IRON AND VITAMIN A NUTRITION OF YOUNG AUCKLAND CHILDREN:

An Investigation into the Methods to Assess the Nutritional Status of Micro-Nutrients in 6-24 Month Olds.

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Abstract

This study validated a food frequency questionnaire specifically for identifying iron and vitamin A intake in thirty 6 to 24 month old children. Children were recruited using the cluster sampling technique, and stratified by ethnicity. Of the thirty children enrolled in this study, 7 (23%) were European, 6 (20%) Maori, 11 (36.7%) Pacific Island and 6 (20%) were of Other ethnic groups. From the results of this validation study, 24.19% (7 of 29) of children were iron deficient, 14% (4 of 29) had iron deficiency anaemia and 14% (4 of 29) had vitamin A deficiency.

This validation study compared a food frequency questionnaire against a four day weighed food record and the biochemical status obtained from a blood sample. The Spearman's ranked correlation values from comparing the food frequency questionnaire administered in the first and second values ranged from 0.132 for chicken to 1 for iron supplements. The limits of agreement method by Bland and Altman tested for the reliability of the food frequency questionnaire and showed good agreement between the two administrations of the food frequency questionnaire. This method was also used to test the validity of this food frequency questionnaire by comparing the differences between the food frequency questionnaire and the four-day weighed food records.

The use of multiple regression analysis of variance was used to identify the contributing variables to iron deficiency, iron deficiency anaemia and vitamin A deficiency. The results of the regression analysis suggested a small significant contributor to the variance in predicting iron deficiency and iron deficiency

anaemia of these children was being Pacific Island and the mean daily iron intake obtained from the four-day weighed food records. The probability values ranged from 0.01 to 0.001 with the greatest level of significance found in the Pacific Island ethnic group.

These findings have important significance in future undertakings of dietary assessment in children and further developments of accurate and reliable dietary tools to assess mean nutrient intake in children.

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List of Abbreviations

IDA	Iron Deficiency Anaemia
VAD	Vitamin A Deficiency
WHO	World Health Organisation
RE	Retinol Equivalents
IU	International Units
NHANES	National Health and Nutrition Examination Surveys
Hb	Haemoglobin
SD	Standard deviation
WFR	Weighed Food Records
FFQ	Food Frequency Questionnaire
RDI	Recommended Dietary Intake

Introduction

Dietary assessment studies provide information on the food we eat, eating patterns and also enable researchers to identify any causal links that may exist between diet and disease. These studies can also determine nutrient inadequacies that may lead to nutrient deficient states. Links between diet and disease can be detected and mean nutrient intakes can be determined for different population groups. These are two key roles of undertaking dietary assessment studies. The tools used in dietary assessment studies include prospective and retrospective methods of measuring the food we eat, anthropometric and biochemical methods.

Validation studies are undertaken to test the dietary tools including food frequency questionnaires, 24hr recalls, weighed food records and diet histories in order to determine how accurate these methods are for the specific aims of a particular study. The value in undertaking validation studies lie in the opportunity it provides to conduct a pilot study, thereby testing to see how reliable and reproducible the particular dietary assessment tool is. The validation study also prepares the researchers as to what to expect, in terms of results, when it comes to undertaking the actual study itself.

There is an increasing need to direct research of public health in children, in order to ensure the health of this future generation. The use of dietary assessment is one method that can be used in this field of research.

Children are most at risk of developing nutrient deficiencies. This due to their physiological vulnerability and demands on their developing body. Many of

the nutrient deficiencies in the world affect mostly children. This is true for protein malnutrition, iron deficiency and vitamin A deficiency.

This study looks at validating the dietary assessment tools that will be tested specifically for children, to enable research and ultimately address the issue of iron deficiency and vitamin A deficiency in young toddlers in Auckland.

Literature Review

The infant's nutritional requirements in the first phase of its life are unique. During this time, it has the greatest amount of linear growth and the development of the major organs of the body, particularly the brain. In order to ensure that the infant's requirements are met, adequate and appropriate nutrients from key foods are essential. The causes and functional effects of nutrient deficiencies, in particular for infants and young children who are at risk of being deficient in many nutrients is a growing field of interest and research. Amongst the many nutrients that are known to affect growth and health of young children, two are of particular current interest - iron and vitamin A.

Iron deficiency remains the single most prominent of nutrient deficiencies affecting a significant majority of the world's population. It poses an enormous public health issue – both in developing countries and developed countries like New Zealand. On the basis of figures from the 1985 World Health statistics quarterly, estimates of the global prevalence of iron deficiency amongst young children was approximately 43% [1, 2]. The First International conference on Nutrition in Rome in 1992 recognised the enormity of this problem; declaring that one of its principal aims was to reduce the global incidence of iron deficiency by a third before 2000 [3]. Updates from the 1993 Proceedings of the XV International Congress of Nutrition in Adelaide [3] indicate that progress has been made. A number of studies have been undertaken to estimate the prevalence of iron deficiency present in the world population. However, detailed studies of prevalence of iron deficiency and iron deficiency anaemia in the various age groups are not available in all countries. New Zealand is no exception. Most of the reported studies concerning iron deficiency and iron

deficiency anaemia have been undertaken in developing countries where the situation is at its worse [1].

The New Zealand Public Health Commission (1995) [4] has identified infants and young children as being at high risk for low iron status. The average incidence rate of iron deficiency anaemia of New Zealand infants of various ethnic backgrounds ranges from 20% to 47% [5]. A pilot study conducted in 1997 found 41% of a random sample of 29 nine month old infants born at the National Women's Hospital to be iron deficient. Table 1.1 shows that whilst the prevalence of iron deficiency is alarmingly high in the New Zealand population of infants and young children, there are some discrepancies in the interpretation of these studies.

Table 1.1 The Incidence of Iron Deficiency Anaemia in New Zealand. [5]

Locality	Date	Age	No.in study	Ethnicity	Prevalence of IDA
Bay of Plenty	1988	6-24mth	123	Maori European	40% 22%
Porirua	1991-2	6-30mth	43	Pacific Is. Maori European Other	42% 35% 16% 7%
Dunedin	1990	3-19 mth	17	Cambodian	47%
Auckland	1993	9-24 mth	50	Not specified	20%

These are as follows: - the sample sizes of these studies are very small, and are therefore not representative of the population of New Zealand, these figures highlight the prevalence of iron deficiency Anaemia, the more extreme form of iron deficiency. More recent studies show that the prevalence of iron deficiency has increased, although not significantly. Wham (1996) reported that 13% of 9-24 month old children had iron deficiency whilst 20% had iron deficiency

anaemia [6]. More recently, Adams (1997) [7] reported that the prevalence of iron deficiency and iron deficiency anaemia in 9-11 month old children (n=29) was 41% and 17% respectively. Secondly, the subjects in these studies were recruited from opportunistic samples and thus do not represent the true prevalence of iron deficiency in the general population. There is currently no published literature reporting the true prevalence of iron deficiency of young children in the healthy population of New Zealand.

Vitamin A inadequacy is a major nutritional concern in the world, particularly in children. Reports of vitamin A deficiency (VAD) date back 3500 years ago, making it one of the oldest recorded medical conditions [8]. The prevalence of this deficiency, with its unique ocular manifestations as night blindness remains high in developing countries with statistics that indicate that this continues to be a problem today.

Vitamin A deficiency is an international public health problem manifesting itself as xeropthalmia in extreme cases (See section 2.31). Xeropthalmia is known to occur in some seventy-three countries in varying degrees of severity, especially in the southern and peri-equatorial regions of the world [9]. The following map (Figure 2.1) shows the regions most affected. "Blank" countries on the map represent an absence of data rather than an absence of risk.

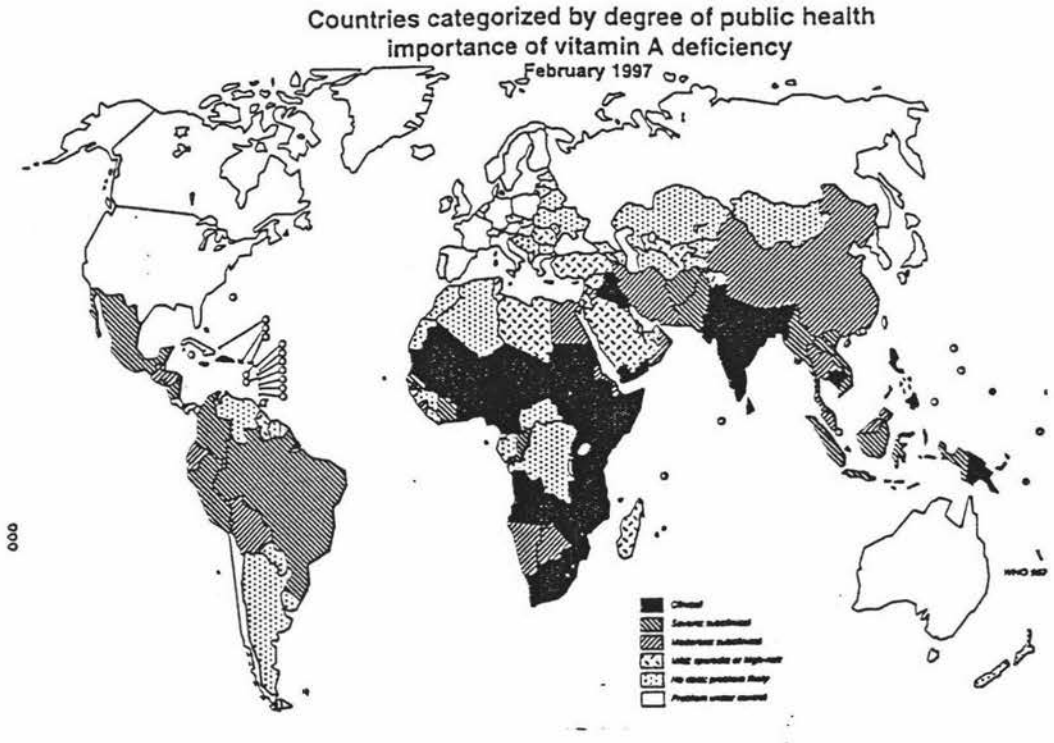

Figure 2.1 The Prevalence and Severity of Vitamin A Deficiency as a Public Health Problem in the World [9]

Statistically, population-based studies record that 5-10 million children develop xeropthalmia each year, with half a million of whom go blind [10]. More recent statistics from the WHO (1996) report that at least 2.8 million – 3 million pre-school children are clinically affected and around 251 million are moderately to severely affected [11]. These statistics highlight only the clinical manifestations of VAD. The prevalence of sub-clinical vitamin A is likely to be higher, and is also likely to include developed countries like the United States [12] and New Zealand.

