is influenced by time of day, and transferrin saturation also decreases in an inflammatory state (Burke et al., 2014). TIBC is a stable measure of the amount of iron bound to transferrin, however, this is not very sensitive as values only decrease when iron stores are depleted (Burke et al., 2014).

3.4.2. Collection of Blood Samples

Venous blood samples were taken by a certified phlebotomist aided by the paediatric registrar. For participants within the Auckland region, blood collection was done at either North Shore or Waitakere Hospitals. The phlebotomist took three plasma samples and two RBC samples from each participant.

Blood collection was undertaken using the Push Button Butterfly method. This method is preferred compared to the syringe method for the paediatric population and those with small or fragile veins. The phlebotomist explained the procedure to the parent or caregiver, and the purpose for collecting a serum sample. Verbal consent was received before any procedures were performed, and identification of the patient was confirmed before continuing. The participant was positioned correctly and an appropriate collection site was identified before a tourniquet was applied approximately seven to ten centimetres above the venepuncture site. After anchoring the vein, the needle was inserted. Blood from the syringe was dispensed into tubes, which were inverted eight to ten times. Correct disposal of materials was performed, and the participant or caregiver was asked to apply pressure to the venepuncture site for several minutes. The site was checked for physical signs of blood leaking into the tissue adjacent to the site, and a piece of tape was applied to retain pressure.

Collected blood samples were labelled with the participant's VIDOMA ID number, plasma or RBC collection number and month number, e.g. VIDOMA000, P1, M1. Each tube was also given a specific sample number which was included on the tube as a barcode. This allowed quick identification of the participant, sample type and month taken by scanning the barcode. All these identification aspects were put on a sticky label and attached to the tube. Blood tubes were handled according to the requirements of the laboratory tests manual specifications. Documentation was completed and

blood samples were put into a biohazard bag, labelled with participants' VIDOMA identification number and NHI number, and sent to the laboratory within 20-30 minutes of blood collection. Parents and caregivers were advised to contact the phlebotomist or paediatrician if they noticed any major signs of bruising, bleeding, swelling, discomfort, tingling or numbness. The child was congratulated for their cooperation and bravery and were given a choice of a sticker, stamp, special plaster or lollipop. A complete detailed version of the blood collection method used by the phlebotomist at WHDB can be found in Appendix F.

3.4.3 Processing Serum Blood Samples

All biochemical analysis of blood samples were performed at North Shore Hospital laboratory. A summary of the serum sample processing methods used at WDHB to determine iron status is outlined in Table 3.1. In addition to the biomarkers in Table 3.1, transferrin saturation was calculated by determining the ratio of serum Fe to TIBC, multiplied by 100. For the analysis of Hb concentration, the blood sample was measured at light absorbance (555nm), and then compared to the absorbance of the diluent.

Table 3.1: Waitemata District Health Board methods to assess biomarkers of iron status

Assay	Machine (brand/name)	Method	Cat. number
Serum Fe	Dimension Vista® System	IRON Flex® reagent cartridge	K3085, KC240
SF	Dimension Vista® System	FERR Flex® reagent cartridge	K6440, KC640
TIBC	Dimension Vista® System	TIBC Flex® reagent cartridge	K3084, KC230

Fe = iron; SF = serum ferritin; TIBC = total iron binding capacity

3.4.4 Iron Depletion, Iron Deficiency and Iron Deficiency Anaemia Definitions

A SF concentration of ≤ 20 µg/L was classified as iron depletion and SF of ≤ 12 µg/L was defined as ID. The definition of IDA used in this study was SF ≤ 12 µg/L as well as Hb ≤ 110 g/L for children between the age of 1-5 years or ≤ 115 g/L for children between ages 5-8 years.

3.5. Measures of Dietary Intake and Dietary Practices

3.5.1. Four-Day Food Diary

Parents were required to fill in an estimated four day food diary for their child. An example of the food diary can be found in Appendix E. Written instructions were provided at the beginning of the diary and verbal instructions were also given via a DVD developed by academics from the School of Food and Nutrition, Massey University. Participants completed four consecutive days: being either Sunday, Monday, Tuesday, Wednesday; or Wednesday, Thursday, Friday, Saturday. This was to account for

differences in dietary intake during weekends compared to weekdays. The allocation of days was achieved based on suitability according to the participants' schedules, convenience, as well as timing of their follow up appointment date. The ratio of food diary allocation was approximately 50:50.

Participants were encouraged to provide as much information as possible in the diaries. This included detail on time of day the food was eaten and the type of food (including brand of product, variety and preparation method). The quantity of food consumed was recorded using household measures, actual weights or by comparing the size to other objects. Participants were also invited to provide the packaging of foods to improve accuracy when analysing the diaries.

Parents or caregivers were asked to bring the food diary to the appointment at Massey University Albany. At this appointment, the diary was checked to ensure completeness and gave the opportunity for the research team to ask the participant for further detail or information if sections of the diary were unclear.

The food diaries were entered into Foodworks7 (Xyris Software (Australia) Pty Ltd, 2012) by two Massey University Masters in Science (MSc), Nutrition and Dietetic students. If a food was not on the Foodworks7 database, the most similar match was used. If there was no comparable food on the database, the nutritional information panel on the company's website or the food packaging was used. This data was used to determine dietary intake, looking specifically at foods rich in iron and foods which may enhance or inhibit iron absorption (iron, vitamin C, calcium, dietary fibre, vitamin A). There is no data for phytic acid in the New Zealand food composition tables, therefore dietary fibre was used in this study to determine the intake of phytic acid as these often occur together in foods.

3.5.2. Dietary Questionnaire

The dietary questionnaire was developed by MSc, Nutrition and Dietetic Massey University students after reviewing the literature (Appendix E). The questionnaire was checked by Massey University Human Nutrition and Dietetic academic staff before being sent to participants. The questionnaire was developed to provide information on specific dietary factors which may influence iron intake and absorption. This included the frequency of consumption of iron rich foods and the frequency and timing of known factors which influence iron absorption such as meat, tea and coffee, and foods high in vitamin C. The questionnaire also provided information on iron supplements, general multivitamin use, allergies, as well as any special diets the child may have been following. The data collected on supplement use, allergies and special diets will be analysed by another MSc Nutrition and Dietetic student at Massey University and presented in a separate thesis. The supplement data collected was not included in dietary analysis using Foodworks7, but was used to determine use of multi-vitamins

and iron supplements. In this study any combination of two or more minerals or vitamins were classified as a multivitamin.

The parents or caregivers of the child completed the questionnaire alongside the food diary and either sent it back to the research team, or brought it to the follow up appointment. If any data in the questionnaire was missing or if the answers were unclear, one of the research team members sought clarification from the parent or caregiver. Each question in the questionnaire was coded to allow statistical analysis to be performed.

3.5.3. Behavioural Paediatrics Feeding Assessment Scale (BPFAS) Questionnaire

The BPFAS is a parent report measure developed by Crist et al. (1994). The scale is comprised of 25 questions about child behaviour around food, and the next 10 questions determine parental feelings or strategies for dealing with their child's behaviour. Each question is presented as a five point Likert scale ranging from never to always. Each of the 35 questions is followed up by a yes or no question to determine if the behaviour is a problem for the parent. Two scores are produced from the BPFAS. The first score is from the Likert scale highlighting the frequency of behaviour, with higher scores indicating a greater number of problems. A score of greater than 84 indicates clinical feeding issues. The second score is from the parents' perception of the problem, with a higher frequency (greater than 9) indicating greater problems from parental perspective. The BPFAS has been proven to be a reliable and valid measure in determining clinically significant feeding problems in both normal population groups as well as in clinical populations (Crist & Napier-Phillips, 2001).

3.6. Anthropometric Data

Anthropometric measures of the children were collected at the follow-up appointment at Massey University, Albany. Weight was taken with the participant wearing light clothing and no shoes on standard electronic scales (Wedderburn). If weight could not be obtained by the child on the scales, the parent stood on the scales with the child and parental weight was subtracted. Height was measured using a portable stadiometer (Holtain Limited). The participant was required to take off their shoes, have their heels against the back of the stadiometer and look straight ahead. Some participants were sensitive to the noise made by the stadiometer. If this was the case, a mark was drawn on the wall and then measured with a tape measure. Only one weight and height measurement were taken.

3.7. Statistical Analysis

Data analyses were performed using IBM statistics package version 22 (IBM Corp, 2013). Each variable was tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk test and for homogeneity using the Levene's test. Normal data is presented as mean ± standard deviation (SD). Data which presented as non-normal was log transformed to obtain normality. If normality was achieved data is

reported as mean (95% confidence interval). Non-normal data is presented as median (25-75th percentile).

Participants were split into two groups depending on their iron status. The iron replete group included participants who had normal iron biomarkers (SF > 20 μg/L and Hb > 110 g/L for 1-5 years or > 115 g/L for 5-8 years) and the iron insufficient group included participants with either iron depletion, ID or IDA. Characteristics between iron replete and iron insufficient groups were compared using independent t-tests or Mann-Whitney tests for continuous variables and a chi-square for categorical variables. A 2-tailed *P* value of < 0.05 was considered statistically significant. Data were normally distributed and the group variances were equal for the independent t-tests. A chi-square test was used for independent data if the expected count for each cell was >5 and none <1. However, if expected count was <5 in a cell, the Fishers exact test was used.

For variables which showed a statistically significant difference between the two groups, an effect size was calculated to determine the importance of the effect. For the Mann-Whitney test, effect size = $Z/\sqrt{n}$; for the independent t-test, $\sqrt{t^2/(t^2+df)}$; and for the chi-square test, an odds ratio was used. An effect size of 0.10 indicates a small effect, 0.3 a medium effect and ≥0.5 indicates a large effect (A. Field, 2009).

Chapter 4: Results

The aim of this study was to investigate the iron status and factors influencing iron status in a cohort of children with ASD. This section will cover the degree of iron deficiency; dietary intake of key enhancers and inhibitors of iron absorption; dietary practices; and feeding behaviours associated with iron status in children with ASD.

4.1. Participant Characteristics

In total 69 participants were recruited to take part in this study. Of those recruited, anthropometric data was collected from 66 participants, 67 participants completed the demographics questionnaire, 59 had blood samples taken, 50 completed the four day food diary and dietary questionnaire, and 62 participants completed the BPFAS questionnaire. A complete set of data regarding blood results and food diaries/dietary questionnaires was available for 42 participants.

Characteristics of the population sample are summarised in Table 4.1. The median age of participants was 5.0 years (4.5, 5.1), mean weight was 21.5kg (20.4, 22.5), and the mean height was 113.7±9.3cm. The prominent gender was male, making up 57/69 (85.1%) of the population sample, and the majority of participants 43/69 (62.3%) were of New Zealand European ethnicity. An equal number of participants had mild to mild/moderate versus moderate, moderate/severe, severe and unknown ASD severity. Nine of 69 participants (13.0%) had ID or IDA, 4/69 (5.8%) had blood loss in the last 6 months, and 29/69 (42.0%) had an additional chronic comorbidity. The mean BPFAS frequency score was 82.0±21.9, and mean BPFAS problem score was 12.2±8.0.The proportion of participants with a frequency score ≥84 in the BPFAS questionnaire, indicating a clinical feeding issue, was 28/69 (40.6%). The proportion of participants with a problem score ≥9 was 41/69 (59.4%), representing parental problems with their child's feeding behaviour.

Table 4.1: Characteristics of participants involved in the study.

Variable		**N=69**
Age (years)‡		5.0 (4.5, 5.1)
Weight (kg) †		21.5 (20.4, 22.5)
Height (cm) §		113.7±9.3
Gender n (%)	Male	57 (82.6)
	Female	10 (14.5)
Ethnicity n (%)	New Zealand European	43 (62.3)
	Māori	1 (1.4)
	Pacific	2 (2.9)
	South Asian	1 (1.4)
	Chinese	2 (2.9)
	Southeast Asian	1 (1.4)
	Other	15 (21.7)
	Missing	4 (5.8)
ASD severity n (%)	Mild and mild/moderate	30 (43.5)
	Moderate, moderate/severe, severe and unknown	30 (43.5)
Previous ID or IDA n (%)		9 (13.0)
Blood loss in last 6 months n (%)		4 (5.8)
Chronic condition n (%)		29 (42.0)
BPFAS frequency score §		82.0±21.9
BPFAS frequency ≥84 n (%)		28 (40.6)
BPFAS problem score §		12.2±8.0
BPFAS problem ≥9 n (%)		41 (59.4)

ASD = autism spectrum disorder; BPFAS = behavioural paediatrics feeding assessment scale; ID = iron deficiency; IDA = iron deficiency anaemia

§ Values are mean±SD

†Mean (95% CI) for the log transformed data values, back transformed to the original scale

‡Median (25^{th}-75^{th} quartiles)

Note: anthropometric data (n=66), demographics data (n=67), BPFAS (n=62)

4.2. Iron Status of Participants

Blood samples were collected from 59 participants, with results shown in Table 4.2. The mean serum iron concentration was 13.4±4.9 µmol/L, and mean SF concentration was 24.2 (21.1, 27.9) µg/L. Mean iron saturation was 0.20±0.07 % and median TIBC was 66.6 (63.1, 69.7) µmol/L. The mean Hb concentration was 129.8±7.4 g/L.