Moreover, variation in the definition of terms and estimates of data raises the question of precision and accuracy of the different studies. Existing population based studies in developing countries like Indonesia, Nepal and Bangladesh point to a figure of about 125 million children globally of pre-school age that are vitamin A deficient [13,14]. These alarming figures indicate not only the enormity of this problem for child health, but the need to urgently address this issue with clear prevention strategies in mind.

The WHO has set a goal of eliminating VAD and its consequences, including blindness by the turn of the century. To achieve this goal, the existing deficiency in the various countries needs to be eliminated. As well as that, identifying and eliminating the prevalence of such a deficiency in countries where there is no known information is crucial. New Zealand is one such country where there is currently no information on the nutritional status of vitamin A and data on the prevalence of VAD.

Measles continue to be a major public health problem in New Zealand (See Section 2.24). Epidemics continue to occur every 5-6 years [15]. There is a known relationship between VAD and the severity of measles. Studies have shown that vitamin A supplementation of vitamin A deficient children with acute measles has been associated with a reduction in morbidity and mortality. Recommendations made on the basis of these findings by WHO and UNICEF are to administer vitamin A to all children diagnosed to have measles in communities where VAD is a recognised problem [16]. Measles epidemics will still exist and it is predicted that the next epidemic in New Zealand will occur in 1997-1998. It has also been reported that the hospitalisation rates for measles varies with ethnicity, with Maori and Pacific Island children having a 19 times higher hospitalisation rate [17].

The aim of this literature review is to address the effects of the consequences of vitamin A deficiency and iron deficiency in children, and to review the dietary assessment methods that have been used to ascertain nutrient intake data in children, with a specific focus on iron and vitamin A.

2.1 Iron

2.11 Biochemical Function of Iron

Iron has a pivotal role in the normal functioning of the body. Iron, with an atomic weight of 55.85 and atomic number of 26, exists in 2 valency states – ferrous (FeII) and ferric (FeIII). Because of this dual valency state, it can take part in the reduction-oxidation process, undergoing reversible valency changes in reduction and oxidation processes. It is also involved in electron transport in the mitochondria at cellular level, participating the generation of energy for the cell. Its ability to bind to oxygen either on its own or as part of a complex gives it another important role – oxygen transport. Iron transports oxygen to cells in the body in haemoglobin proteins. Oxygen is stored in the muscle cells as a component myoglobin, creating the red pigment of muscle [18]. The known key functions of Iron in the human body are highlighted in table 1.2.

Figure 1.2 The Functions of Iron Containing Complexes and Enzymes

Function	Iron Storage Protein
Oxygen Transport	Haemoglobin, Myoglobin
Iron Transport	Transferrin, Lactoferrin
Ferritin	Iron Storage
Mitochondrial Electron Transport	Cytochrome C family
Reduction of H2O2	Catalase
Regulator of Iron Binding proteins	Iron responsive element binding protein
Co-Enzymes in Metabolic Pathways	Succinic Dehydrogenase, Aconitase,

Ionic iron is toxic to the living cells. Therefore, it is bound to various complexes like transferrin, iron porphyrin complexes of myoglobin, haemoglobin and haem-containing enzymes for transport and function. Iron is stored in the body in the liver as ferritin and haemosiderin and is transported in the interstitial spaces as transferrin complex [19].

Other iron complexes present in the body are the electron transport enzymes known as cytochromes. These enzymes are located in the microchondria and

are involved in electron transport. These enzyme complexes undergo the reversible reduction of Fe(II) to Fe(III) transitions as iron sulphur proteins. The enzymes include flavo-proteins like NADH dehydrogenase, succinic dehydrogenases and acconitase, which are also involved in the Tricarboxylic Acid Cycle, a metabolic pathway [20].

The haem enzymes catalase and peroxidase reduces H_2O_2 in the body while lactoferrin, found in human milk acts as a cationic carrier similar to transferrin. These complexes and enzymes clearly demonstrate the many diverse functions iron has in the body.

2.12 Iron Homeostasis

Excessive intakes of iron can lead to overloading and can be highly toxic to the body. On the other hand, insufficient amounts of iron can lead to a deficiency. The balance or equilibrium that controls the iron intake, iron absorption and iron utilisation needs to be maintained. This is known as Iron Homeostasis. Iron homeostasis demonstrates the body's mechanism of maintaining the equilibrium of iron loses versus iron uptake. The disruption of this balance for any prolonged period of time will have consequences [19].

Iron is utilised in the body for haemoglobin and all the enzymes mentioned in table 2.2, whilst iron losses occur in women during menstruation, and some through cell shedding in the Gastrointestinal Tract. There are also increased demands for iron during growth. The body is able to regulate this balance, matching iron absorption to its need. However, the body's demand for iron increases at certain times, especially during the infant and pre-adolescent growth spurt and in women during pregnancy. This places pressure on the body to meet such needs. Thus, these groups are at risk of being iron deficient.

2.121 Iron Needs of Infants

Due to the increasing growth requirements, the growing infant is in potential risk of being iron deficient. A full term breastfed infant has sufficient iron stores to last around 4 – 6 months after birth [21,22]. Iron in breast milk supplements the iron stores that the infant has from birth. This is all the iron a baby needs for the first four months following birth. Thus, the need for iron

from other sources is very low, as most of the iron is reutillised. However, at around four months, the iron stores in the infants body begin to reach marginal levels, whilst the iron requirements continue to grow to meet the intensive growth requirements of the infant. At this age, the balance of iron can no longer maintained without an additional source of iron during this rapid growth phase. Approximately 0.8mg of iron from the diet is required to maintain the balance of iron being utilised and to maintain iron stores [21]. Of the 0.8mg of iron from an additional source, 0.2mg of iron is used to replenish any possible losses that may have occurred, and the remaining 0.6mg is utilised for growth alone [21,23]. The demands for iron in the infant reflect the combination of a larger body size and the increased red blood cell volume. Figure 2.3 illustrates the increased need for iron by the marked increase of haemoglobin that a four-month-old infant needs [21].

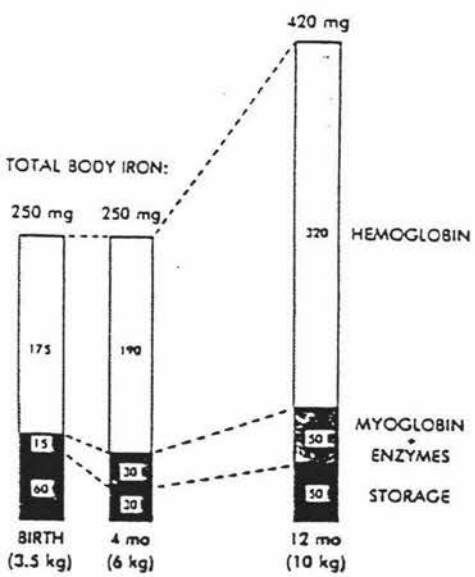

Figure 2.3 Changes in the Body During Infancy [21]

2.122 Known Causes of Iron Deficiency in the Infant

As previously stated, the increased requirement of iron to meet needs of growth places the infant at risk of iron deficiency. There are other factors that increase this risk. It has been proven that the introduction of whole cow's milk at an early age places the infant at a greater risk of developing iron deficiency. This is due to the low content of iron in cow's milk that is poorly absorbed [24-26]. Other factors that increase the risk of iron deficiency in infants are the consumption of foods that are low in absorbable iron. (See section 2.13)

2.13 Sources of Iron in Food

Iron in the body is supplied primarily from the diet in 2 forms: haem iron and non-haem iron. Sources of haem iron are derived mainly from the haemoglobin and myoglobin of meat and fish, while non-haem iron comes from iron in other products and iron salt such as in supplements and fortified foods.

Absorption of iron is affected by many factors. There are different mechanisms that operate in terms of absorption of haem and non-haem iron, with haem iron being better absorbed. The difference in the bioavailability is essential in understanding how a deficiency may exist. The term "bioavailable" iron refers to the proportion of total iron in the food or formula that is utilised by the body[27]. Very little is known about the mechanism of absorption of the haem iron except that it is not influenced by the iron status of the subject. Currently, what is known is that haem iron is absorbed from receptors found in the cells of the brush border in the small intestine. Haem iron is then chelated once it is absorbed and transferred to the liver in this form. Similarly, non-haem iron is transported across the intestinal brush border by iron free carrier protein, but it is highly dependent on body iron status and other constituents in the diet[28]. Various enhancers and inhibitors also play a role in the absorption of non-haem iron [27 29-31].

2.14 Functional Consequence of Iron deficiency

Iron deficiency is common during the infant years. As mentioned previously (See section 2.12) the rapid growth spurt places increased requirements on the body's iron stores. There is increasing evidence that iron deficiency may have many implications – short and long term.

2.141 Features of Iron Deficiency

Inadequate intake of iron over a long period of time results in iron deficiency. General symptoms of iron deficiency are constant fatigue, lack of stamina, pale complexion, apathy, and pallor and poor resistance to cold temperatures [32].

Iron deficiency is characterised by 3 stages. These are generally measured by different haematological and biochemical tests. Depending on the aspect of

iron metabolism that is being studied, different tests are used. The three stages of iron deficiency are as follows :-

Figure 2.4 Stages of Iron Deficiency [33]

During the iron depletion stage, the amount of storage iron is progressively being depleted, which leads to iron deficient erythropoiesis, where the iron stores are exhausted. Iron deficiency anaemia occurs when the haemoglobin falls below normal levels [33].

2.412 Iron Deficiency and Motor Function

The crucial years of growth and development are generally recognised to be from birth to two years of age. The increase in the physical size of the infants' body and the acquisition of various functions as the child develops is most visible in early childhood. It is therefore likely that a consequence of iron deficiency may prevent this growth spurt from taking place. A study in Indonesia of pre-school children [34] reported that stunting has been corrected with iron supplementation, whilst a study in Danish infants reported a negative association between serum ferritin levels and growth velocity, indicating a need for iron during growth [25].

The most rapid growth period of brain growth takes place 9 months before birth and during the first months of postnatal life. During that time the brain grows to about 80% of its adult size [35]. Traverse sections of monkey's brains

stained for iron reveals that the area where iron gets deposited in is the central part of the brain [36]. This part of the brain is known as the extra – pyrimidal tracts and is where the substantia niagra and hippocampus is located [37]. The main function of this part of the brain is language acquisition and dexterity. Most of the iron that is stored in the brain gets deposited in the first year of life through what is known as the blood brain barrier. At the end of the first year, the barrier becomes less porous and traps the iron in is the brain, and stops further iron from entering the brain [37].

With the greatest increase in brain size occurring from birth to two years of age, any nutrient deficiencies that may occur during this crucial development period may have significant effects. There is increasing evidence that iron deficiency during this critical period may have long term implications on development and learning. Lansdown and Wharton [38] have undertaken a comprehensive review of the link between iron deficiency and cognitive development in infants. The studies can be grouped into several categories of population studies – observational, interventional and prospective studies.

On reviewing the various observational studies, there is conclusive evidence of a link between iron deficiency, and mental and motor development in young children. There have been seven recent studies that have found no or insignificant associations whilst there have been 18 studies that indicate that there is a significant association with motor development [38]. The different methodologies, variations in measurements and sample sizes may have confounded the discrepancies in the findings between studies [23]. However, even with the discrepancies, there is still substantial evidence to support the conclusion that iron deficiency is associated with impaired cognitive performance in children from the observational studies.

Lozoff and colleagues, and Walter undertook the key studies that link iron deficiency and motor development in infants in South America [39-43]. Results report measures of mental development scores that point to a small but statistically significant difference between normal infants and IDA infants.

These cross-sectional studies showed that infants at 9 and 12 months showed the most significant differences with iron deficiency anaemia and their mental and motor skills. The extent of the loss was dependent on the length of the iron deficiency anaemia[44]. The areas that were significantly affected in these infants were those of language acquisition and body balance. Other measures showed that the fine and gross motor skills of the iron deficient child were also affected[39-43]. The studies of infants with IDA at 6 months using various measures point to a delay in motor development. Overnight sleep studies and ECG measurements also show variations in their sleep-wake cycles, particularly in variable respiration and increasing motor activities [38].

Short and long intervention studies in children in developed and developing countries reveal short-term improvements on mental scores of these children. Significant improvements in Bayley Mental Scores were found in several short-term interventional studies using standardised tests of development or intelligence. In longer-term studies, those that support the link of iron and motor development outnumber those that did not find a substantial link by four to one. On the basis of this ratio, it can be concluded that there is a causal link between the two. Further intervention studies can confirm the strength of this theory.

Longitudinal studies documenting the long term functional consequences of iron deficiency in South America [41,44] with similar results in Indonesia [45] have found that the consequences of iron deficiency in infancy affect the learning abilities of the same children at 5, 8 and 10 years time. Studies by Walter et.al [44] on the same group of iron deficient infants studied at 2 years of age reported that though they had normal iron status at five years old, there was still a small deficit in IQ points, poor language and fine and gross motor skills. At 10 years of age, the IQ differences of the same children had been lost although the language differences and dexterity scores continued to be lower than the children without IDA. These affects are seen in infants who had the IDA corrected at the age of two. From the basis of these studies, it could be assumed that the difference in language and dexterity scores could be a permanent consequence of iron deficiency in infancy. These may be the long-term effects of a short-term deficiency. Should these results prove to be true, it provides a further reason to determine and eliminate the prevalence of such a deficiency in the infant population.

2.413 Iron and Infection.

Laboratory experiments in animals and humans have provided evidence of a decreased resistance to infection as a characteristic of iron deficiency. Some studies have shown an increase in infection when iron is given. However, a direct association between iron deficiency and increased rate of infection has not been found [46,47]. Conflicting results and problems with experimental design of various clinical studies have provided inconsistent results. Several studies reported that the incidence of respiratory infections in iron deficient children decreased after oral administration of iron [48,49]. Conversely, a later study by Burman (1972) [50], reported no difference. Hersko (1988) proposed that iron deficiency decreased the incidence of infection, and thought that the mechanism involved withholding iron from the bacteria [51]. As IDA and infections are common amongst the socially disadvantaged and poor populations, there needs to be further research undertaken to establish or confirm a cause and effect relationship between iron and infection.

2.2 Vitamin A

2.21 Biochemical Function of Vitamin A

The term "Vitamin A" is a generic term for a group of long chain hydrocarbon compounds. The compounds in the group are retinol and its esters (retinyl palmitate) and retinoic acid. They represent the three oxidation states of alcohol, aldehyde and an acid. It is via these 3 different states that vitamin A carries out its function. Vitamin A is involved in 5 main areas of the human body – vision, differentiation of epithelial tissues, morphogenesis, growth and reproduction.

2.211. Retinol and Vision

The human eye retinal has two types of receptor cells known as cones and rods. The cones provide colour vision, whilst the rods are used in low light intensities. The outer segments of the rod are made up of membrane vesicles serving as light receptors. Vitamin A's role in vision revolves around these membrane vesicles. Embedded in these membrane vesicles is a ~ 30 000 dalton protein known as opsin. These vesicles contain among other compounds the vitamin A derivative as 11-cis retinol. Opsin combines with 11-cis retinol upon exposure to light to form Rhodopsin that sets off a biochemical cascade involving the conversion of cyclic guanosine monophosphate to guanosine monophosphate. Membrane hyperpolarisation, or the closure of the sodium channel of the outer segment of the rod occurs, transmitting an electrical signal to the optic nerve[32,52]. As this rhodopsin system is involved in the perception of light at low intensities or at night, vitamin A deficiency in the form of a lack of the vitamin A derivative will affect this vision system leading to night blindness.

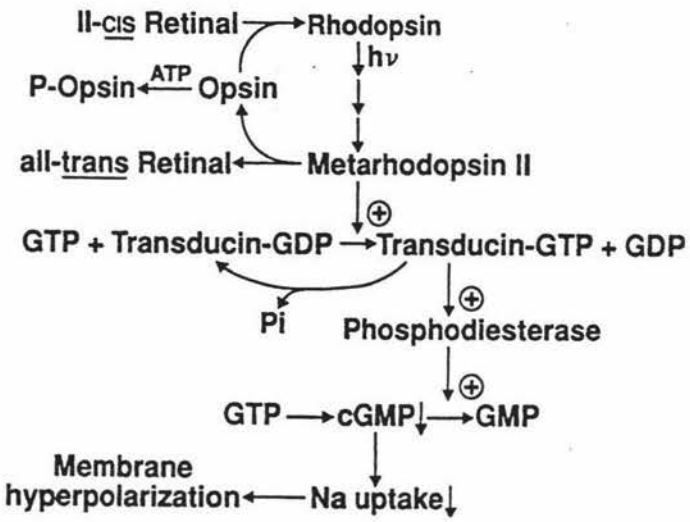

Figure 2.5 The Biochemical Pathway Involved in Vision [52]

2.212. Vitamin A and Cellular Differentiation

Another role that vitamin A has is in the maintenance of epithelial tissue and mucous production. "Keratomalacia" or blindness due to the changes in the conjunctival corneal epithelium of the eye is one of the more prominent effects of VAD. In a vitamin A deficient state, keratin producing cells replace the mucous producing cells in many tissues of the body. The biochemical pathway that vitamin A is involved in is the sulphur transfer reaction pathway, which affects the synthesis of mucopolysaccarides and the generation of mucous and mucous producing membranes [18]. Vitamin A is required for the synthesis of the "active sulphate" Adenosine 3`phosphate 5`phosphosulphate (PAPS).

Vitamin A and its related compounds also have a considerable influence in the way cells differentiate. The mechanism by which this takes place is linked to the gene regulation by retinoic acid receptors (RAR). All trans-retinol is oxidised to all trans-retinoic acid, which is then isomerized from 9-cis retinol to 9-cis-retinal. This is then further oxidised to 9-cis retinoic acid, which is transported by specific retinoic binding proteins (RBP) to the nucleus. In the nucleus, it is bound to one or more of the 3 RAR or RXR (a,b,c). Activation of

retinoic acid responsive genes – a heterodimer of either RAR or RXR initiates transcription. There are also other different mechanisms involved in gene regulation, but it is clear that retinoids play an important role in the development and maintenance of many tissues [53].