Table 4.2: Biomarkers of iron status

Variable	N=59
Serum Fe (µmol/L) §	13.4±4.9
SF (µg/L) †	23.5 (20.6, 26.6)
Fe saturation (%) §	0.20±0.07
TIBC (µmol/L) ‡	66.6 (63.1, 69.7)
Hb (g/L) §	129.8±7.4

Fe = iron; SF = serum ferritin; TIBC = total iron binding capacity; Hb = haemoglobin
§ Values are mean±SD
†Mean (95% CI) for the log transformed data values, back transformed to the original scale
‡Median (25^{th}-75^{th} quartiles)

4.2.1. Prevalence of Iron Deficiency and Iron Deficiency Anaemia

Iron depletion was classified as having a SF concentration of ≤ 20 µg/L; ID as SF ≤ 12 µg/L; and IDA as SF ≤ 12 µg/L as well as Hb ≤ 110 g/L for children between the age of 1-5 years or ≤ 115 g/L for children between ages 5-8 years. Table 4.3 outlines the proportion of iron depletion, ID and IDA in children with ASD. The prevalence of iron depletion amongst children with ASD in this sample population was 32.9%, ID was 4.3%, and 0.0% of participants had IDA.

Table 4.3: Prevalence of iron depletion, iron deficiency and iron deficiency anaemia

Variable	Definition	n(%)
Iron replete	SF > 20 µg/L	33 (55.9)
Iron depletion	SF ≤ 20 µg/L	23 (32.9)
ID	SF ≤ 12 µg/L	3 (4.3)
IDA	SF ≤ 12 µg/L plus Hb ≤ 110 g/L (1-5 years) or Hb ≤ 115 g/L (5-8 years)	0 (0.0)

ID=Iron deficiency; IDA=Iron deficiency anaemia; SF = serum ferritin; Hb = haemoglobin
Note: blood sample (n=59)

4.3. Dietary Intake

A summary of participant dietary intake from the four day food diary and a comparison to the NRVs (Ministry of Health, 2006) for age is found in Table 4.4 The mean energy intake for participants was 7011.9±1454.8kJ. The mean protein intake was 61.1±12.8g, contributing 15.1±3.0% to energy intake. The mean carbohydrate and sugars intake was 208.6±53.2g and 90.7±43.3g respectively, with total carbohydrates contributing 49.3±7.2% towards energy. Mean dietary total fat intake was 62.9±22.4g, with saturated fat intake being 24.5±10.0g. Total fat contributed 32.7±7.2%, and saturated fat 12.7±4.0% towards energy intake. Mean dietary fibre intake was 20.4±7.4g, which contributed 2.3±0.8% towards energy intake.

All participants met the estimated average requirement (EAR) and the recommended daily intake (RDI) (Ministry of Health, 2006) protein values for age. The proportion of participants meeting

the adequate intake (AI) for dietary fibre (Ministry of Health, 2006) for 1-3 years was 60.0% and for 4-8 years 59.0%.

The mean dietary iron intake for ASD children in this cohort was 11.2±3.7mg. When the population was split by age group, participants aged 1-3 years had mean dietary intake of 10.8±4.4mg and children aged 4-8 years had intake of 11.3±3.6mg. Overall, 8 (80.0%) participants aged 1-3 years and 20 (54.1%) participants aged 4-8years met the RDI for iron. All participants aged 1-3 years met the EAR for iron, and 38 (97.4%) of those aged 4-8 years met the iron EAR.

The number of children aged 1-3 years meeting the RDI for vitamin C was 70.0% and for participants aged 4-8 years was 74.4%. A similar proportion of participants in each age group meet the EAR for vitamin C (80.0% in those aged 1-3 years and 79.5% aged 4-8 years). The proportion of participants meeting the RDI for calcium was 70.0% and 43.6% for ASD children aged 1-3 years and 4-8 years respectively. Seven (70.0%) and 29 (74.4%) participants aged 1-3 years and 4-8 years respectively met the EAR for calcium. The proportion of participants meeting RDI for vitamin A was 60.0% for 1-3 years and 56.4% for 4-8 years. The proportion of participants reaching the EAR was 70.0% for 1-3 years and 76.9% for 4-8 years.

Table 4.4: Macronutrient and specific micronutrient intake of children with ASD with comparisons to RDI and EAR for age.

Nutrient	Intake (All participants)	Age Range (years) ₣	Intake (age range years)	RDI/AI¥	N(%) met RDI	EAR¥	N(%) met EAR
EnergyDF (kJ) §	7011.9±1454.8	1-3	6080.3±1470.0	-	-	-	-
		4-8	7234.2±1355.6	-	-	-	-
Protein (g) §	61.1±12.8	1-3	53.9±10.6	14	10 (100.0)	12	10 (100.0)
		4-8	63.4±12.9	20	40 (100.0)	16	40 (100.0)
CHO (g) §	208.6±53.2	1-3	191.9±63.6	-	-	-	-
		4-8	211.1±50.9	-	-	-	-
Sugars (g) §	90.7±43.3	1-3	99.0±52.1	-	-	-	-
		4-8	88.5±41.3	-	-	-	-
Fat (g) §	62.9±22.4	1-3	48.9±18.7	-	-	-	-
		4-8	66.6±21.9	-	-	-	-
SFA (g) §	24.5±10.0	1-3	17.4±7.8	-	-	-	-
		4-8	26.5±9.6	-	-	-	-
DF (g) §	20.4±7.4	1-3	16.8±8.9	14	6 (60.0)	-	-
		4-8	21.2±7.1	18	23 (59.0)	-	-
Iron (mg) §	11.2±3.7	1-3	10.8±4.4	9	8 (80.0)	4	10 (100.0)
		4-8	11.3±3.6	10	20 (54.1)	4	38 (97.4)
Calcium (mg)	611.6 (537.1, 696.7) †	1-3	650.0±378.4§	500	7 (70.0)	360	7 (70.0)
		4-8	677.3±268.1§	700	17 (43.6)	520	29 (74.4)
Vitamin C (mg) †	60.2 (47.8, 76.0)	1-3	66.7 (33.4, 133.4)	35	7 (70.0)	25	8 (80.0)
		4-8	58.7 (45.6, 75.5)	35	29 (74.4)	25	31 (79.5)
Vitamin A equivalents (µg) †	422.4 (346.3, 515.1)	1-3	350.9 (209.1, 588.9)	300	6 (60.0)	210	7 (70.0)
		4-8	420.9 (337.2, 525.3)	400	22 (56.4)	275	30 (76.9)
% energy from protein §	15.1±3.0	1-3	15.6±3.7ĸ	-	-	-	-
		4-8	15.2±2.9	-	-	-	-
% energy from fat §	32.7±7.2	1-3	29.9±7.7	-	-	-	-
		4-8	33.8±7.1	-	-	-	-

% energy from SFA §	12.7±4.0	1-3	10.5±3.1	-	-	-	-
		4-8	13.4±3.9	-	-	-	-
% energy from CHO §	49.3±7.2	1-3	51.2±9.1	-	-	-	-
		4-8	48.8±6.7	-	-	-	-
% energy from DF§	2.3±0.8	1-3	2.2±0.9	-	-	-	-
		4-8	2.4±0.8	-	-	-	-

DF = dietary fibre; CHO = carbohydrate; SFA = saturated fatty acids; RDI = recommended daily intake; AI=adequate intake; EAR = estimated average requirement

§ Values are mean±SD

†Mean (95% CI) for the log transformed data values, back transformed to the original scale

‡Median (25^{th}-75^{th} quartiles)

¥ Values from (Ministry of Health, 2006)

₣ Age range: 1-3 years (n = 10), 4-8 years (n = 40)

₭ Not appropriate to compare with AMDR as these are aimed at adults and adolescents over 14 years of age.

Note: data from four day food diary (n=50)

4.4. Comparisons between Iron Replete and Iron Insufficient Groups

Participants were categorised into two groups depending on their iron status, with comparisons between descriptive characteristics summarised in Table 4.5. The iron insufficient group (n=26) included those who had iron depletion, ID and IDA, and the iron replete group included those who had normal iron biomarkers (n=34). Comparisons between these two groups showed no difference in age, weight, height, gender, ethnicity, ASD severity, previous ID or IDA, and blood loss. Participants in the iron insufficient group had a significantly higher BPFAS frequency score (t = 2.362, 46 df, $P = 0.022$, medium effect size, $r = 0.33$), and significantly more had a frequency score ≥ 84 ($X^2 = 5.371$, $P = 0.020$), when compared with the iron replete group. This indicates those who had iron insufficiency were more likely to have clinical feeding issues. The odds for iron insufficient children to have high risk of clinical feeding issues were 3.78 times more likely than for iron replete children to have feeding issues. However, no differences were identified in whether the child's feeding behaviour was a problem for the parent.

Only 42 participants completed the dietary questionnaire, food diary, and also had a blood sample taken. Participants who completed both were split into iron insufficient (n=17) and iron replete (n=25) groups. Table 4.6 summarises comparisons of dietary factors from the dietary questionnaire which affect iron status between iron replete and iron insufficient group. No significant differences were seen between the two groups when comparing dietary factors associated with iron status.

Table 4.5: Comparison of participant characteristics between the iron insufficient and iron replete group.

Variable		Iron Insufficient N=26	Iron Replete N=34	P-value
Age (years)‡		5.0 (4.2, 5.3)	5.0 (4.3, 5.3)	0.888
Weight (kg) †		21.5 (20.0, 23.2)	21.3 (19.6, 23.2)	0.873
Height (cm) §		113.6±8.1	113.8±10.7	0.908
Male n (% per group)	Male	24 (92.3)	27 (81.8)	0.446
	Female	2 (7.7)	6 (18.2)	
Ethnicity n (% per group)	New Zealand European	21 (80.8)	16 (50.0)	0.192
	Māori	0 (0.0)	1 (3.1)	
	Pacific	1 (3.8)	1 (3.1)	
	South Asian, Korean	0 (0.0)	0 (0.0)	
	Chinese	0 (0.0)	2 (6.3)	
	Southeast Asian	0.0 (0)	1 (3.1)	
	Other	4 (15.4)	11 (34.4)	
ASD severity n (% per group)	Mild and mild/moderate	11 (50.0)	15 (51.7)	0.903
	Moderate, moderate/severe, severe and unknown	11 (50.0)	14 (48.3)	
Previous ID or IDA n (% per group)		5 (20.0)	3 (9.4)	0.280
Blood loss in last 6 months n (% per group)		0 (0.0)	4 (12.1)	0.126
Chronic condition n (% per group)		8 (30.8)	16 (48.5)	0.169
BPFAS frequency score §		88.4±11.9	74.2±25.9	0.022*
BPFAS frequency ≥84 n (% per group)		15 (68.2)	9 (34.6)	0.020*
BPFAS problem score §		11.9±7.5	12.3±7.5	0.478
BPFAS problem ≥9 n (% per group)		16 (61.5)	20 (60.6)	0.957

ASD = autism spectrum disorder; ID=iron deficiency; IDA=iron deficiency anaemia; BPFAS = behavioural paediatrics feeding assessment score

§ Values are mean±SD

†Mean (95% CI) for the log transformed data values, back transformed to the original scale

‡Median (25^{th}-75^{th} quartiles)

*Significant differences between the two iron groups (P<0.05) (Chi-Square)

Note: anthropometric data (n=66), demographics data (n=67), BPFAS (n=62)

Table 4.6: Comparison of dietary factors between iron insufficient and iron replete group that may impact iron status.