2.213. Vitamin A and Morphogenesis.

There is an adverse effect of an excess or a deficiency of vitamin A and embryogenesis. Summerbell and Maden (1990) [54] put forward a hypothesis that all-trans retinoic acid could be one of many morphogens that controls embryonic development. This was demonstrated by their experiment of the presence of trans-retinoic acid in the anterior of a developing chick limb bud that mimicked the occurring polarizing activity of a group of cells in the polarizing region [55]. Two possible hypotheses have been put forward to explain this effect. First, retinoic acid induces differentiation of a specific group of cells leading to the development, and second, that a morphogenic gradient of retinoic acid existed in the developing chick limb bud. No direct conclusions have yet to be proven [56].

2.214. Vitamin A and Growth

Vitamin A has also another function in growth, more specifically in the modulation of growth of the bones. The epiphyseal cartilage requires vitamin A for the activity of the cells that undergo the cycle of growth., maturation and degeneration to allow the bones to grow. The role that vitamin A plays is thought to revolve around the regulation of 1,25 –dihydroxy Vitamin D receptors on the surface of osteoclasts. Osteoclasts are involved in the demineralisation of bone[18]. Field studies in Nepal indicate that moderate to severe vitamin A deficiency, marked by xeropthalmia is likely to impair normal physical growth in children [14].

2.215. Vitamin A and Reproduction

Animal studies have also shown a correlation between vitamin A and fertility. In a vitamin A deficient state, spermatogenesis is arrested at the spermatid stage in rats, chickens and cattle. This effect is reversed when the deficiency is

addressed [18]. Further studies in the female rat have identified the effect of VAD – interfering with the oestrus cycle and placental production. These effects have not been observed in humans and the mechanisms involved remain unclear.

2.216. Vitamin A and Immune Function

Among other functions of vitamin A, there is an increasing evidence of its role in the immune function. It has been shown that there is a strong correlation between vitamin A deficiency and increased severity of infection, and mortality from respiratory tract infection. Specific and non-specific protective mechanisms are impaired in a deficient state. This includes the humoral response to bacterial, parasitic and viral infections, cell mediated immunity, mucosal immunity, natural killer cell activity and phagocytosis [52] .The number of infections in vitamin A deficient animals and humans indicate a link that has now been supported by clinical studies. Furthermore, it has been reported that requirements for vitamin A increased during infection. Significant amounts of retinol and retinol-binding protein (RBP) were found in urine of subjects with serious infections. In contrast, only trace amounts were found in urine of healthy subjects. This suggests that the excretion of vitamin A in urine during infection may be linked to a depleted state of vitamin A, and lead to a deficient state [57].

Sommer (1996) put forward a hypothesis that vitamin A deficiency increased the incidence of infection and childhood mortality. Numerous cross-sectional studies from developing countries found a strong association between mild vitamin A deficiency and the presence or recent history of diarrhoea [58]. In a controlled morbidity intervention trial that was undertaken in Ghana, the vitamin A supplemented group experienced less diarrhoeal episodes. Hospitalisation rates and clinic attendance of this group reduced by 38% and 12% respectively. Further studies detailing the link between the role of vitamin A and immune function are highlighted in community studies looking at the severity of measles (See section 2.244).

This relationship between vitamin A deficiency and immune function can also be seen in its role in childhood mortality. Community mortality prevention

trials have reported a mortality reduction from 6% to 45% in young children after vitamin A supplementation [58]. (See section 2.243)

2.22 Vitamin A Homeostasis

One of four fat-soluble vitamins, vitamin A along with vitamin D, E and K are absorbed in the small intestine with the aid of bile. They are transported across the membrane by membrane receptors [53]. Vitamin A in the form of retinyl palmitate and β–carotene is hydrolysed in the cells of the small intestine, and is reduced to retinol. The absorbed retinol is re-esterified and makes its way into the blood stream via the lymphatic system to be stored in the liver. The esterified retinol is transported to the liver by chylomicrons. Vitamin A can be stored for long periods of time in the liver until it is transported to the retina target cells or adipose tissue by retinol binding proteins (RBP)[53]. Oxidation of retinol takes place in the liver and in target cells, where it is then reutilised as required (See section 2.21).

2.23 Sources of Vitamin A in Food

Vitamin A activity comes in 2 forms – pre-formed vitamin A and selective carotenoids. Preformed vitamin A is generally known as retinol, and its related compounds, which are usually 20 carbon atoms in length. Carotenoids and β–carotene are among a number that are 40 carbon atoms in length. There are hundreds of carotenoids, but only few have any potential vitamin A activity. The most recognised carotenoid is β-carotene, which also has the greatest vitamin A activity [19,52].

Retinol is found in animal products and carotenoids in plants. Common sources of vitamin A in plants are in carrots, pumpkin, margarine and many of the yellow and orange vegetables. Vitamin A from animal sources is found in fortified milk, eggs and especially in liver [52]. As the source of vitamin A can be derived from retinol or β-carotene, Retinol Equivalents (RE) are used as a standard to express vitamin A present in food. 1 RE is equivalent to 1μg of retinol or 6μg of β-carotene or 12μg of other vitamin A derivatives. Historically, International Units (IU) were used to express vitamin A activity, but it is gradually being phased out. The non-equivalence of retinol and β-carotene was

the reason for the introduction of the new standard of using retinol equivalents. One RE is equivalent to 3.33IU from animal sources or 10IU from β-carotene [32].

2.24 Functional Consequences

2.241. Features of Deficient State

Vitamin A is essential for maintaining the integrity of epithelial tissue and specifically the surface lining of the eye, respiratory, urinary and intestinal tracts. Therefore, the eye's sensitivity provides the first warning signs of vitamin A deficiency. The stages of xeropthalmia or dry conjunctiva are classified below.

XN Night Blindness
XIA Conjunctival xerosis : drying of the conjunctival surface
XIB Bitot's spots : bubbly, cheesy or foamy patches of keratinised cells
X2 Corneal xerosis: drying keratinisation of cornea surface; haze, opaque appearance
X3 Corneal ulceration : keratomalacia, softening of the cornea

Figure 2.5 Different Stages in the Development of Xeropthalmia [59]

The stages up to XIB are reversible if sufficient vitamin A is given. However, further stages of deficiency are associated with more severe and irreversible lesions. These extreme cases are clearly visible in developing countries where the prevalence of malnutrition is wide spread. In developed countries, only sub-clinical deficiencies have been detected [59].

2.242. Factors Affecting Adequate Vitamin A Intake

The single largest cause of vitamin A deficiency is malnutrition or inadequate dietary intake of vitamin A rich foods. This deficiency is widely prevalent in developing countries and in the minority populations of developed countries, where the income levels of these people are classified as being of the low socio-economic levels [60]. There have been several studies attempting to identify the bioavailability of vitamin A in common foods that are abundant in order to address the vitamin A deficiency present in developing countries [61-63].

Biochemically, vitamin A metabolism may be affected by inadequate intake of protein. Protein supplies among others, the precursors for the synthesis of retinol binding protein which are crucial transport proteins for retinol [52,53].

Absorption of vitamin A can be impaired in a fat malabsorptive state. Vitamin A is dependant on the absorption of fat in order to be absorbed through the intestinal membrane. Thus, fat malabsorption would restrict and limit the amount of vitamin A available to be absorbed.

2.243. Studies and Epidemiological Evidence

Clinical studies suggest another role for vitamin A. It is possible that a vitamin A deficiency is associated with increase susceptibility to infection, as well as increased rates of morbidity, particularly in respiratory tract infections. Studies from developing countries have highlighted this relationship between sub-clinical vitamin A deficiency and increased morbidity and mortality from common infectious diseases [64,65]. Vitamin A supplementation has also been shown to reduce incidences of clinical attendance and hospital admissions in Ghana [66]. In Indonesia, 4000 pre-school children were involved in a study to identify dietary factors, and other factors responsible for night blindness and xeropthalmia. It was found that child mortality was directly related to severity of xeropthalmia [64,65]. Further studies in Africa and Asia have demonstrated a similar relationship, with a strong correlation between vitamin A deficiency and the rate of infection and mortality in pre-school aged children [58].

2.244 Vitamin A and Measles (see Introduction)

The association between sub-clinical vitamin A deficiency and increased morbidity from infectious diseases is most evident in children with measles. This relationship was first discovered in 1932. Where children hospitalised with measles were given cod liver oil, mortality was reduced by 58 % [67]. In 1987, the possibility that a relationship between vitamin A and measles emerged when a group of scientists in Tanzania highlighted the significant protective effects of supplementing vitamin A to children with measles [68]. Subsequent studies in South Africa found that a sub-clinical vitamin A deficiency existed amongst children. Those who had received large doses of vitamin A (400 000 IU) had 33% lower complication rates and also a 50% lower mortality rate than those who didn't [69].

Randomised clinical trials, especially in developing countries have found consistent and significant results indicating that mortality was much higher in undernourished and vitamin A deficient children [65,68]. There have also been studies undertaken in the US, where the studied the serum vitamin A levels of children with measles was examined [12]. Majorities of the children were from low-income families, with incomes below the poverty levels. Reports that 70% - 72% of these children had deficient vitamin A levels. It was also found that the severity of the deficiency was directly correlated to the severity of the illness and complications [70]. Such findings have prompted the WHO and UNICEF to issue a joint statement with the recommendations mentioned earlier. (See Introduction of Literature Review, p3)

2.3 Link between Iron and Vitamin A

Animal studies undertaken by Findlay and McKenzie in 1922 [71] suggested an interdependent relationship existed between vitamin A and Iron. Later studies in animals also reported anaemia being present in vitamin A deprived animals[72]. These animal studies concluded that vitamin A deficiency produces anaemia, increased iron absorption, and hemosiderosis of the spleen and liver[73]. Recent reports by Roodenburg in an intervention trial revealed that vitamin A supplementation improved the iron deficient state in rats [74].

Studies in humans confirm an association between vitamin A and anaemia. Observational studies found varying results from a positive correlation between serum retinol and haemoglobin in 5-12 year old children to a strong correlation between serum vitamin A [75] and haemoglobin in 15-45 year old women [76]. Further cross sectional studies in Thailand [77,78], Ethiopia [79] and Indonesia [80] confirm that an association exists between vitamin A and anaemia. Bloem et al (1989)[77] in Thailand found a strong association between the levels of serum retinol with packed cell volumes, serum Iron, ferritin and transferrin. However, there was no correlation between serum retinol and haemoglobin in these studies.

A protective effect was observed when the vitamin A status of Pakistani infants supplemented with iron improved, alongside an improvement on iron status of these children. No vitamin A supplements were given with the main source of retinol coming from the diet [81].

The causal link between iron and vitamin A is strongest in the intervention studies. Supplementation of vitamin A increased levels of haemoglobin, packed cell volume and serum iron significantly, over a period of 2-3 weeks [72]. Mejia and Arroyave (1982) also reported after 6 months of vitamin A fortification in young children, iron status had improved. In another controlled intervention trial with Guatemalan children, monosodium glutamate (MSG) was fortified with vitamin A [72]. The group that received the supplementation demonstrated a 10 g/L rise in haemoglobin [82].

In a well-designed study by Meija and Chew (1988)[83], vitamin A supplementation in anaemic children produced significant increases of serum retinol and iron, haemoglobin, packed cell volumes, erythrocyte count and percentage transferrin saturation. It is interesting that vitamin A supplementation had no effect on total iron- binding capacity or serum ferritin levels. This study confirmed animal studies undertaken by Roodenburg [74] that in humans with a marginal vitamin A intake, supplementation of vitamin A and iron is more effective in normalising iron status than iron supplementation alone.

In Thailand, it was reported that high doses of vitamin A supplementation of anaemic children did not alter haemoglobin levels, but showed increases in serum iron and transferrin at 2 months[77]. However this effect was not seen at 4 months. Another intervention trial in Indonesia of 236 pre-school children demonstrated that vitamin A supplementation was associated with large increases in plasma ferritin levels. It was suggested that these increased ferritin levels may have been a secondary effect of improved iron uptake[84]. The most recent intervention study found that combined supplementation of vitamin A

and iron in anaemic women led to the reversal of anaemia in 97% of these women[85].

The underlying mechanism of how vitamin A and iron are linked remains unknown. However, it is clear from the intervention studies that vitamin A supplementation with iron may make iron deficiency anaemia control programmes much more effective. A potential benefit would be that this would also ensure that vitamin A deficiency is addressed.

The first part of this review has highlighted that nutritional iron anaemia and vitamin A deficiency are two of the most prevalent nutritional deficiencies in the world. This is particularly true in the developing countries and to a lesser, though significant extent in the developed countries. The biochemistry provides further understanding into the mechanisms of metabolism and absorption. The severe long-term functional consequences of these 2 nutrient deficiencies highlights the urgent need to address this issue.

2.4 Dietary Methodology.

Since the main source of iron and vitamin A comes from the food we eat, the key to identifying the dietary causes of iron and vitamin A deficiency lie in analysing the foods consumed. Dietary assessment is one of the tools required to provide information of nutrient intake and eating patterns that may lead to the existence and causes of these deficiencies.

2.41 Summary of Various Dietary Methodologies

Dietary assessment is a component of nutritional assessment whereby the regular nutrient intake and eating patterns is used as one of many indicators for ranking the nutritional status of the individuals in populations. The goal of dietary assessment is to screen for groups of individuals that are at risk of nutrient or nutritional deficiencies and to determine any causal effect that food or a specific nutrient has on a disease. This form of research has been used in large-scale epidemiological studies of the nutritional status of the general population to selected small groups of individuals, as well as identifying foods that prevent or have a causative effect to health.

There are two general categories of dietary methodology that are used in dietary assessment. The retrospective method and the prospective method each provide a different perspective to dietary assessment. All dietary methods have a degree of error in them. No method will be able to measure the exact and correct intake of nutrients of a subject. However, measuring the extent of the error and precision, enable corrections and considerations to be made in order to provide a valid conclusion. The precision of the instrument determines how reliable or reproducible the tool is for use in population studies [86,87].

2.411 Retrospective Methods

Retrospective methods rely on recollection to obtain data. These methods focus on what has been eaten in the past, over a set period of time. Examples of these are the diet history, 24-hour recall and the food frequency questionnaire.

1 Diet Histories

The weakness of diet histories the need for a trained interviewer to observe the situation as well as prompting the respondents in a way that doesn't affect the results or make the respondents uncomfortable about their food intake [88]. Thus, errors in memory and response bias add to the inaccuracy of this method. Aspects that affect the precision of using this method for assessing usual mean intakes of individuals or groups, depends on the time frame and time lag of this method, the technique of measuring the amounts of food and the population being studied [86,89].

2. 24 Hour Recall

The 24-Hour recall is one of the more popular methods of assessing individual food intake. Strengths of this method include the low respondent burden and minimal literacy on the part of the respondent. Respondents are asked to remember and report all food and drink consumed in the 24 hours preceding the interview. The structured interview with some prompting and visual objects may aid the respondent to remember the food eaten over that period as well as the approximate amounts. The interviewer can then probe further to discover details about food preparation and brand names of the foods [86].

Large studies favour the use of this method for its relative ease, low cost and low respondent burden. However, there are also limitations for this method. An interview situation may affect the respondent to under or over report their food intake by the way the questions are asked. Foods that may be considered to be bad may be under-reported whilst "healthy" foods may be over-reported. Secondly, a single 24-hour recall doesn't provide appropriate information for comparison of group food intake. Much variation exists not only in the variation between individuals, but also in the day to day variation for the individual respondents [90]. A single day of the respondent's diet does not

necessarily reflect normality. Thus, the use of multiple 24 hr recalls improves the precision of this method [91].

3. Food Frequency Questionnaires

A food frequency questionnaire is another retrospective method that is generally used to assess food intake. This approach asks respondents about the 'frequency' of consumption of a group of foods over a set period of time. Information obtained can be quantitative or qualitative depending on the specific aims of the food frequency questionnaire. If only frequency of food consumption is collected this is known as a qualitative food frequency questionnaire. The inclusion of portion sizes to estimate relative intakes makes the food frequency questionnaire a quantitative questionnaire [92].

The biggest limitation of the food frequency is the lack of details of dietary intake that may remain unmeasured. Recall of estimates from the respondent will generate large variability and discrepancies over the reporting of amounts. Food frequency questionnaires are best used in ranking individuals in populations according to food or nutrient intake rather than determining individual intakes [93].

Each one of these dietary methods provides a tool to assess food intake. However, with the strengths and limitations of each method – it is clear that these methods have a specific use – either to determine individual food intake, or the average food or nutrient intake of a population or group of individuals. These retrospective methods provide a useful information on the history and background of an individual.

2.412 Prospective Methods

With retrospective methods relying on the recollection of food intake lies several difficulties and sources of error. Assumptions that respondents have remembered exactly what, as well as how much they have eaten places considerable responsibility for accurate information on the part of the respondents. Circumstances may provide results that may not be consistent with the individual's eating patterns and average food consumption. An example of this in New Zealand is the traditional feasting-fasting cycle of the

Pacific Islanders. Due to circumstances, whether it may be poverty or celebration, the availability of food may be spontaneous. Retrospective dietary methods will fail to detect the rises and fluctuations of food intakes. As a result, a grossly inaccurately under-reported or over-reported food intake will be identified. (See section 2.43)

This is where prospective methods play an important role. Not only do these methods provide a useful tool in determining nutrient intake; they also provide a different perspective that may overcome any limitations that retrospective methods may have. Prospective methods rely on respondents reporting their food and nutrient intakes as they are consuming them. There are different sets of limitations that exist for these methods.

1. Weighed Food Records

A weighed food record is used to minimise the possible errors arising from the memory lapses and inadequate estimates of portion sizes that retrospective dietary assessment methods are confounded with. As a dietary method, it has been widely accepted in the nutrition research community as a "gold standard". "Gold standards" are methods that the research community has considered being the most accurate. However, there are differing opinions as to which is the true gold standard, depending on the nutrient of interest.

Weighed food records have the potential for obtaining quantitative data on food consumed in the recording period. This method requires the respondent to weigh, measure all the food and drink, recording these over a period of days. Generally, a set period of 3-7 days with the inclusion of weekend days is used. The precision of this method is dependent on the number of days of records that are taken. Insufficient days may not provide data that represents the mean nutrient intake of the subject, whilst prolonged measuring may lead to respondent fatigue [86,94].

Though considered a 'gold standard', this method has its limitations. Respondent burden is much greater, with the need to train the respondents to

measure and accurately record information regarding food intake and food preparation. An added weakness of this method and prospective methods in general is temptation for the respondent to alter their eating patterns and food choice throughout the period they are recording their food intake. Respondent burden is considerably greater with this method, which can affect the data collected.

A further limitation of using the weighed food records is its accuracy in determining the mean daily nutrient intake of specific nutrients. This method is able to provide a relatively precise record of most nutrient intakes. However, several studies have observed poor correlation values of vitamin A, vitamin C and cholesterol. Due to the restricted and limited availability of vitamin A in food sources, accurate individual and group measures are difficult to obtain, with very large differences in the day to day intake of vitamin A in a diet, and also the large difference between individuals [94,95].

The differences of both the retrospective and prospective dietary methods highlight the fact that the sources of error and variation of both methods differ. A combination of both methods may reduce the margin of error and inaccuracy considerably.

2.42 Issues of Reproducibility and Variability

Sources of error and variation exist in every method that is used to measure dietary intake. There is no absolute measure to determine nutrient intake of humans. Comparison between different methods provides a degree of accuracy in any conclusions that are made. Often, the correlation between the "gold standard" and the chosen method is used to determine the degree of accuracy of the "test" method.