Variable n (%)	Iron Insufficient N=17	Iron Replete N=25	P-value
Breastfed	16 (94.1)	23 (92.0)	1.000
Vegetarian or vegan	2 (11.8)	2 (8.0)	1.000
Vegetarian	1 (5.9)	1 (4.0)	1.000
Vegan	2 (11.8)	1 (4.0)	0.556
Eats any meat (red meat, chicken, other)	13 (76.5)	23 (92.0)	0.212
Eats red meat	12 (70.6)	22 (88.0)	0.235
Eats beef	12 (70.6)	14 (56.0)	0.288
Eats pork	4 (23.5)	6 (24.0)	1.000
Eats lamb	3 (17.6)	4 (16.0)	1.000
Eats chicken	8 (47.1)	14 (56.0)	0.654
Eats other meat (goat, rabbit, duck)	0 (0.0)	0 (0.0)	1.000
Eats fish (fresh, dried, canned)	3 (17.6)	4 (16.0)	1.000
Eats other seafood (prawns, shrimps)	2 (11.8)	0 (0.0)	0.166
Eats offal (liver, kidney, heart)	1 (5.9)	0 (0.0)	0.405
Eats high vitamin C fresh fruits (oranges, mandarins, kiwifruit or juice from these fruit)	7 (41.2)	16 (64.0)	0.145
Drinks tea or coffee	1 (5.9)	0 (0.0)	0.405
Likes taste Fe rich foods	7 (41.2)	15 (60.0)	0.485
Skips main meals	10 (58.8)	13 (52.0)	0.491
Follows a special diet Ŧ	5 (29.4)	8 (32.8)	0.859
Fe supplement	1 (5.9)	1 (4.0)	1.000
Multivitamin supplement	4 (23.5)	5 (20.0)	1.000

Fe=iron

Ŧ Special diets: gluten free, casein free, yeast free, sugar free, egg free, specific carbohydrate diet, low glycaemic index diet, additive and preservative free, caffeine free

Note: Participants having blood sample and completing food diary/dietary questionnaire (n=42)

The differences in dietary intake between iron replete and iron insufficient groups are outlined in Table 4.7. No significant differences in were seen in energy, carbohydrate, sugars, total fat, saturated fat and dietary fibre intake. Iron replete children had a significantly higher protein intake than those in the iron insufficient group (t = -2.254, 38.8 df, P = 0.003, medium sized effect r = 0.34). The iron replete group also had a significantly higher vitamin A intake than the iron insufficient group (U = 127.0, P = 0.036, medium sized effect r = -0.323).

Table 4.7: Comparison of macronutrient and specific micronutrient intake between iron insufficient and iron replete groups.

Variable	Iron Insufficient N=17	Iron Replete N=25	P-value
EnergyDF (kJ) §	6900.2±1763.6	7.140.2±1408.7	0.653
Protein (g) §	56.9±6.9	64.0±15.5	0.030*
CHO (g) §	208.7±63.1	214.4±51.0	0.866
Sugars (g) §	90.9±49.4	94.1±44.0	0.825
Fat (g) §	62.0±27.4	62.1±21.7	0.943
SFA (g) §	22.2±10.4	25.1±10.4	0.348
DF (g) §	19.9±8.1	20.7±7.0	0.749
Iron (mg) §	11.5±3.7	11.6±3.7	0.907
Calcium (mg) §	673.1±302.2	669.1±317.8	0.968
Vitamin C (mg) †	50.1 (30.7, 81.6)	72.9 (53.8, 99.0)	0.178
Vitamin A equivalents (µg) †	298.2 (191.7, 464.0)	473.4 (387.5, 578.2)	0.036*
% energy from protein §	14.8±3.7	15.3±2.9	0.460
% energy from fat §	32.7±7.7	31.8±7.0	0.799
% energy from SFA §	11.6±3.8	12.7±3.8	0.283
% energy from CHO §	49.7±7.8	50.0±7.3	0.896
% energy from fibre §	2.3±0.9	2.3±0.7	0.972

DF = dietary fibre; CHO = carbohydrate; SFA = saturated fatty acids

§ Values are mean±SD

†Mean (95% CI) for the log transformed data values, back transformed to the original scale

* Significant differences between iron insufficient and iron replete groups (P<0.05) (independent t-tests, Mann-Whitney U test)

Note: Participants having blood sample and completing food diary/dietary questionnaire (n=42)

A comparison between iron insufficient and iron replete groups meeting the RDI/AI and EAR for various nutrients was undertaken (Table 4.8). No significant differences were seen in the number of participants meeting the RDI for protein, dietary fibre, iron, calcium and vitamin C. Significantly more iron replete participants met the RDI for vitamin A compared to iron insufficient participants (X^2 = 4.627, P = 0.031). The odds of iron replete participants meeting the RDI for vitamin A was 2.09 times more likely than iron insufficient participants.

Table 4.8: Comparison between iron replete and iron insufficient groups meeting the RDI/AI and EAR for specific nutrients.

Variable	Met RDI/AI n(%)		RDI P-value	Met EAR n(%)		EAR P-value
	Iron Insufficient N=17	Iron Replete N=25		Iron Insufficient N=17	Iron Replete N=25	
Protein	16 (100.0)	27 (100.0)	1.000	17 (100.0)	28 (100.0)	1.000
DF	9 (56.3)	16 (61.8)	0.735	-	-	-
Iron	10 (62.5)	15 (62.5)	1.000	16 (100.0)	26 (100.0)	1.000
Calcium	9 (56.3)	12 (46.2)	0.525	11 (68.8)	18 (69.2)	1.000
Vitamin C	10 (62.5)	21 (80.8)	0.281	12 (75.0)	21 (80.8)	0.711
Vitamin A equivalents	5 (31.3)	17 (65.4)	0.031*	10 (62.5)	21 (80.8)	0.281

RDI=recommended daily intake; EAR=estimated average requirement; DF=dietary fibre

* Significant differences between iron replete and iron insufficient groups (P<0.05) (Chi-Square)

Note: Participants having blood sample and completing food diary/dietary questionnaire (n=42)

Chapter 5: Discussion

5.1. Introduction

The aim of this study was to investigate the iron status and determine factors affecting iron status in a cohort of children with ASD in New Zealand. As previously discussed, children with ASD between the ages of 3-8 years completed a four day food diary, dietary questionnaire, BPFAS questionnaire and had a blood sample taken to determine iron status. To our knowledge, this is the first study to investigate iron deficiency amongst children with ASD in New Zealand. The findings will be discussed, followed by methodological considerations, final conclusions and recommendations for future research.

5.2. Summary of Findings

5.2.1 Iron Status of Children with Autism Spectrum Disorder

Results from the present study indicate that iron depletion (SF ≤ 20 µg/L) was present amongst 32.9% of children with ASD in this population sample. This is much higher than the 3.0% iron depletion (SF < 10µg/L) seen in Australian children with ASD (n=122) (Sidrak et al., 2014), and 8% (SF < 12µg/L) in American children with ASD (n=368) (Reynolds et al., 2012).

ID without anaemia (SF ≤ 12 µg/L) in the present study was seen in 4.3% of the population sample. Studies using the same cut-off value found 12% of children with ASD (n=52) and 13.6% of children with Asperger's (n=44) in England had ID (Latif et al., 2002); and the prevalence of ID in American children with ASD (n=368) was lower with 1% (Reynolds et al., 2012). Other studies in children with ASD using a cut-off value of SF < 10 µg/L found 7.5% of Australian children (n=122), and 32.2% of Turkish children had ID (Bilgic et al., 2010; Sidrak et al., 2014). Other studies assessing iron status in children with ASD used SF < 10µg/mL for children under 5 years, and < 12µg/mL for school-aged children. They found 24.1% of Turkish children with ASD (Herguner et al., 2012), and 7% of Canadian children with ASD had ID (Dosman et al., 2006). Results from the National Children's Nutrition Survey (Ministry of Health, 2003) found 0.1% of males and 0.0% of females aged 5-6 years had ID, and 0.7% for both males and females aged 7-10 years. The survey did not include iron status on children aged 2-4 years. When comparing results of the present study with prevalence of ID in New Zealand neuro typical children, levels of ID appear higher in children with ASD. This is similar to a conclusion by Sidrak et al. (2014) who also found children with ASD in Australia had higher prevalence of ID than neuro typical developing children.

IDA (SF ≤ 12 µg/L plus Hb ≤ 110g/L for 1-5 years; or Hb ≤ 115g/L for 5-8 years) was not observed in any of the participants in our sample of children with ASD. Studies using similar cut off values to define IDA (Hb < 110g/L for children under 5 years, and < 120g/L for school aged children) showed one

participant out of 368 American children with ASD had IDA (Reynolds et al., 2012); 11.5% of English ASD children (Latif et al., 2002); and 15.5% of Turkish children with ASD had IDA (Herguner et al., 2012). The American study also used SF to determine iron status, but did not measure any biomarkers of inflammation, which may have masked some ID due to SF being an acute phase protein (Worwood, 1997). Other countries which used Hb < 110g/L to define IDA found prevalence was 6.5% in Turkish children (Bilgic et al., 2010), and 3.0% in Australian children with ASD (Sidrak et al., 2014). When comparing results from this present study to neuro typical developing children in New Zealand, similar results are seen. The National Children's Nutrition Survey (Ministry of Health, 2003) found 0.0% of children aged 5-6 years had IDA, and in children aged 7-10 years 0.2% for males and 0.1% for females. As discussed above, children aged 2-4 years were not included in the iron status analysis.

Comparison of prevalence of iron depletion, ID and IDA in children with ASD between studies should be interpreted with caution as they have used different biomarker cut off values to define each stage of ID (see section 5.3.3). Although the level of IDA in this study is low, it is important to note the high prevalence of iron depletion and ID in this population sample. ID is characterised by depletion of iron stores with reduced iron in the serum as well as decreased transportation of iron (P. R. Dallman, 1986). Without increased dietary iron intake or alteration of dietary factors impacting iron absorption, this may develop into IDA. As discussed earlier, IDA can impair brain development and cognitive performance, behaviour, growth and immunity and may mean children with ASD won't reach their full potential due to ASD being a neurodevelopmental condition.

5.2.2. Dietary Intake of Children with ASD and Associations with Iron Status

As previously discussed, the dietary intake of various macronutrients and micronutrients were investigated in our study by analysing four-day food diaries on Foodworks7 (Xyris Software (Australia) Pty Ltd, 2012). The mean energy intake for ASD children in this cohort was 7012.0±1454.8kJ. The following macronutrients contributed the following towards energy intake: protein (15.1±3.0%); carbohydrate (49.4±7.1%); total fat (32.7±7.2%); saturated fat (12.7±4.0%); and fibre (2.3±0.8%).

The dietary intake of children with ASD was compared to the NRVs set by the Ministry of Health for New Zealanders (Ministry of Health, 2006), the values used in this analysis are defined below. Protein, iron, calcium, vitamin C and vitamin A intake were compared the RDI and EAR per age group for 1-3 year olds and 4-8 year olds. Dietary fibre does not have a RDI, so therefore comparisons were made to the AI, which is used when an RDI value cannot be determined (Ministry of Health, 2006). The AI was determined from the median dietary fibre intake from Australian (Australian Bureau of Statistics: Commonwealth Department of Health and Aged Care, 1998) and New Zealand children (Ministry of Health, 2003).

Estimated Average Requirement (EAR)
A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

Recommended Daily Intake (RDI)
The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98 per cent) healthy individuals in a particular life stage and gender group.

Adequate Intake (AI)
The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

Definitions taken from Ministry of Health (2006).

The mean dietary iron intake for participants in this cohort was 11.2±3.7mg. The majority (77.8%) of participants aged 1-3 years met the dietary iron RDI whereas only 54.3% of participants aged 4-8 years met the RDI. Results from a Chinese study determining nutritional adequacy in children with ASD (n=111) found 80-90% of participants met the daily recommended intake range for iron (Xia et al., 2010). This differs to results seen in other studies on children with ASD which showed inadequate iron intake compared to recommendations (Cornish, 1998; Herndon et al., 2009). No differences in dietary iron intake were observed between the iron insufficient and iron replete groups. This is also seen in other literature which suggests dietary iron intake does not necessarily reflect iron status as associations between dietary iron intake and measures of iron status are relatively weak (Gibson, 1999). The UK National Diet and Nutrition Survey (NDNS) reported a weak correlation between iron intake and Hb concentrations, and no significant correlation with SF concentrations (Gregory et al., 1995). Iron status is dependent on a several factors which may be an explanation for this weak relationship (Gibson, 1999). Iron status is influenced by dietary iron intake as well as the form of iron that is ingested, being haem or non-haem. It also depends on the existence of factors which may enhance or inhibit the absorption of non-haem iron, which typically makes up more than 90% of iron in the human diet (Thane et al., 2000). Therefore this present study also determined the dietary intake of known enhancers and inhibitors of iron such as phytates, calcium, vitamin A, vitamin C and tannins.