The "gold standard" is the method that is considered to give the most accurate measure of nutrient intake. For example, the standard for energy intake is currently considered to be the use of doubly labelled water[1] as a biomarker. A

[1] Doubly Labelled Water : Water containing a stable isotope deuterium (^{2}H) and $^{18}0$ used to measure energy expenditure in humans

validation study of energy intakes in children used doubly labelled water to validate a 7 day weighed food record against a diet history. The results showed that there was good agreement between the weighed records and doubly labelled water in the younger children (96.9% and 107.9% of weighed records over doubly labelled water), with an increasing divergence in adolescents (88.7%- 72.7%). Similar results were found in diet histories in children (112.5% - 97.6% of diet histories over doubly labelled water) [96]. However, the cost and also the demands that this method places on subjects have meant that this method is not readily used as a comparison with other methods. This method is also only limited to measuring energy. The weighed food record has been adopted as a "gold standard", particularly in validation studies. There are problems in using the weighed food records to measure nutrient intake, as there have been reports of large variation in assessing micro-nutrient intake [86,95].

Relativity is measured by the source of error. The source of error can come from various places. These generally include the shortcomings of the method to measure what is intended to measure and over or under-reporting. Over and under-reporting cannot be measured directly but it can point to certain discrepancies that may cause the results to be misinterpreted [97]. This highlights the importance of undertaking validation and reproducibility studies. Validation and reproducibility studies are used to identify the possible sources of errors and the extent that these errors may affect the results.

2.422 Issues of Validity

Before a dietary method can be used to measure food or nutrient intake, it needs to be 'calibrated' to measure specifically what it is supposed to measure. This is known as validation. Validation studies compare different dietary methods in order to determine the accuracy and precision of a specific method. As mentioned before, the weighed food record method has been accepted as the "gold standard". In 'validation studies, of retrospective methods, the weighed food records provide a useful source of information to identify if the respondents recollection is reliable. In addition, validation studies may also highlight potential sources of error or loopholes that may be present [98].

The main purpose in carrying out validation studies is to improve the accuracy and precision of the dietary method, and ensure that the "dietary tool" is measuring what it should. This also provides opportunities to test for difficulties that may be encountered in coding the results, or the appropriateness of the dietary method for measuring what is in question. Validation studies are crucial in conducting a well-designed study. It is the next logical step after the development of questions and questionnaires [99].

No dietary method is going to assess nutrient intake either of an individual or a population without error. However, addressing the issue of the presence of error, and minimising the source of error means that the data is more likely to be accurate and acceptable.

Measurement error can then be used to correct the misinterpretation of resulting data. There are many statistical measures that are applied in validation studies in order to determine if the intended dietary method is as accurate as the "gold" or reference method. Correlation between methods of individual nutrients is the most popular statistical measure, with Pearson's and Spearman's correlation coefficients well recognised in validation studies. More recently, measures of limits of agreement by Bland and Altman have been recognised to be a better measure of how reproducible a method is [100] (See section 4.4 and section 5.53). However, there are currently very few reported or published dietary studies that use the Limits of Agreement method to validate a dietary method.

2.421 Issues of Reproducibility

Reproducibility, or also known as reliability is a test to see if the method in question will produce a similar answer when administered repeatedly. Precision is also another name for reproducibility that is commonly used. Results of reproducibility studies may reveal problems in instrument design, respondent instructions or quality control. Reproducibility studies are increasingly becoming recognised as a necessary part of instrument design and development [91,101].

Caution is needed in undertaking reproducibility studies. The re-administering of the same questionnaire to determine if a similar answer is generated can generate a conclusion that is desired, but also systemic error. This potential problem is overcome by increasing the time interval between the administration of the first and second questionnaire [102]. Studies have shown that this time interval needs to be long enough for the subject to forget what was answered in the previous questionnaire, and short enough for their intakes not to have changed considerably [102]. Two key factors affecting reproducibility include the inability to estimate diet, and secondly, in an elapsed time, dietary changes would have taken place [91].

There are two types of measurement error that needs to be identified in any reliability study. Systemic bias refers to errors such as respondent fatigue, or general learning. This is also known as bias in reporting. The other type of measurement error is the random error that is due to the biological or mechanical variation [100]. Taking into account these two main sources of error in the analysis of reliability studies may increase the true accuracy of the dietary assessment tool being tested.

2.43 Dietary Assessment in Children

Difficulties arise when the subjects of dietary assessment are children. Children have the additional factor of being unable to record and remember their diets accurately. For younger children, the responsibility of recording and remembering the infant's diet falls on the main caregiver [103,104]. Large day to day variation of a child's eating habits and tiny quantities of food that a toddler eats increases the level of error in the measurement of nutrient intake. This extends a challenge for researchers to explore different ways to minimise these sources of error and overcome the difficulties that each method poses, when the subjects are children.

In addressing the difficulties and barriers mentioned above, dietary studies in children will provide invaluable information on the nutritional status of children. There have been several large-scale nutritional studies undertaken in

the United States that have involved children over the past three decades. The Ten State Nutrition survey was a study assessing the nutritional status of children and adolescents in the United States, using 24 hr recalls. Other studies involving children include the NHANES and the Nation-wide Food Consumption Survey (NFCS). These studies were done in the 1970's. Subsequent studies include the 1987-88 NFCS and NHANES III [105]. There will undoubtedly have been a significant number of dietary studies undertaken in developing countries that have not been covered in this review.

In the case of pre-school children and toddlers, there has been lesser studies undertaken. Issues of ethics and the difficulties mentioned earlier have posed a barrier. There are however, an increasing number of studies examining at the dietary intake of infants and toddlers. Several studies have been undertaken countries to determine the diets of healthy under two year old children in Sweden, Norway, the UK and the US [106-110]. These are general dietary studies involving children, providing information of the children's eating patterns and the food children eat.

There have been numerous studies specific to identifying nutrient intake undertaken in children. Many of these studies are from developing countries like Indonesia, Nepal, and India, as the extent of nutrient deficiencies are more severe in these nations. Field studies undertaken in Egypt, Kenya and Mexico have provided data for examining the diets for the probability of nutrient inadequacies [111]. Food records from toddlers aged from 18-30 months were compared with 3 anthropometric measurements to estimate the prevalence of inadequate vitamin intakes. This method predicted the prevalence of vitamin inadequacies for vitamin A (32%), riboflavin (20%) in Egypt, vitamin A (68%) and Vitamin C (63%) and riboflavin (52%) in Mexico and Vitamin B-12 (44%) in Kenya [111].

The national nutrition survey in the United Kingdom has successfully managed to determine the nutrient intake in children under two. The National Diet and Nutrition survey looked at the diets of children aged 1½ to 4½ years of age

using the weighed food records as a measure [110]. However, Iron and vitamin A intake from food was not related to iron and vitamin A status, nor was the four-day weighed food records compared or validated with any other method.

In a longitudinal study of healthy Norwegian and immigrant children, dietary interviews were carried out with the care-giver completing a food frequency questionnaire. Comparison with the biochemical status of children was made to determine the iron status and diet in children under two years of age [109]. This is one of the few published dietary studies focussing on dietary iron intake and its relationship with the prevalence of iron deficiency in a population. This study showed a positive correlation suggesting that the differences in food intake may be a significant factor in the lower Iron status of the immigrants [109].

Smaller sub-groups of children have also been studied. A study undertaken by Boutry and Needlman (1996), where the diet history was used to screen iron deficiency in young children indicate that the diet history method was able to correctly identify children with microcytic anemia 97% of the time [112].

2.431 Validation Studies in Children

Blom et al validated a food frequency questionnaire in a group of children aged between 2-16 years. The food frequency questionnaire was validated against a 7 day food record. In this study the comparison of the two methods showed a correlation of 0.52 –0.76, regarding foods high in sucrose, protein, fat, fibres and Vitamin C. However, the questionnaire showed a lower sensitivity (0.38 –0.5) with a high specificity (0.86 -1.0) in identifying high or low consumers of foods high in sucrose, protein, fat, fibres and Vitamin C [113].

Another validation study in children was undertaken by Livingstone et.al (1992) [96], where weighed dietary records were validated against a diet history, and doubly labelled water. This validation study's aim was to determine the estimates of energy expenditure in children aged 3 – 18 years. From the results of this study, it was found that weighed dietary records underestimated food intakes of adolescents, whilst the diet histories over-estimated in most age groups.

There have been no reported studies undertaken to validate a dietary method to assess food intakes of young children and toddlers, especially under the age of two. Validation studies that were mentioned above were undertaken in older children. The lack of general validation studies in young children means that validation studies in children of this age group specific to Iron and vitamin A are non-existent. Currently, there are no reported studies on the validation of dietary assessment methods that determine vitamin A and Iron intake in young children. Studies that focus on iron or vitamin A are mostly intervention studies, with a dietary intake dimension added into the study. As the link is proven between diet and preventable diseases that affect young children, there will be an increasing need for accurate dietary assessment.

2.45 The New Zealand Situation

In New Zealand, iron deficiency anaemia has been identified as a problem in Maori infants since the 1960's [114,115]. Many of the studies that were done then were obtained from opportunistic samples, either from hospitals or intervention studies. True prevalence studies have not been conducted to determine iron deficiency and vitamin A status in the general community.

Results of a study in the Bay of Plenty confirm the ethnic imbalance in the prevalence of anaemia. The community study reported that 34% of Maori compared to 6% of Europeans had haemoglobin levels below 110 g/L [116]. More recently, the paediatric acute assessment unit at Middlemore hospital reported that one in three children aged 6 –23 months that was seen at the hospital was anaemic [117]. Of the sixty seven children enrolled in the study, fifty five of the subjects were of Maori and Pacific Island descent. Of these 21% had blood results suggestive of iron deficiency, of which 14% had iron deficiency anaemia. The results of this study highlight a pressing public health problem in Auckland itself. These children were in the paediatric acute assessment unit with infections. How much greater is the iron deficiency in the well children population of New Zealand that still remains undetected?

Wham (1996) [6] undertook a study to identify the prevalence of iron deficiency in 9-24 month old children that were recruited from Plunket Child centres. However, this sample was not a random sample. A single 24 hour recall and a diet history questionnaire were used in this study. The results revealed that the mean iron intake for infants in this age group ranged from 0.66 of Recommended Dietary Intake in 9-12 month olds, to 0.8 of the Recommended Dietary Intake in 12-24 month olds. Further studies that have been considered have been highlighted in the table on page 3 (See Introduction to Literature Review).

A review of the various studies indicates that whilst the prevalence of iron deficiency is alarmingly high in New Zealand infants and children, sample sizes of these are very small, and are not true random samples. This raises the need for true prevalence studies to be undertaken in the wider healthy population of children in New Zealand. Furthermore, these studies have been based solely on the biochemical status of the children obtained from a blood sample, with very little reference to their diets and causal effects. Very few studies have been dietary studies assessing iron status from the nutritional point of view, especially in a true randomly sampled population. As mentioned previously, (See Section 2.4) assessing the diets of the subjects is the first step in identifying inadequate sources of a nutrient that could potentially lead to a nutrient deficiency.

There is also a need for an effective tool to measure the prevalence of iron deficiency and vitamin A status specifically for Auckland infants. Auckland is ethnically very different from the rest of New Zealand. The population of Auckland represents almost 1/3 of the New Zealand population. It is the multi-cultural capital of New Zealand, ethnically comprising of people from all over the world. Of particular interest is the Pacific Island population. Hospital statistics show this population to have higher admission rates for measles and a higher incidence of iron deficiency [17].

In order to address this public health issue and develop ethno-specific nutrition strategies to address this problem, ethnic specific research methods are a vital key. The need for culturally sensitive dietary methods is crucial if the alarming situation of such toddlers is to be addressed, and the message passed on to the different ethnic communities in Auckland.

2.6 Overall Conclusion and Inferences from the Review of the Literature

Given all the information that has been presented in the literature review on iron and vitamin A nutrition in young children, several key points have been identified. These are:

- Iron and vitamin A deficiencies are two major key issues in child health today, affecting children from developing countries, but also developed countries like New Zealand.
- Children of ages 6 – 24 months are at greater risk of developing iron deficiency and iron deficiency anaemia and vitamin A deficiency due to the increased requirements for growth and diets which may be low in the nutrients.
- Data on the prevalence of these two nutrient deficiencies are incomplete and inconclusive. The true prevalence of these two micro-nutrients Iron and vitamin A in New Zealand remains unknown or unpublished.
- The functional consequences of these nutrient deficiencies are being confirmed by observational, longitudinal and intervention studies world-wide.
- The effects of these two micro nutrient deficiencies are well recorded. The lack of iron during the crucial months can lead to an impaired motor and cognitive development.
- The severity of measles is known to be associated with low levels of vitamin A.
- Validation studies of dietary methods young children of this age group are few.
- There is a need for New Zealand and Auckland specific data and dietary tools to assess this potential problem.

Aims

This study's overall aim is to validate methods to obtain data on the dietary iron intake and vitamin A intake of 6 - 24 month old Auckland children. This will be achieved by validating a food frequency questionnaire against weighed food records. This study was a part of a pilot study, with the overall aim to assess methods to obtain data on iron and vitamin A status of 6-24 month old children.

The practical aims of this study are to collect nutrient intake data of 9-24 month old infants. Methods used include: -

- A food frequency questionnaire
- 4 day weighed food record diaries
- Anthropometric measurements
- Blood samples to measure iron and vitamin A levels
- General questionnaire

Dietary data collected will be analysed by ranking the individual nutrient intake obtained from a food frequency questionnaire, and weighed food records to identify characteristics and relationships of iron deficient and sufficient Auckland children.

Methodology

4.1 PARTICIPANTS AND METHODS

4.11 Subjects

Thirty children of ages ranging from 6 to 23 months inclusive were enrolled in this pilot study. This age group (6 - 23 months) was targeted for several reasons. The functional consequences of iron and vitamin A deficiencies would be a major public health concern, should the prevalence of these deficiencies be found in Auckland children. Six to twenty three month old children have also been identified to be at a high risk of iron deficiency (See Section 2.121). It has also been observed that the majority of children hospitalised with measles in Auckland are under 24 months of age.

4.12 Ethical Consent

This research study was approved by the Northern Regional Health Authority.

4.13 Ethnicity of the Subjects

The multicultural demography of Auckland has been taken into consideration, with all ethnic groups eligible for this study. Eligible children were assigned to one of the four main ethnic groups Maori, Pacific Islander, European or Other, depending on the ethnicity that was identified by their care-giver. Children with multiple ethnicity were assigned to an ethnic group based on the priority system from the 1993 NZ standard classification of ethnicity by the Department of Statistics [118].

4.14 Selection Criteria

Children of all ages between 6 months and 23 months, from all ethnic groups were eligible to be in this study. Children who were recovering from a recent

acute infection were excluded from participating in this study. Acute infections however mild, raises serum ferritin concentrations and there is a corresponding fall in serum iron concentrations as part of the acute phase response [119,120]. Due to this physiological reaction to infection, there would be difficulties in determining iron status in the presence of an acute inflammatory process, and differentiating iron deficiency anaemia from the anaemia of acute infection. Children with acute infection were screened by asking the main care-giver about the child's health status in the past three months before enrolling them as subjects, and were excluded from the study if infection was detected.

4.2 STUDY DESIGN

This was a population-based study. Research literature and Intervention studies undertaken at Starship Hospital and also at National Women's Hospital in the past have indicated a high prevalence of iron deficiency amongst children of this age.

4.21 Recruitment Strategies

The children that made up the study sample were recruited by cluster sampling using random address start points in Central Auckland. The borders of central Auckland were determined as the area in which the Auckland City Council services. Children that were eligible were identified using the cluster sampling technique, a method that has been previously piloted in Auckland [121].

It is important to note that whilst all children aged 6 – 24 months in Central Auckland are eligible to be in the study not all who were recruited were enrolled in the study. Each recruited child was randomised after they were recruited to stratify the sample, ensuring equal ethnic participation. Children with acute infections were also not enrolled.

4.22 Cluster Sampling Technique

The cluster sampling technique ensures that a random sample of children was enrolled for the study. Children who were eligible to participate in the study were identified from homes at random start points. Ten houses were visited at each start point, and each eligible child had a chance of being enrolled. Repeat

visits to the area were made at different times of the day and different days to ensure that all houses in the start points were contacted. For households with eligible children, information about the study was given, and the care-givers were given approximately one week to make a decision to participate in the study. Recruiters followed up these children by telephone and answered any further questions the care givers had. An appointment was scheduled at this time to conduct the interview once consent was given.

4.23 Stratification of Sample

As the children were enrolled based on their ethnicity, they were assigned to one of the four ethnic groups based on the group that they or their caregiver identify most. Children with multiple ethnicity were allowed to specify the ethnic group with which the child or care-giver identified most with. If there was a difficulty, ethnicity was assigned using a priority system from the Department of Statistics [118].

After obtaining consent from the care-giver, each child was then selected to be in the study using a sampling ratio based on the child's ethnicity. This "stratification" of the sample ensured the desired numbers from each of the four ethnic groups were enrolled. Based on the 1991 census, of the Auckland population of children aged between 0 to <24 months, the ethnic composition was 20% Maori, 21% Pacific Island, and 59% European/other. To ensure that each eligible child identified had a chance of being enrolled; these percentages have been used. All eligible Maori children have been enrolled, 91% of eligible Pacific Islanders and 34% of eligible European/Other children were enrolled. In households with more than one child eligible, one child was randomly selected to participate.

The interviews were conducted as each eligible child was enrolled. The timeline for each enrolled infant from identification to completion of data collection was four to five weeks.

4.24 Interview Process

Conducting the interviews at the home of the infant was primarily to ensure that the child was in its normal environment. This minimised the effort required by the care-giver, with the hope of encouraging higher response rates.

4.41 Study Protocol

Week 1:	Identification of eligibility by recruiter, application of sampling ratio based on ethnicity.
Week 2:	Family invited to enrol in study
Week 3:	Interviewer visit #1 Informed consent obtained, food frequency questionnaire completed, collection of anthropometric data and blood sample. Caregiver is instructed on how to complete four day weighed food record.
Week 3-4:	Caregiver completes Weighed food Records. Telephone follow up by interviewer.
Week 4:	Interviewer Visit #2. Demographic questionnaire completed. Completed four-day weighed food records checked.

Figure 4.3 Timeline for each Enrolled Subject

During the weeks that the care-givers were completing the weighed food intake diary, the interviewer telephoned the care-givers prior to the days the care-givers were weighing the infant's food to give encouragement. It has been reported that this may increase the compliance and the accuracy of the weighing the foods the infant eats [110].

The second interview was arranged when the four day weighed food records were completed. During this interview, the food frequency questionnaire was administered for a second time and the corresponding general questionnaire was also administered.

4.3 MATERIALS AND METHODS

The principal aim of this pilot study was to identify an effective tool for determining dietary intake of vitamin A and iron. Broad categories of measurements include dietary assessment, physical measures, biochemical markers and blood samples. In this study, a combination of dietary assessment, physical measures and blood samples were used to assess the infant's nutritional status.

All measurements were collected from the infant, primarily via their care-givers in two interviews. There were five categories of information required from each infant : a food frequency questionnaire, a four day weighed food intake record, anthropometric measurements, analysis of a blood sample, and an interview with the caregiver to complete a general questionnaire.