The most common definition of dietary fibre is “the oligosaccharides, polysaccharides and the (hydrophilic) derivatives which cannot be digested by the human digestive enzymes to absorbable components in the upper alimentary tract, which also includes lignins” (Trowell et al., 1976). Dietary fibre was used in this study as an indicator of phytic acid intake as these often occur together in foods. Additionally food composition values for phytic acid are not available in the New Zealand Food Composition tables. Phytic acid found in high fibre foods may increase the risk of nutritional

deficiencies by chelating with minerals in the intestinal lumen, reducing solubility and therefore preventing iron absorption (Sandberg & Svanberg, 1991). Studies have shown impaired iron absorption with intake of wholemeal bread (Widdowson & McCance, 1942), bran and other wholemeal products (Jenkins, Hill, & Cummings, 1975; Persson, Raby, Fonss-Bech, & Jensen, 1976), which are all phytic acid containing foods. However, some evidence suggests dietary fibre can bind iron to the cell wall, and therefore also inhibit iron absorption. Some researchers suggest using dietary fibre when determining the bioavailability of dietary iron, as it encapsulates both inhibitory mechanisms (Cummings, 1978). However, phytic acid can be removed during the processing of fibre ingredients which has been shown to improve the bioavailability of iron (Torre, Rodriguez, & Saura-Calixto, 2009). Another study done in children with ASD found they had inadequate fibre intake when compared to recommended levels for age. Interestingly the age-matched controls also had inadequate fibre intake (Herndon et al., 2009). Results from the present study show no differences in dietary fibre intake between iron insufficient and iron replete groups. .

Dietary calcium inhibits both haem and non-haem iron absorption in the intestinal lumen. Studies on human subjects indicate there is a moderate inhibitory effect of calcium on non-haem iron absorption (Reddy & Cook, 1997). The inhibition of iron absorption by calcium has a dose-response relationship with additions up to 300mg calcium inhibiting iron absorption by 50-60% (Hallberg et al., 1991). Additionally, the same level of inhibition was seen when adding 165mg calcium in the form of milk, cheese or calcium chloride to wheat rolls (Hallberg et al., 1991). Another study looked at a meal as a whole to determine the inhibitory effects of calcium. They found when milk or cheese were not served with lunch and dinner, approximately 30-50% more iron was absorbed (Gleerup, Rossander-Hulthen, Gramatkovski, & Hallberg, 1995). This highlights the importance of consuming calcium-rich foods away from meal times to improve iron bioavailability. The calcium intake for both age groups did not significantly differ from the RDI in this present study and no differences were seen in calcium intake between iron replete and iron insufficient groups. However it is likely that the inhibitory effects of calcium will be present, particularly if calcium-rich foods are consumed alongside iron-rich foods. Results from a small study conducted in England on children with ASD (n=17) found calcium intake exceeded 200% of the recommended level due to excessive milk consumption. This differs to the Chinese ASD study (n=111) which found inadequate intake of calcium in their diets (Xia et al., 2010). Another study (n=77) also found children with ASD had lower calcium intakes when compared to aged-matched neuro typical control group (Herndon et al., 2009). It is not certain if the negative association of calcium on iron status is due to dairy foods displacing iron rich foods in the diet or due to the inhibitory effect of calcium on iron absorption (Heath, Skeaff, Williams, & Gibson, 2001). A British

study (n=1859) in preschool children found those consuming >400mg per day of milk or cream were less likely to eat a variety of food from each food group (Thane et al., 2000).

In the present study those in the iron replete group had significantly higher vitamin A intake than those in the iron insufficient group, with more participants in the iron replete group meeting the RDI than the iron insufficient group. Results in this study differ from that found in the Chinese study, which showed children with ASD had inadequate intake of vitamin A (Xia et al., 2010). There is controversy surrounding the potential beneficial effects of vitamin A on promoting iron absorption. Studies have found vitamin A counteracted the inhibitory iron absorption effects of tea or coffee and prevented the inhibitory effects of phytates from bread (Garcia-Casal et al., 1997; Layrisse et al., 1997). It was proposed that vitamin A binds iron in the intestinal lumen, forming a soluble complex, thus preventing iron interaction with polyphenols and phytates (Layrisse et al., 1997). However another study found that vitamin A had no enhancing iron absorption effects (Walczyk et al., 2003).

It is well known in the literature that vitamin C enhances the absorption of iron by reducing iron from ferrous (Fe^{3+}) to the ferric iron (Fe^{2+}). Results from one study showed 70mg vitamin C was associated with a five-fold increase in iron absorption (Layrisse, Martinez-Torres, & Gonzalez, 1974). Another study showed 280mg supplement taken at breakfast increased daily iron absorption two-fold, but taking 280mg as split doses increased daily iron absorption three-fold (J.D Cook & Monsen, 1977). Vitamin C can also reverse the inhibiting effects of calcium and tannins (Lynch & Cook, 1980). No differences were seen in dietary vitamin C levels between iron replete and iron insufficient groups. This is similar to a study conducted by Herndon et al. (2009) which found 89.1% of participants met the vitamin C recommended daily level, and there were no differences in vitamin C intake between children with ASD and neuro typical children. Our study differs from the study conducted in China which showed children with ASD had inadequate vitamin C intake (Xia et al., 2010). A small study done on ASD children in England (n=17) found one or more participants had inadequate vitamin C intake (Cornish, 1998).

In this study, in addition to nutrient intakes, dietary patterns that may impact the iron status of the sample population were investigated. No differences were seen between the iron insufficient and iron replete groups in terms of the number of vegetarians or vegans. Although there was no difference between the iron replete and iron insufficient groups in the consumption of red meat, with a larger sample size this may have reached significance level. This differs to a study by Sidrak et al. (2014) which found one of the biggest risk factors for ID and IDA in children with ASD was consuming inadequate amounts of chicken, meat, eggs or fish. Another study showed 35% of children with ASD did not consume red meat or meat products (Cornish, 1998). In adult population groups, the majority

of studies show consumption of red meat is associated with higher SF concentrations, whereas there is less convincing evidence for the effect of other nutrients on iron status (K. L. Beck, Conlon, Kruger, & Coad, 2014). Poor red meat consumption is of concern as it is a good source of haem iron which is more bioavailable than non-haem iron found in plant sources, and is less influenced by inhibitory dietary factors (Hallberg et al., 1991). Red meat also contains the meat, fish, poultry (MFP) factor which has been seen to improve iron absorption, although the mechanism is unclear (Monsen et al., 1978).

Tannins are naturally occurring polyphenols found in plants, seeds, bark, wood, leaves and fruit skins, as well as beverages such as tea and coffee. Foods high in tannins inhibit the absorption of iron by forming insoluble complexes with iron in the intestinal lumen, therefore reducing iron bioavailability (Disler et al., 1975). In this present study, consumption of tannin-containing beverages such as tea and coffee was only reported in one participant. This is of interest as results from the National Children's Nutrition Survey showed 21% of New Zealand children drink tea and 6% drink coffee (Ministry of Health, 2003). A study in infants found drinking tea was a major determinant of poor iron status (Grant, Wall, Brunt, et al., 2007). Due to the inhibitory effects on iron absorption and providing little nutritional value, the Ministry of Health recommends tea and coffee should be avoided in children under 13 years of age (Ministy of Health, 2012). However if groups at risk of ID do consume tea or coffee, it is recommended to drink between meals and to wait at least one hour after meals before drinking tannin-containing beverages (Nelson & Poulter, 2004).

The use of iron supplements was low in our population sample, with only two participants taking iron supplements. One participant taking iron supplements was in the iron insufficient group and the other was in the iron replete group, therefore iron supplementation did not have any effect on iron status. The use of iron supplementation in children with ASD has not been assessed, however, one study found iron supplementation for eight weeks improved SF, mean corpuscular volume, Hb levels, and sleep quality in 77% of children with ASD (n=33) (Dosman et al., 2007). Although the use of iron supplements was low in this study, the data collected did not indicate frequency of use or dosage. This is a major limitation of our study as this information could further explain iron status and practices of iron supplementation in children with ASD, which is a relatively unknown area of research.

5.2.3. Feeding Behaviours Associated with Iron Status in Children with Autism

Feeding issues among children can range from 20 to 40% (Laud, Girolami, Boscoe, & Gulotta, 2009), however, some reports suggest in populations with neurodevelopmental issues prevalence is much higher, ranging from 13 to 80% (Coffey & Crawford, 1971; Jones, 1982; Perske et al., 1977). In our study the proportion of participants which had a BPFAS frequency score ≥84 was 39%. The iron insufficient group had a significantly higher BPFAS frequency score than the iron replete group. In

addition, significantly more participants in the iron insufficient group had a BPFAS score ≥84. This indicates children who had sub-optimal iron status were more at risk of clinical feeding issues (Crist et al., 1994).

The association between feeding behaviour and iron status in children with ASD has not been researched. However, many anecdotal reports from parents, teachers and health professionals indicate children with ASD have challenging feeding behaviours. One study using the BPFAS questionnaire to identify feeding issues found children with ASD aged 2-12 years (n=82) were less likely to eat unaided and accept new foods (Martins et al., 2008). The high proportion of feeding issues in our study is comparable to other research indicating children with ASD have significantly more feeding problems than neuro typical children (Ahearn et al., 2001; K.A Schreck et al., 2004). The reason for the high prevalence of feeding issues was not explored in this thesis, however other studies suggest children with ASD may restrict intake due to dislike of texture, appearance, taste, smell, and temperature of the food (Williams et al., 2000).

5.3. Methodological Considerations

There are a number of methodological considerations that must be taken into account when analysing the results of this study. These are outlined in this section.

5.3.1. Study Design

This study is the first to investigate the iron status of children with ASD in New Zealand. As ASD is a neurodevelopmental disorder and iron is an essential mineral in brain development, it is useful to determine the prevalence of ID and IDA in this population group. Although the number of participants with ID and IDA in this sample was low compared to studies in some other countries, the high iron depletion rate seen in the study is of clinical importance. If iron depletion is not treated appropriately this is likely to develop into ID or IDA. Children with ASD in New Zealand do not come to the attention of health professionals unless management of comorbidities is required, therefore the iron status of children with ASD is not routinely tested. The Centres for Disease Control Prevention suggest population groups at risk of ID or IDA should be screened (Centres For Disease Control Prevention, 2002).

A strength of this study design was dietary intake (both nutrients and patterns) which may impact iron status were also investigated. Only two other studies have explored dietary intake as well as iron status in children with ASD (Cornish, 1998; Sidrak et al., 2014), therefore this study provides additional information regarding dietary intake associated with iron status in children with ASD. This study also investigates the relationship between feeding behaviours and iron status in children with ASD. To the best of our knowledge, this is the first study investigating this association.

A paediatrician and certified phlebotomist were employed to collect the blood sample from participants in this study. Children with ASD are a challenging population group to obtain a blood sample from due to behaviour and communication difficulties, therefore having trained professionals involved was important. Collecting blood samples from this population proved to be difficult with only 85.5% of the population having a sample taken. As children with ASD typically prefer repetitive routines and have difficulties adapting to new social situations, an unfamiliar environment, surrounded by strange people may increase stress on the child. This explains why blood collection was not successful in all participants. Alternative methods in determining iron status in population groups have been investigated. One such method is the spot ferritin method which involves the analysis of serum ferritin from a 20µL blood sample, such as a finger prick. This method has been proven reliable and accurate when compared to venous blood samples (Ahluwalia, Lonnerdal, Lorenz, & Allen, 1998). The spot ferritin method may have been more appropriate than venous blood samples to determine iron status in this population sample as a finger prick is less invasive.

This study was limited by low subject numbers. For the study to have statistical power to determine ID prevalence to within 5% based on an expected prevalence of 8%, 114 children needed to be recruited. As the study is using data collected from participants of a larger RCT, recruitment was not complete at the time of data analysis due to time constraints. Difficulties experienced in recruiting this population sample meant only 69 participants were recruited. Some results from this current study have trends towards being significant, and may have been if an adequate sample size had been achieved. This study does however provide valuable insight into the iron status, dietary patterns and behaviour of children with ASD, and provides a good basis for additional, larger studies to be completed.

In addition to the small sample size there were large amounts of missing data. Out of the 69 participants recruited, data on iron status and dietary intakes was only available for 42 participants. As this study was part of a larger 12 month RCT participant burden was high, particularly on parents who had to complete a variety of questionnaires, a food diary, and attend appointments. This is on top of caring for a child with ASD which comes with its own challenges.

Another limitation of the study is the lack of a control group of neuro typical developing children of similar age and gender. A control group would have allowed comparisons of dietary intake and iron status to be achieved. Other studies have used food diaries to compare dietary intakes of children with ASD to a neuro typical control group (Herndon et al., 2009; Lockner et al., 2008; Raiten & Massaro, 1986; Schmitt et al., 2008). Although this study did compare dietary intake to the RDI and

EAR for age and gender set by the Ministry of Health (Ministry of Health, 2006), it does not enable comparisons to be made with dietary intake of a typical New Zealand child.

Cross-sectional studies are limited in the fact they cannot determine a causation relationship as the exposure and outcome are measured simultaneously (Pynaert, 2007). In order for causation to be determined a longitudinal study where the same cohort is followed up over a period of time should be conducted. However, longitudinal studies are more expensive and require more burden on both the participants and research team (Pynaert, 2007).

5.3.2. Study Population

The study population was recruited for the VIDOMA RCT, and subsequently for the 'Iron status in young children with ASD' study through the WDHB developmental paediatrician and ASD co-ordinator, as well as advertisement on media platforms such as social media and radio. The use of a volunteer sample population is likely to attract parents or caregivers of participants who are health conscious, who have experienced previous nutrition deficiencies, or who are proactive in finding additional treatment options which may benefit their child's development and behaviour. A disadvantage of using a volunteer sample is that it may not be representative of all children with ASD living in New Zealand.

The majority of the participants recruited for this study were male (85%). ASD is four times more common in males than females (American Psychiatric Association, 2013). One study found females diagnosed with neurodevelopmental disorders had a greater number of harmful copy-number variants and single-nucleotide variants than males. This suggests the female brain requires more mutations than the male brain to produce symptoms of ASD and other neurodevelopmental disorders (Jacquemont et al., 2014). A high proportion of participants (40%) had an additional comorbidity, and 12.5% of participants had a previous diagnosis of ID or IDA. The majority of participants were of New Zealand European (62.3%) ethnicity, with only 1.4% Māori and 2.9% Pacific. Results from the National Children's Nutrition Survey showed Pacific children had greater prevalence of inadequate intake of iron, and in addition, Māori and Pacific had greater prevalence of ID compared to New Zealand European children (Ministry of Health, 2003). A larger sample size with more ethnic diversity could have allowed comparisons between differing ethnicities to assess if this is a determinant of ID amongst children with ASD.

5.3.3. Assessment of Iron Status

Due to the existence of multiple biomarkers, the optimal method to assess iron status is not clear. The current recommendation is to use a combination of different iron biomarkers (Burke et al., 2014; Clark,

2009). To determine the iron status of children with ASD in this study, a combination of biomarkers were used, being SF, serum iron, iron saturation, TIBC, and Hb.

To determine the stage of iron deficiency in this population group, SF was used to determine the amount of iron stored in the body. This is the preferred method to assess depleted iron stores (World Health Organisation, 2001). As discussed earlier in Chapter 2, each biomarker of iron status has various advantages and disadvantages. When inflammation is not present, The World Health Organisation (WHO) suggests SF as the best test for determining ID (World Health Organisation, 2001). The cut off-value used to define iron depletion in this study was ≤ 20 µg/L. This differs to other studies in children with ASD which used a level of <10µg/L (Sidrak et al., 2014), or <12µg/L (Reynolds et al., 2012). In the present study the cut off values used to define ID was a SF value of ≤ 12 µg/L. This is similar to the level suggested by the WHO of < 12 µg/L for children under 5 years of age, and <15 µg/L for children older than five years (World Health Organisation, 2001). Other studies defining ID in children with ASD used SF cut off values of < 10 µg/L (Bilgic et al., 2010; Sidrak et al., 2014), < 12 µg/L (Latif et al., 2002; Reynolds et al., 2012), or < 10 µg/L for children under five years and < 12 µg/L for school-aged children (Dosman et al., 2006; Herguner et al., 2012).

When determining IDA in population groups and individuals the most commonly used biomarker is Hb concentrations (World Health Organisation, 2001). This done in isolation is not specific to IDA as anaemia can be caused by other nutritional deficiencies such as low folic acid and vitamins A, B12 and C (World Health Organisation, 2001). Anaemia can also be caused by infectious diseases such as malaria and hookworm, although these are uncommon in New Zealand (Kline, McCarthy, Pearson, Loukas, & Hotez, 2013; Ministry of Health, 2012). Hb is also not a very sensitive measure of IDA as levels only drop when ID is already severe, therefore it is important to measure other sensitive iron indices such as SF (World Health Organisation, 2001). In this study the cut-off values when determining IDA was a SF concentration ≤ 12 µg/L, as well as Hb ≤ 110 g/L for children between the age of 1-5 years or ≤ 115 g/L for children between ages 5-8 years. This is similar to the Hb cut-off values recommended by the WHO with Hb values < 115g/L indicating anaemia in children aged 5-11 years. Other studies determining the degree of IDA in children with ASD have used Hb cut-off values of < 110g/L (Bilgic et al., 2010; Sidrak et al., 2014); < 110g/L for children under five years of age (Herguner et al., 2012; Reynolds et al., 2012), children between 2-4 years (Dosman et al., 2006) and children under six years of age (Latif et al., 2002); Hb < 120g/L in children aged five years and older (Dosman et al., 2006; Herguner et al., 2012), and children aged six years and older (Latif et al., 2002; Reynolds et al., 2012).

As well as reflecting iron stores, SF is an acute phase protein, so serum concentrations will rise during infection or inflammation (Worwood, 1997). One major limitation in our study was that no marker of inflammation was measured. The study was originally designed to measure C-reactive Protein (CRP) to determine the inflammatory state of the participants in order to determine true iron status when using SF. However, blood samples and analyses were performed off-site and this was not measured. This may have caused some participants to have normal SF levels when they were in fact in a state of ID.

Limitations also exist for other iron biomarkers used in this study. Serum iron and transferrin saturation measure of the amount of circulating iron and can help to differentiate between ID and anaemia caused by chronic disease or inflammation. However serum iron fluctuates depending on the time of day and it doesn't detect iron bound to Hb, and decreases in transferrin saturation are also seen during an inflammatory state. TIBC determines the amount of binding sites on transferrin, the iron transport protein, however this is not a sensitive measure of ID as levels only change when iron stores are depleted (Burke et al., 2014).

A biomarker that would have been useful to measure in this study is soluble transferrin receptor (sTfR), which concentration increases during ID. Additional benefits to this biomarker include good accuracy in the paediatric population and it is less sensitive to inflammation than SF (Beguin, 2003). However, due to budgetary constraints of this study this test was not used as it is expensive.

5.3.4. Other Determinants of Iron Status

Other determinants of iron status such as socio-economic status were not investigated in this study. Some studies have found children and adolescents of lower socio-economic status have increased prevalence of ID than those from higher socio-economic backgrounds (Keskin et al., 2005; Soekarjo et al., 2001). Possible explanations for differences in iron status in socio-economic groups include the lack of food security with less money spent on animal sources of iron (meat, fish, and poultry) (Bhargava, Bouis, & Scrimshaw, 2001), limited dietary diversity (Lynch, 2011), and reduced access to health care (Looker et al., 1997). Genetic factors can also influence iron status such as the genetic disorder haemochromatosis where iron is absorbed and stored in excess amounts, or alternatively other genetic conditions can limit RBC production or result in chronic haemolysis (Baker & Greer, 2010). These were not investigated in this study.

5.3.5. Assessment of Dietary Intake

Estimated four day food diaries completed by the participant's parents were used in our study to determine dietary intake of macronutrients and micronutrients, specifically protein, dietary fibre, iron,

calcium, vitamin A and vitamin C. Food diaries are widely used in literature to assess diet quality in children, however, there are both strengths and limitations in using this method.

The gold standard in determining dietary intake is a weighed food diary, where the participant records the time, food and weight of food consumed. Although this method is preferred, it is time consuming and requires high participant commitment and motivation, leading to high drop-out rates and poor compliance. An estimated food diary involves the participant recording quantities of food eaten based on household measures, or comparing to other objects and weight. This method of assessing dietary intake is less time consuming and offers flexibility, therefore less participant burden and lower drop-out rates. Estimated food records were found to have the highest correlation with weighed food records when compared with other methods of dietary assessment such as 24 hour recalls and a FFQ (Bingham et al., 1994).

Children under seven years of age are unable to reliably report their dietary intake due to: (i) over and under-reporting (Baranowski et al., 1986; Domel, Thompson, Baranowski, & Smith, 1994; Emmons & Hayes, 1973; Meredith et al., 1951; Samuelson, 1970); (ii) incorrect identification of foods (Emmons & Hayes, 1973; Meredith et al., 1951; Samuelson, 1970); (iii) decreased recall ability when the number of foods eaten increases (Baranowski et al., 1986; Meredith et al., 1951); (iv) distractions (Baranowski et al., 1986); (v) and the fact that main course items may be easier to remember than secondary items (Emmons & Hayes, 1973). Therefore it is essential for parents to accurately report their child's food consumption in order for analysis of children's diets to be feasible (Livingstone & Robson, 2000). Evidence from the literature suggests in the home environment parents are able to reliably report their child's dietary intake (Baranowski et al., 1991; Basch et al., 1990; Eck, Klesges, & Hanson, 1989; Klesges, Klesges, Brown, & Frank, 1987), particularly if both parents are involved in the recording process (Eck et al., 1989). Participants in these studies were present at the time their child was consuming the food being recorded. Parental reports of food intake can be used to determine means of energy intake and nutrients at a population level, however should not be used to interpret individual data due to inaccuracies in determining portion sizes and misreporting (Baranowski et al., 1991; Basch et al., 1990; Eck et al., 1989; Klesges et al., 1987). Additionally, food diaries in the present study were completed prospectively, therefore eliminating error due to memory (Candilo et al., 2007).

There are however, certain limitations in using an estimated food diary completed by participants' parents. Inaccuracies in parental food diary reporting occur when the child is away from home such as when they are attending pre-school and school (Baranowski et al., 1991). Mothers of pre-school children are less accurate in reporting dietary intake if their child is away from home for more than four hours per day (Baranowski et al., 1991). Therefore it is important that other adults

caring for the child aid in recording dietary intake. This may also pose problems as they may be less motivated than parents to record accurately (Candilo et al., 2007). Thus, having parents as the only reporters of dietary intake can impair the quality of the data collected, and this is also a limiting factor in our study.

Under-reporting or over-reporting is also an issue in our population group, with mothers more likely to under estimate their child's dietary intake than over estimate (Baranowski et al., 1991). Another limitation with estimated food records is the ability of the participant to quantify food eaten, this creates a large unknown component of error (Livingstone & Robson, 2000). This error can be reduced by training the parents to accurately estimate portion sizes prior to study commencement (Livingstone & Robson, 2000). In our study parents received training on how to fill in the food diary, the level of detail required, as well as how to estimate portion sizes. Although the food dairies were completed prospectively in this study, reviewing the food dairies with the researcher was done retrospectively. This could cause memory related limitations such as forgetting, mistaking intake at the time of food diary with current intake, and difficulty in recalling portion sizes (Livingstone & Robson, 2000). In addition, estimated food diaries require high literacy and numeracy levels as well as having a large financial cost (K.L Beck & Health, 2013).

5.4. Conclusion

Results from this study suggest that children with ASD are at a higher risk of developing iron depletion and ID compared to neuro typical children in New Zealand, however this needs to be confirmed in a larger population sample. No differences in dietary iron intake were seen between iron sufficient and iron replete participants. However iron replete participants had significantly higher vitamin A and protein intake than iron insufficient participants. Additionally participants in the iron insufficient group had significantly more feeding behavioural problems than those in the iron replete group. Due to many other factors affecting iron absorption further investigations are needed to determine what factors are the largest determinants of iron status in this population.

5.6. Recommendations for Future Studies

1. Additional studies should recruit a larger sample size in order for statistical power to be achieved and therefore obtain more meaningful results.
2. With a larger sample size multiple or logistic regression analysis could be done to determine which factors have the biggest influence on determining iron status of children with ASD.
3. Studies should include a control group of neuro typical developing children in order for comparisons between iron status, dietary intake, and feeding behaviours which may influence iron absorption to be made.
4. Studies should use an inflammatory biomarker, such as CRP, to control for effects of inflammation on SF. Additionally studies determining the iron status in paediatric populations should use soluble transferrin receptor as a biomarker.
5. Routine screening of iron status should be done in populations at risk of ID and IDA, including children with ASD. Studies should investigate alternative iron screening methods, such as finger pricks to measure serum ferritin concentrations.
6. Future research should also investigate the use of iron supplementation in this population, including dose, frequency of use and duration of supplementation.

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Appendix A

First Contact Pack

StudyID: VID_____

The VIDOMA Study
CONTACT DETAILS SHEET

Parent/ guardian name: _____

Child's name: _____

Child's gender (Please tick): Male □ Female □

Child's Date of birth: _____

Child's age: _____

Child's NHI numbers: _____

ASD Severity rating: _____

Daytime telephone number: _____

Mobile number: _____

Email address: _____

Address (for delivery of supplements and information – if you move house during the trial, please contact)

House Number: _____

Street Name: _____

Suburb: _____

City: _____

Postcode: _____

GP Name: _____

GP Address: _____

GP Contact Number: _____

OK for us to send results: O

The VIDOMA Study
Medical History

Confidentiality

Your personal information will be kept in a secure location separate from the main questionnaire data. Your answers are completely confidential. No personal information such as your name or address will be shared with any other individual or agency.

1. Does your child have or has your child had any chronic (that is ongoing) medical conditions? E.g asthma, eczema, allergies, ear or throat infections

If yes, please describe:

_____

2. Is your child currently taking any medication or supplementation? Brand/dose/ frequency

If yes, please list (medication name & dosage, reason for taking)

_____

_____

3. Is your child currently having any therapy related to autism, such as behavioural or speech therapy?

If yes, please describe it (what type of therapy, who with, how often)

_____

_____

4. Has your child ever had or been treated for low iron stores, iron deficiency or iron deficiency anaemia?

Diagnosis, date, diagnosed by, any further details:

_____

_____

5. Has your child had any blood loss in the past 6 months e.g. from medical conditions, injuries, nose bleeds, etc

If yes, please describe

_____

_____

_____

6. During pregnancy, did the mother of the child experience any infections or illnesses? e.g gestational diabetes, iron-deficiency anaemia, severe morning sickness

Yes □ No □ If yes, please specify condition

_____

7. After birth, did the child experience any infections, illnesses or anything else of note? e.g jaundice, respiratory tract infections, ear infections, rickets

Yes □ No □

If yes, please specify condition _____

8. Which ethnic group or groups does your child belong to?:

□ New Zealand European

□ Maori

□ Pacific Please specify _____

□ South Asian

□ Chinese

□ Korean

□ Southeast Asian Please specify _____

□Other ethnicity Please specify _____

9. In the last 12 months what was your annual household income (after tax)?

□ Below $60,000 □ $60,000 – 140,000 □ Over $140,000

10. How many other children live in your household?

_____

11. What are their ages?

_____

12. Have any of your other children been diagnosed with autism?

Yes □ No □

---

13. If your child was breastfed, how long were they exclusively breastfed for?

Not breastfed □ Exclusively breastfed for _____ months

14. During the first 18 months of life, which, if any, of the following did your child regularly consume:

Infant formula □

Follow-on formula or toddler milk □

Blue top cow's milk □

Orange top cow's milk □

Water □

Juice □

VIDOMA Study

First contact to caregiver

INTERVIEWER NOTES

Questions?
Introduce team
Info sheet questions

Next steps

Verbal consent – return envelope

Medical history

Demographics and contact info sheet

If twin – fraternal or identical?

How is your child during a blood test?

How easily does your child adapt to new environments?

Ask parent what material may be required to more successfully familiarise the child with the proceedings

Does the parent require any additional information or help in completing the questionnaires?

Genetic component? Consent form

Day/ time preference?

Introduction parcel with contents – consent forms, social stories, food diary, return envelope etc.

Appendix B

Information Sheet

The VIDOMA Study
A nutrition intervention in children with ASD
Information sheet for study participants

This information sheet provides you with the background to the research and other important details about what is involved, so please read carefully before deciding whether or not to participate.

We are currently recruiting children with Autism Spectrum Disorder (ASD) aged 2.5 - less than 8 years old to take part in this research. The study is nicknamed the VIDOMA study, short for VItamin D and OMega-3 in Autism. As a parent/guardian, it is important for you to understand why we doing this research and what it will involve for you if you decide that your child can participate. This information sheet tells you about the purpose of the study and what will happen to if you choose for your child to take part. Please take time to read it carefully and discuss it with others if you wish. Please ask if anything is not clear, or if you would like more information.

Introducing the researchers

This study involves a team of researchers from Massey University and from Waitemata District Health Board (WDHB). The principal investigator is Dr Pamela von Hurst, Co-director of Massey's Vitamin D Research Centre. The study manager is Mr Owen Mugridge whose contact details are at the end of this information sheet. The psychologist on the team is Ms Lindy Thomas and the Paediatrician is Dr Bobby Tsang from WDHB. A number of other staff and students from the Division of Human Nutrition and Dietetics are also involved.

What is the purpose of this study?

Children with ASD often have deficiencies in their diet due to physical and behavioural issues related to the condition. Some of the nutrients which can be deficient in children with ASD are known to affect brain development and function. The two nutrients that this study is going to investigate are vitamin D and omega-3. Recent studies have shown that there are a variety of ways by which both these nutrients can affect the function and connectivity of the developing brain.

If shown to be effective, increasing the vitamin D and omega-3 status of children with ASD may be a powerful, noninvasive and low cost strategy for improving some of the symptoms of ASD and improving the quality of life for children and their families.

Why have I been chosen?

You have been invited to participate because your child has been diagnosed with Autism Spectrum Disorder.

Does my child have to take part?

No, it is completely up to you and your child to decide whether or not to take part. If you do decide to enter your child into the study you will be asked to sign a consent form. You are free to withdraw your child at any time without giving a reason. Your child will also be given a sheet describing what

will happen (very simply) so that he or she has the opportunity to ask questions and agree to take part.
If you decide to withdraw your child from the study, data obtained to date may be kept and used to contribute to the overall results. However, if you request that your child's data and other information relating to your child are destroyed. Taking part in the study does not affect any aspect of the routine care your child received, or could receive from the local DHB.

What do I have to do?
You and your child will attend 5 appointments during the 12 month period. Your first visit will be at North Shore Hospital or Waitakere Hospital where your child will have a blood test. The blood test will look at vitamin D, iron, vitamin B12, folate, and magnesium. If your child is deficient in iron, vitamin B12 or magnesium, the study doctor will prescribe supplements to correct this, then your child can continue with the study. If your child has especially high vitamin D, he or she will not be able to continue with the study. However, we would like to continue with the collection of their dietary information which we will analyse and report back to you.
The second visit will be at Massey University, Albany. This visit will last for approximately 1.5 hours. Before you come, we will send you a "Preparation Kit" to prepare your child for all aspects of the study visit, to help familiarise them with the new surroundings and people. We will also send you a food diary to record 4 days of your child's total food intake. At this visit you will be asked to complete some questionnaires about the symptoms of your child's condition such as behaviour, sensory issues and socialisation. There will also be some questions regarding sun exposure habits of you and your child. We will measure the height and weight of your child and they will have time to play with some toys until you are finished.

As part of the study your child will have to take supplements. The supplements are in the form of a tasteless, colourless oil, and can be mixed in with your child's food or drink, or administered with a medicine syringe. Your child will be randomised into a study group and you will be given the supplements that your child will take for the next 12 months. The supplements will either be vitamin D, Omega-3, a combination of vitamin D and Omega-3 or a placebo. The study is double-blinded meaning that neither you nor the researchers will know what your child is taking until the end of the study.

Six months after starting the study, another blood test will be required, at either North Shore Hospital or Waitakere Hospital. We will send you all the instructions for this at the time. Similarly, at the end of the 12 months there will be another blood test, and a visit to Massey University (and North Shore or Waitakere Hospital) for the same tests as were done at the beginning.

Altogether, you will make 5 trips to either the hospital and/or Massey University over the year of your child's participation in the study. You are welcome to bring supporting friends or Whanau with you to any of the appointments. Your child will have three blood tests during this period, and will have to take the supplement every day for 12 months. There will also be questionnaires for you to complete at the beginning and end of the study.

What will happen to the blood samples?
Nearly all of the blood samples will be processed immediately at the North Shore Hospital Laboratory. A small sample of red cells will be saved and frozen for later analysis of red cell fatty acids. This analysis will be completed in a laboratory at the University of Wollongong in Australia. Some of the samples may not be analysed immediately after the study and will remain stored at the

Massey Nutrition Laboratory in the meantime. These samples may be used for further analysis, including genetic analysis. There is a separate information sheet and consent form relating to these samples. If you are not comfortable with these aspects of the study, you do not have to consent to them. This will in no way affect your child's participation in the rest of the study.

Will I be reimbursed for my time?
You will not receive reimbursement for your time. However, we will support you with travel for the trips made during the study, if requested.

What are the possible risks and disadvantages of taking part?
There are no reported risks of taking either vitamin D or Omega-3 at the dose we will be using. There is a very small risk that vitamin D supplementation will increase calcium levels in the blood. A safety check blood test will be carried out at the 6 month stage to ensure that all is going well with blood levels of vitamin D, Omega-3 and calcium.
It is quite possible that the blood test will cause your child (and you) some distress. The hospital staff are very experienced at conducting blood tests, and the Paediatric Registrar will be on hand if any further help is required. If you know that your child gets very distressed by blood tests, let us know and we can discuss with you the option of some light sedation which will be administered by the Paediatrician.

What are the advantages to taking part in the study?
As a participant in this study, your child will have a number of assessments which are not usually available through standard care from the District Health Board. Any nutritional deficiencies will be identified at the beginning of the study and addressed. You will receive a wealth of information about your child's responses to the psychological assessments which are normally valued at approximately $400. You will also be helping with research which, if successful, could make an important difference for many other children and families like yours.

What will happen if anything goes wrong?
The risks involved in this study are very small and all of the measurements are routinely made. If you have any concerns during the study you can discuss these with a member of the study team. If your child has any other problems, illnesses or concerns you should discuss these with a member of the study team.
Any complaints you have will be fully investigated. If you have a concern about any aspect of this study, you should speak to a member of the study team who will do their best to answer your questions.

Will my taking part in the study be kept confidential?
Yes. All information collected about you and your child during the study will be kept strictly confidential. Information will be entered into a protected database at Massey University. Massey University code all data so that your names and address are kept separate from any other information about your child.
Information collected about you will be kept strictly confidential and secure in locked filing cabinets and/or electronic files on computers with passwords and restricted access. Each participant is assigned a unique code which is used on all data collected. Only the specified research team will have access to personal identifying information.

Massey University maintains a central record of all research projects but this does not include any personal information about participants. We will store the data for 10 years, at which point it will be destroyed.

What will happen to the results?
You will receive all the results that apply to your child. At the end of the study, we will also be in touch to let you know the results of the study. The overall results may be presented at scientific meetings or published in scientific journals to ensure that the wider community including health professionals know about the findings. The findings may also be featured in the media. Your child will not be identified in any of these presentations or publications.

Who is organising and funding the study?
This study is being co-ordinated by Massey University's College of Health in collaboration with Waitemata District Health Board. The Principal Investigator is Dr Pamela von Hurst. The study is funded from a number of sources including Douglas Nutrition Ltd who provided the supplements free of charge, and Massey University.

Who has reviewed the study?
This project has been reviewed and approved by the Health and Disability Ethics Committee: 14/NTA/113.

Contact for further information:
If you have any further questions or if you have any concerns whilst participating in the study then please contact Owen Mugridge – 09 213 6650.
Massey University,

Albany College of Health
Gate 4 – Building 80
Turitea Place
Albany 0632
Auckland
09 414 0800 Ext 43650

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.

Cultural Support
If you require Maori cultural support, talk to your whanau in the first instance. Alternatively you may contact the administrator for He Kamaka Waiora (Maori Health Team) by telephoning 09 486 8324 ext 2324

If you have any questions or complaints about the study you may contact the Auckland and Waitemata District Health Board's Maori Research Committee or Maori Research Advisor by telephoning 09 486 8920 ext 3204

Appendix C

Consent Form

The VIDOMA Study

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.

I agree for my child to participate in this study under the conditions set out in the Information Sheet.

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

Full Name of Parent/legal caregiver Please print .....

Full Name of child Please print .....

Appendix D

Social Stories

My Grand Day Out at Massey!

We travel to Massey. This is where I will play with toys.

First I will meet some of the people that work there

This is Owen

This is Hajar

We can relax and play some games in this room.

Or play on the trampoline! There are lots of toys and pictures to colour too.

We'll see how tall I am and see if I'm growing strong!

This looks like a robot!

Can you see Elsa and the Minions?

We are finished now. I can choose a balloon. I wave goodbye and we leave.

My Grand Day Out at the Hospital

Can you find the entrance?

Here it is!

Down the corridor….

I can sit here while I wait for the nurse

Here's the nurse!

Can you see the toys?

Will you get the Bravery Award?

My Grand Day Out at the Hospital

Can you find the entrance?

Here it is!

Where is the nurses' room? Follow the penguins!

All penguins to the left!

Down the corridor….

Now the penguins are going right!

Where can we sit whilst we're waiting?

Can you spot the penguins?

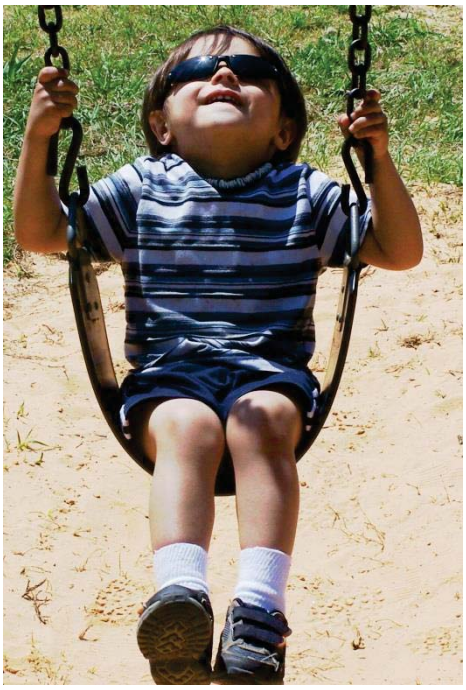
Here's the nurse!

Can you see the toys?

Appendix E

Food Diary and Dietary Questionnaire

The VIDOMA Study



4 Day Food Record

Thank you very much for taking part in this study. We are extremely grateful for your time, effort and commitment

If you have any questions, please contact Owen Mugridge on 09 213 6650

All information in this diary will be treated with the strictest confidence. No one outside the study will have access to this.

Please bring the food diary with you when you bring your child in for assessment at Massey University.

4 day food diary - what to do?

- Record all of the food that your child eats and drinks on the following dates.

- **Please complete the diary on consecutive days for 1 weekend day and 3 week days. For example, Sunday, Monday, Tuesday and Wednesday OR Wednesday, Thursday, Friday and Saturday.**

- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.

- Include all meals, snacks, and drinks, even tap water.

- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.

- Write down any information that might indicate size or weight of the food to identify the portion size eaten.

- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.

- Use as many pages of the booklet as you need.

- You can also save any packets such as muesli bar wrappers and bring them in with your child's food diary

- Please answer the short questionnaire at the back of this booklet regarding your child's diet

Describing Food and Drink

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	Food record description
Breakfast example – cereal, milk, sugar	2 Weetbix (Homebrand) 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Lunch – Ham sandwich	2 slices of wholegrain bread (Vogels) 1 slice ham 2 slices edam cheese 2 tsp flora margarine Water 1 cup to drink
Dinner – Spaghetti Bolognese	½ cup mince sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand) Milk 1 cup Pam's whole milk
Snacks	Flemmings apricot chocolate chip muesli bar (35g) 1 small banana 2 Salada crackers with 1 tsp peanut butter Small packet of Bluebird salt and vinegar chips

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	Food record description
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g white fish pan-fried

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	Food record description
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	½ cup of casserole beef or 5 chicken nibbles in honey soy marinade
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

General description	Food record description
Apple	1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

General description	Food record description
Milo	I x cup Milo made with Milo powder and 150mls Calci-trim milk, 100 ml hot water. No sugar

- **Record recipes** of home prepared dishes where possible and the proportion of the dish your child ate. There are blank pages for you to add recipes or additional information.

Recording the amounts of food your child eats

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. Eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.

- By weight marked on the packages – e.g. a 425g tin of baked beans, a 32g cereal bar,

- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.

- For bread – describe the size of the slices of bread (e.g. sandwich, medium, toast) – also include brand and variety.

- Using comparisons – e.g. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.

- Use the food record instructions provided to help describe portion sizes.

General description	**Food record description**
Cheese	1 heaped tablespoon of grated edam cheese 1 slice cheese edam (8.5 x 2.5 x 2mm) 1 cube edam cheese, match box size

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please try to have your child eat as normally as possible – ie. Don't adjust what he/she normally eats just because you are keeping a diet record and be honest! This record will give us important information about your child's diet, and help us identify any possible deficiencies which we can then help you correct.***

Example day

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)
Example 7:55am	Sanitarium Weetbix	2 weetbix
" "	Anchor Blue Top milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	6 x chocolate covered Girl Guide biscuits (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Cookies and Cream ice cream	1/2cup (g) (125g)

Date_____ **DAY 1**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ DAY 1 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 1)

Recipes (Day 2)

Date_____ **DAY 2**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ **DAY 2 continued**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ **DAY 3**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ DAY 3 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 3)

Recipes (Day 4)

Date_____ **DAY 4**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ **DAY 4 continued**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ **(spare pages)**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date_____ (spare pages)

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Dietary Questionnaire

Please answer the following questions regarding your child's diet:

1. Is your child a vegetarian?
   - ☐ Yes
   - ☐ No

2. Is your child a vegan?
   - ☐ Yes
   - ☐ No

3. Does your child eat red meat?
   - ☐ Yes
   - ☐ No

4. Food intake practises:
   Yesterday, during the day and night, did your child eat any of the following foods on their own or as part of a dish.
   Read the list of iron-rich foods below and tick either yes or no for each food item.

   Organ meat

Liver	☐ Yes	☐ No
Kidney	☐ Yes	☐ No
Heart	☐ Yes	☐ No

   Fish meat

Beef	☐ Yes	☐ No
Pork	☐ Yes	☐ No
Lamb	☐ Yes	☐ No
Goat	☐ Yes	☐ No
Rabbit	☐ Yes	☐ No
Chicken	☐ Yes	☐ No
Duck	☐ Yes	☐ No

   Fish and seafood

Fresh fish	☐ Yes	☐ No
Dried fish	☐ Yes	☐ No
Canned fish	☐ Yes	☐ No

Prawns ☐ Yes ☐ No

Shrimps ☐ Yes ☐ No

5. Consumption of vitamin-C rich fruits:
   Does your child usually eat fresh fruits high in vitamin C such as oranges, mandarins, kiwifruit, or drink juice made from them?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

If yes:

Every day?

- ☐ Yes
- ☐ No
- ☐ Don't know

When does your child usually eat fresh fruits high in vitamin C?

- ☐ Before a meal
- ☐ During a meal
- ☐ After a meal
- ☐ Other *(specify)* _____
- ☐ Don't know

6. Consumption of tea or coffee: Does your child usually drink tea or coffee?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

If Yes:

Every day?

- ☐ Yes
- ☐ No
- ☐ Don't know

When does your child usually drink tea or coffee?

- ☐ Two hours or more before a meal
- ☐ Right before a meal
- ☐ During the meal
- ☐ Right after a meal
- ☐ Two hours or more after a meal
- ☐ Other *(specify)*_____
- ☐ Don't know

7. How much does your child like the taste of foods rich in iron such as beef, chicken or liver?
    - Dislike
    - You're not sure
    - Like

8. Does your child skip meals?
    - Yes
    - No

If Yes:

What meal is the most commonly skipped?

- Breakfast
- Morning tea
- Lunch
- Afternoon tea
- Dinner

The following questions will enable us to find out information about any special diets and supplements you might have your child on.

1. Is your child on any of the following diets? If the diet is not listed please provide detail of what kind of diet your child is on. (Tick all those that apply)
    - Gluten free
    - Dairy free
    - Yeast free
    - Sugar free
    - Egg free
    - Gluten-free/casein free
    - Specific Carbohydrate diet
    - Feingold diet
    - Low GI diet
    - Additive and preservative free
    - Caffeine free
    - Other_____
      _____
    - Not on any special diet

2. If you selected any of the above diets, what were your reasons for adopting the use of a special diet?
    - ☐ To improve your child's health and behaviour
    - ☐ To improve your child's heath
    - ☐ To improve developmental levels
    - ☐ Health professionals recommendation
    - ☐ Other_____
      _____
      _____
    - ☐ N/A

3. What changes did you observe, if any, in regards to the change in your child's diet?
    - ☐ Improved behaviour
    - ☐ Improved communication skills
    - ☐ Improved sleep pattern
    - ☐ Improved social interaction
    - ☐ Other_____
      _____
      _____
    - ☐ No notable changes
    - ☐ N/A

4. If you selected any of the above diets, please advise where you received your information regarding the special diet. (Tick all those that apply)
    - ☐ TV
    - ☐ Website
    - ☐ Magazine
    - ☐ Book: please specify name -
      _____
    - ☐ Parent support group
    - ☐ Relatives and friends
    - ☐ Your GP
    - ☐ Dietitian
    - ☐ Autism NZ
    - ☐ Celiac Society
    - ☐ Alternative health professional – Naturopath
    - ☐ Other_____

5. Have you had your child tested for allergies?
    - ☐ Yes
    - ☐ No

If yes, please give information on where you had your child tested to confirm the allergy?

_____

6. Please give information on any supplements your child is on. (Tick all those that apply)
   - ☐ Omega 3 capsules
   - ☐ Probiotics
   - ☐ Vitamin C
   - ☐ Vitamin B12
   - ☐ Vitamin B6
   - ☐ Vitamin D
   - ☐ Selenium
   - ☐ Iron
   - ☐ Magnesium

   - ☐ Melatonin
   - ☐ General mutli-vitamin
   - ☐ Iron
   - ☐ Calcium
   - ☐ Echinacea
   - ☐ Other_____
   - ☐ N/A

7. Where have you received information from regarding the use of supplements? (Tick all those that apply)
   - ☐ TV
   - ☐ Internet
   - ☐ Magazine
   - ☐ Parent support group
   - ☐ Relatives and friends
   - ☐ Your GP
   - ☐ Dietitian
   - ☐ Autism NZ
   - ☐ Celiac Society
   - ☐ Other_____

8. What were your reasons for using supplements?
    - To improve your child's health and behaviour
    - To improve your child's heath
    - To improve developmental levels
    - Health professionals recommendation
    - To prevent nutritional deficiencies
    - Other_____
    - N/A

9. What changes did you observe, if any, in regards to the use of supplements that your child is taking?
    - Improved behaviour
    - Improved communication skills
    - Improved sleep pattern
    - Improved social interaction
    - Other_____
      _____
    - No notable changes
    - N/A

Appendix F

Blood Collection at Waitemata District Health Board

Venepuncture using Evacuated Tube System

Step	Action
1. Greet Patient	• Greet the patient and family/whanau in a friendly manner. • Introduce yourself and state your purpose. Explain the procedure that you are about to perform. • Gain the confidence of the patient and their verbal consent by checking that the patient will allow you to collect the blood specimen.
2. Perform Hand hygiene	• Hand hygiene must be performed before and after any patient contact.
3. Identify Patient	• Identify all patients by asking him/her to state their full name and date of birth. Confirm their verbal response matches the details on the request form. • Do not proceed if their name and date of birth do not match the request form. • Confirm that the in-patient's ID bracelet, attached to the patient, matches the request form details. Perform hand hygiene before and after patient contact. • If the in-patient has no ID bracelet, do not proceed until the patient's nurse has put the correct ID band on the patient. • If the patient is unable to verbally confirm their identity, ask the patient's nurse to identify the patient for you. Use the stamp "Patient identified by Nurse X" and obtain their signature.
4. Check Request Form & special requirements	• Check the request form for any special test requirements such as fasting, diet restriction, trough/peak drug levels. • For Drug Level collects, record the time of previous dose. This can be obtained from patient's nurse or medication chart. • Check to see whether a patient has latex sensitivity; most patients will know this and will inform the phlebotomist. In the wards a prominent notice is displayed at the bedside.
5. Assemble supplies	• Select the blood tubes required, placing them in the correct order of draw ready for use. • Refer to the Laboratory Test Manual to confirm correct tubes and collection requirements are selected for all requested tests. • Place tourniquet, alcohol wipe, gauze, tape, needle and tube holder in kidney dish or paper towel.

6. Perform Hand Hygiene Before Gloves	• Perform hand hygiene before patient contact and putting on gloves. Allow hands to air dry before putting on gloves. Select the correct size & type of gloves: gloves for venepuncture should be latex or nitrile and powder-free.
7. Position the Patient	• Position the equipment beside the patient. Ensure the sharps disposal bin is within safe access. • Ensure that the patient is positioned in a comfortable phlebotomy chair or bed. • Position the arm to form a straight line from the shoulder to the wrist in a downward direction to prevent reflux or “backflow”. • In the phlebotomy chair, support the arm with the armrest; in the bed, the arm may be supported by placing a pillow underneath the arm. • A slight bend in the arm is helpful if the arm hyper-extends. • Ensure that the patient does not have any foreign objects in their mouth during the collection procedure e.g. food, liquid, chewing gum, thermometer.
8. Select a suitable collection site	• Check the arm for suitability as a site for venepuncture. • Avoid areas with scarring, mastectomy, oedema, haematoma, intravenous therapy, cannula, fistula, vascular graft.If patient has an IV drip or a luer, collection from the other arm is preferable. • If the only suitable vein is in the “drip” arm, ask the patient’s nurse to turn off the drip for a minimum of 2 mins before blood collection. • Inform the nurse to restart the drip when you have finished. Phlebotomists must not stop nor restart the drip. • If the patient is a diabetic do not collect from the feet or legs. If there is no alternative site, permission must be obtained from the Doctor to use the leg or foot. • If patient is currently receiving a blood transfusion, check with medical staff before taking the specimens. In most cases the collection may need to be deferred until the transfusion is completed.
9. Apply the Tourniquet	• Apply the tourniquet 7 -10 cm above the intended venepuncture site. • The tourniquet should not be tightened on the arm for more than 1 minute during preliminary vein selection. If necessary, release the tourniquet, let blood flow for a minute or two before retightening. • If the patient has a skin problem, the tourniquet should be applied over the patient’s clothing, or a paper tissue or towel. • It is recommended that the patient avoids making a fist or doing vigorous hand exercise (“pumping”). This can elevate some levels in the blood.

10. Select Most Prominent Vein	• Examine the antecubital region. First choice is the median cubital vein. • Second choice is the cephalic vein (outer aspect of the arm). • Exercise caution if choosing the basilic vein on the inner aspect of the arm, to avoid damage to the brachial artery & median nerve. • Palpate the vein to judge depth and thickness of vein and surrounding tissue so that only appropriate force is used to insert the needle through the layers into the lumen. • If the veins on that arm are unsatisfactory, release the tourniquet and apply to the other arm. • Veins in the forearm and back of hand may be used if a suitable vein is not found in antecubital area. • Veins on the plantar side of the wrist must not be used. • Take notice of intended puncture site, to avoid need to repalpate. • Loosen tourniquet.
11. Assemble Blood Collection Equipment	• Select the most appropriate needle gauge based on the physical characteristics of the vein, location of the vein and the volume of blood to be withdrawn. Hold opposite ends of the sealed needle with both hands and twist the two sections apart. Discard the shorter of the two plastic sheaths. • Thread the exposed end of the needle into the open end of the threaded tube holder, using aseptic technique.
12. Prepare Venepuncture Site	• Cleanse the site with an isopropyl alcohol wipe using several firm strokes until clean (3-5secs). • Allow the alcohol to air dry (30secs). • If there is a need to re-palpate, then the site must be re-cleansed with isopropyl alcohol and allowed to air dry again. • Tighten the tourniquet in preparation for puncture.
13. Inspect Needle, Anchor Vein	• Grasp the holder firmly using a safe and secure technique by placing the thumb on top and 2 or 3 fingers underneath. • Place the first tube into the tube holder, without engaging it into the interior needle. • Remove the outer sheath from the needle. • Visually examine the needle to ensure that it is sharp and free from burrs. • Hold the patient’s arm firmly distal to the intended puncture site. • Place the bevel of the needle face up at a 15-30’ angle close to the intended puncture site. • Ensure that the open end of the hub remains accessible for exchanging the tubes.

	• Anchor the vein by stretching the skin (pulling downward) on the arm, 2.5 to 5 cm below the intended puncture site. This technique is the most effective way to minimise the pain of venepuncture, it anchors the vein to prevent it from rolling away from the needle, and is less likely to cause needle-stick injury to the phlebotomist. • Inform the patient of the imminent puncture.
14. Insert Needle	• With a steady hand use a forward motion to guide the needle through the skin and into the vein. • Avoid a slow, timid puncture. • Do not stab nor use a rapid, jabbing motion, which may cause the needle to pass completely through the vein.
15. Engage and Fill Tube, Loosen Tourniquet	• Position the index and middle finger of the free hand on either side of the hub and push the tube forward so that the stopper is punctured by the interior needle. • Once the blood has started to flow, request the patient open their hand. • Loosen the tourniquet, while allowing the blood to continue to flow. • Fill the tubes to the appropriate level.
16. If Blood Does Not Flow	• If blood is not obtained, the needle may be improperly positioned in the vein or the tube may have lost vacuum. • Firstly, gently reposition the needle by moving it forward. • If you suspect that the needle has already punctured the distal wall of the vein, then move the needle gently back until the blood begins to flow. • Avoid side-to-side manipulation of the needle as an injury can result. • Try changing the tube to see whether this makes any difference to the blood flow. • If unable to obtain a sample, or an inadequate sample, then remove the needle. • If the procedure needs to be repeated then a fresh needle must be selected. • No more than 2 attempts are to be made. If unsuccessful, label form as “difficult” collect, record your phlebotomy number, then strike it out. Refer the form on to another experienced phlebotomist to perform the collection.
17. Exchange Tubes, Follow Order of Draw, Mix Tubes	• Exchange tubes following the proper order of draw. • Hold needle and holder assembly steady to avoid displacing the needle during tube exchange by counteracting pulling pressure against tube holder. • Give each tube several inversions as it is removed.

	• Remove the last tube from the holder. • Release the tourniquet. • Complete mixing of tubes by gentle inversion 8 - 10 times total (3 - 4 times only for coag tubes), ensuring the bubble travels from one end of the tube to the other, so that mixing of additives is complete.
18. Remove and Dispose of Needle	• Place gauze lightly over the venepuncture site • Withdraw needle, then immediately apply pressure to site. • Activate any safety feature on the needle according to the manufacturers' instructions. • Discard the needle and single-use holder into the sharps bin or • Never re-cap needles.
19. Post Venipuncture Care	• Ask the patient to press firmly on the gauze for several minutes until the bleeding has stopped, discourage the patient from checking the site repeatedly, or • Relocate the tourniquet over the gauze, tighten slightly to apply a light pressure. • Complete closure of the puncture site may take several minutes. • The arm must remain straight (not bent at elbow) • Lift the gauze pad and check the puncture site to ensure that it has sealed and that bleeding has stopped. • Observe the tissue around the site for any raising or mounding, which may suggest that blood is leaking into the surrounding tissue. If you suspect this to be the case, reapply pressure for several more minutes then recheck. • Replace the gauze over the site and cover with a strip of non-allergenic tape. This maintains pressure to the site helping to prevent any further bleeding and minimising bruising. • Always be on the lookout for signs that the patient might faint during or after a venepuncture.
20. Hand Hygiene	• Remove gloves • Perform hand hygiene
21. Label Tubes	• The tubes must be labelled immediately after collection and in view of the patient. • Label either by using the patient identification sticky labels or by handwriting the family and first names and either date of birth or NHI number on the tubes. • If using the patient labels take care to ensure that they are the correct labels and the details match both the request form and the patient identification bracelet. • One of the main errors in labelling is failure to correctly identify the patient and/or specimens.

	• Place the labels along the full length of the tubes and immediately below the tube stopper. • Labels on Blood Bank specimens (Group and Hold and Cross-matching) must be hand-written, to include last name, first name, NHI, date of birth, date, time and signature of collector on the tubes. • All blood collection specimens should be labelled by the person performing the blood collection. • The labelling must be done immediately after collection and before leaving the bedside/collection area. • Labelling occurs in front of the patient, thus providing the patient with a sense of confidence in the phlebotomist and the laboratory.
22. Special Handling Requirements	• Handle the specimens according to the requirements in the Laboratory Tests manual e.g. › place on ice – make ice slurry in biohazard bag › maintain at 37'C – prepare thermos › room temperature - include "do not refrigerate" note › fragile - hand deliver PFA (coag) tube. › whole blood - include "do not spin" note. › protect from light - wrap in foil.
23. Complete Documentation	• Complete the "collectors" sections on the Request Form • Record the "Sample Date/Time" of collect • In the "Taken By" box record your phlebotomy identification number • The number and type of tubes collected • The technique used; V for vacutainer, S for syringe • If the patient has been fasting tick the "fasting" box • If the test is usually a "fasting" test, e.g. lipids, but the patient has not fasted for 8 – 12 hours, tick the "non fasting" box • If the blood is collected into microtainers ensure that the "venous" box is ticked. These were designed for capillary collects and lab staff may wrongly assume that the collect is capillary. • If sample was from arm with drip, record whether collection was proximal or distal to drip, length of time drip discontinued before draw, and type of fluid being infused.

24. Transport Specimen to Laboratory	• Perform a final check on the labelling of the specimens and request form to ensure that the details are matching. • Place the specimens in a biohazard bag and the request form in the outside pocket. • If the specimen or request is “Urgent” place an urgent sticker on the request form so that it is visible to Specimen Reception staff as it arrives in the lab. • Send specimens to laboratory in a timely manner – 2-4 samples in a lamson tube together, or within 20-30 minutes of collection. Avoid transport delays.
25. Farewell Patient	• Ask the patient to leave the bandage on for 1-2 hours, and then remove. • Return patient’s environment if moved e.g. bedside table, bed rail, light. • Advise the inpatient to inform their nurse if they experience further significant bruising or bleeding, swelling, discomfort, tingling or numbness in the limb. • Advise the outpatient to contact phlebotomy if they experience further significant bruising or bleeding, swelling, discomfort, tingling or numbness in the limb. Give an “After Venipuncture Care” card, record notes on request form and write in phlebotomy incident record book. • Ask “is there anything else I can do for you while I am here?” This is part of our service to our patients and follows our WDHB core values.
26. Perform Hand Hygiene	After the procedure and all contacts with the patient and their environment are complete, wipe down any surfaces on the trolley with a Viraclean wipe Then perform hand hygiene.

Butterfly Technique

While the syringe technique is useful in the collection of blood when the patient has “difficult “veins, the butterfly collection device is particularly useful on very young children and adults with extremely fragile and small veins.

It is also the recommended technique for the collection of blood cultures.

The wings of the butterfly allow the fingers of the phlebotomist to be closer to the needle so there is greater control and easier positioning when performing a challenging venepuncture.

The butterfly used for blood collection has a flexible tube attached with a needle or luer adapter on the opposite end. This allows for blood collection using either an evacuated blood collection device or a syringe. Butterfly needles are available in 21, 23 or 25 gauge.

The Push Button Butterfly Set has a safety feature that is activated while the needle is in the vein. It is recommended to tell the patient they will hear an audible click as the needle is removed.

The preliminary steps for greeting the patient, patient identification, checking request form, selecting and cleansing the site etc, as outlined in the previous section entitled “Venepuncture using Evacuated Tube System” are to be followed.

This section outlines the assembly and use of the equipment.

Step	Action
1. Assemble and check the equipment	Inspect the package of the butterfly set to ensure that it is intact. The Push Button Butterfly set has a preattached holder for use with vacutainer tubes. If using with a syringe, unseat the plunger and advance it fully forward, expelling the air from the barrel of the syringe. Remove the holder and attach the syringe.
2. Technique	• Grasp the wings of the set • Anchor the vein • With bevel facing up slide the needle into the vein at shallow angle 10 – 15 degrees. • A flash of blood will appear in tubing when needle is in vein • Seat the needle in the vein, hold wing with thumb against arm • Push tubes into holder in downward position so tubes fill from bottom first or • Slowly pull back on plunger of syringe and allow barrel to fill. • Place gauze over site, activate the push button to withdraw the needle, apply immediate pressure to site. • Dispose of butterfly set in sharps bin. • Attach the syringe to luer lock access devise (red tip) to transfer blood to tubes.

Label the blood tubes, perform the paperwork, checking, puncture site care etc as outlined in the previous section.

Paediatric venepunctures

The Phlebotomist ***mus***t obtain the consent of the parent or guardian before taking blood from a child ***aged 15years and under.*** In the case where a person 15 years and under arrives with the ***original laboratory request form*** i.e. not a photocopied form, it is assumed that the doctor has discussed the requirement for a blood test, therefore tacit consent has been obtained.

Prepare all equipment and collection tubes before bringing child into the collection cubicle. Ensure that you are familiar with any special collection protocols – temperature requirements, minimum volumes, protection from light.

Introduce yourself in a warm and friendly manner. Establish eye contact, show concern for the child's health and comfort. Correctly identify the patient. Find out about the child's past experiences with blood collection.

Talk directly to the child about the procedure, don't talk exclusively to the parents or care-giver. Gain their confidence by explaining and demonstrating each step. Be honest with the child who asks whether the puncture will hurt. Most children can be reasoned with and patience is imperative. Use toys as distractions.

Do not attempt to bleed a small child or baby on your own. Gain the parent/caregiver's co-operation by explaining how they can assist. The child usually sits on the knee of the caregiver and holds the child with careful restraint to ensure safety of child, parent and phlebotomist.

Engage the assistance of another phlebotomist to immobilise the arm while the venepuncture is performed. It may be preferable to use a 23 gauge butterfly needle and syringe for paediatric collects.

Reward a child at the end of the procedure. Acknowledge their efforts to co-operate and to be brave. Bravery awards, stickers, stamps, special plasters or lollypop (check with parent first) are available. This will have benefits for the next time the child requires a blood test.