The interviewers were chosen and trained to interview care-givers of the children from their own ethnic-group. These interviewers were trained to ask questions in the interview in a non-misleading way, to take anthropometric measurements and to conduct play therapy whilst the phlebotomist took the blood sample.

4.31 Food Frequency Questionnaire:

A 63 item semi-quantitative food frequency questionnaire was designed for this study, specifically to measure the usual intake of the infant's food high in iron and vitamin A over the past three months. The food items targeted in this food frequency questionnaire were chosen by referring to the NZ Food Composition tables [122], for the content of iron and vitamin A equivalents in these foods.

4.311 Food Frequency Questionnaire Design.

This food frequency questionnaire is unique to this study because it is culturally relevant to the four main ethnic groups that are involved in this study - Maori, Pacific Islander, European and Other. Inclusion of foods that each ethnic group consumed was vital in conducting this study amongst the Maori, Pacific Island, European and Other groups. All attempts were made to determine the cultural foods that were specifically high in Iron and vitamin A, by consulting the Pacific Island Food Composition tables[123], and also consulting various members of each community. This was crucial in determining foods that children of this age group were fed.

It is also unique, in that it is specific only to the nutrients of interest, iron and vitamin A. It concentrates on the foods that are especially high in iron, both haem iron and non-haem iron sources, and vitamin A. In addition to that, there are several other inclusions to this food frequency questionnaire. Foods that

may have an enhancing effect on the absorption of Iron, for example Vitamin C and also inhibitory effects on absorption have been included (See Appendix I)

The food frequency questionnaire was interviewer-administered in the care-giver's home. This questionnaire consisted of 3 parts (See Appendix E). The first section was comprised of questions concerning the subjects eating habits and eating patterns. The second asked about the frequency of specific foods eaten, and the third asked for approximate amounts and ways the food was cooked.

Questions in the first section involved ticking appropriate boxes describing the child's eating patterns, and the eating patterns of the main food groups (See Appendix E). An example of one such question is illustrated below.

1. How would you describe your child's eating pattern? *(Please mark only one)*

- ❑ Eats a variety of all foods, including animal products
- ❑ Eats eggs, dairy products, fish and chicken but avoids red meat
- ❑ Eats eggs, dairy products, but avoids all meats and fish
- ❑ Eats eggs, but avoids dairy products, all meats and fish
- ❑ Eats dairy products, but avoids eggs, all meats and fish
- ❑ Eats no animal products
- ❑ Other *(please specify)*_____

Figure 4.2 Eating Patterns Section of the Food Frequency Questionnaire

In the second section, there were 8 categories of consumption. This was adapted from a combination of food frequency questionnaires.

	Never	< once per mont	1-3 times per mont	Once Per Week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
DAIRY FOODS Unflavoured milk as a drink Cow's Milk as a drink								

Figure 4.3 Frequency of Foods Section of the Food Frequency Questionnaire

Only the foods of specific interest were looked at in the third section. Foods of interest include those that may provide the main source of iron and vitamin A. Amounts of these were recorded in millilitres for volumes of liquids and in cups or grams for the solid foods. In the table, spaces for the other foods were shaded out to remind the interviewer which foods needed this information. In this section, a comment's column was included, with reminders for the interviewer to specify brand names and cooking methods. Interviewers also carried a set of measuring cups that were used as a prompt and as standards for the estimation of approximated quantities consumed.

Amount	Comments
	Please specify type of Milk

Figure 4.4 Quantitative Aspect of Food Frequency Questionnaire

The same food frequency questionnaire was administered in both interviews to test for reproducibility.

Data from the food frequency questionnaire was coded and entered into MS Excel 97 [124] spread sheet, and analysed in SAS systems for Windows (ver 6.12) [125].

4.32 Weighed Food Records

All the food and drink consumed by the child was weighed on a digital electronic scale with tare and recorded in a Home Record Diary (See Appendix C). All records were collected over four *non-consecutive* days, randomly chosen to include three-week days and one weekend day. It has been reported that non-consecutive days were more accurate in determining nutrient intake [126]. Provision was made if the child was at day care, or when there were meals away from home by including an eating out diary, which didn't require the weighing of the food. (See Appendix D)

The design of the Home Record Diary has been validated in other studies [110]. The table constructed provided care-givers a systematic way of working through the diary. These columns also provided further information as to how the foods were cooked, amounts consumed and amounts left over.

During the weighed food intake period, the interviewer telephoned the care-giver to check if there were difficulties encountered and also to provide some encouragement.

The weighed food records were analysed using FoodWorks version 2.00 [127]. Nutrients analysed were both macro-nutrients and micro-nutrients and comparisons were made with the RDI for 6 – 23 month old children.

4.33 Anthropometric Measurements

Three sites were selected to take the skinfold measurements from - triceps, biceps, and subscapular. For each of the three sites, the average of three measurements was taken. Other physical measurements that were taken included the weight, height measured in the supine position and mid-upper arm circumference. For children in this age group, height is only accurately measured in the supine position [128]. All measurements were taken from the child, wearing light clothing and were taken by interviewers who had received relevant training. These measurements were taken to assess the overall nutritional status of the child. (See Appendix G)

4.34 Blood Parameters of Iron and Vitamin A Status

An experienced paediatric phlebotomist collected a venous blood sample (10ml). Prior to the interview, Diagnostic Laboratory was informed of the time of the interview appointment, and an arrangement made for the phlebotomist to arrive 45 - 60 minutes *later.* Upon commencement of the interview, Diagnostic Labs were telephoned to confirm that the interview was taking place. Emla cream was placed under a transparent dressing on both infant's hands. Play therapy, (a strategy of using toys to distract the child) was used to minimise the trauma the child may have experienced during the blood sample collection. Whilst the child was distracted with bubbles, toys and music, blood was collected by venipuncture into a trace element free vacutainer. The sample collected was measured for serum ferritin, serum iron, total iron binding capacity and serum retinol. A full blood count and c-reactive protein was also measured to determine the presence of infection. Results of these provided a measure of Iron status and vitamin A status of the child.

Measurements were determined by automated biochemical analysis with blood parameters measured as follows :

- A full blood count was determined using light scatter and cytochemical straining on a Bayer H2 analyser. Haemoglobin concentration, haematocrit, red cell count, platelet count, total and differential white cell count, and three red cell indices : mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentrations were also determined from the blood sample.
- C-reactive protein concentrations were determined nepholometrically on a Beckman analyser.
- Plasma ferritin concentrations were determined using an immunoassay on an Abott Axsym Analyser.
- Iron saturation was calculated by dividing the serum iron by total iron binding capacity.
- Serum Iron and total iron binding capacity were measured by the Ferrozine method on a Hitachi 747 analyser.
- Serum retinol quantification was obtained by high performance liquid chromatography using UV detection at 325 nm.

All the laboratory work and analysis was undertaken by Diagnostic Labs and Auckland Healthcare Limited.

4.341 Definitions of Iron and Vitamin A Status

In this study, the definition of iron deficiency and iron deficiency anaemia is taken from the National Health and Nutrition Examination Surveys (NHANES II and NHANESIII). These surveys have determined the prevalence of iron deficiency of children in this age group in the United States. Both surveys determined the prevalence of iron deficiency from 3 laboratory tests of iron status: serum ferritin, transferrin saturation and the free erythrocyte protoporphyrin. Iron deficiency was present if the values for two of three variables were abnormal and iron deficiency anaemia, if low haemoglobin as well as iron deficiency was detected. Cut off values of measures of iron status were the same values used in the NHANES III study [129]. The cut off values that were used to determine iron deficiency and iron deficiency anaemia were: Hb,< 110g/L, MCV <72fl, MCH <24pg, SF <10µg/L. Iron deficiency anaemia was defined as Hb<110g/L and SF <15 µg/L.

Vitamin A deficiency was defined as serum retinol levels less than or equal to 0.70 µmols/L. The definition that WHO uses to define vitamin A deficiency in a population is based on the proportion of individuals in the sample who have a low serum retinol level. If 2%-10% of the population sample have serum retinol levels of less than or equal to 0.70 µmol/L, it is considered to be a mild public health problem and if between 10-20% of the sample, it is a moderate problem, and finally a severe problem if greater than 20% of the sample have a low serum retinol level [11].

4.35 General Questionnaire and Interview

A general questionnaire was constructed to collect data on demographic characteristics of the study sample. These included: care-giver's education, household demographics, family characteristics, socio-economic and smoking status, maternal parity, early infant feeding practices, family vitamin/mineral supplement use and food security. This questionnaire was administered in the

second interview, after the food frequency questionnaire has been administered for the second time. (See Appendix F)

4.37 Limitations and Feasibility of Study Design

The limitations of this study design lie in the recruitment strategies. Although it is probably the most accurate way of obtaining a true random sample, there were many keen care-givers excluded from this study. In this pilot study, communication was a major barrier, where a level of English competency was required to conduct the interview. This issue was solved by matching the interviewer from the same ethnic group as the family with the family involved. The enrolment of children was entirely voluntary. Therefore, it is very likely that the care-givers that consented to undertaking this study were ones that were interested in the nutrition and improving the health of their child, providing a bias sample.

There were problems with the dietary methods in this age group. At this age, the infant is likely to be mobile and in its early stages of walking. To monitor the infant, and what it was given was a major challenge of undertaking weighed food records in children of this age group. Furthermore, the tiny amounts and the snack-like meal patters meant that weighing the food was more burdensome. Large day to day variation of vitamin A intake between individuals has been reported. This large variation did not change regardless of time [95].

4.4 STATISTICAL ANALYSIS

Data collected consisted of the consent form, two food frequency questionnaires, a four day weighed food diary (Home diary and eating out diary), a general questionnaire, anthropometric measurements and blood results. All statistical analysis and calculations were performed using Microsoft Excel 97 [124]and SAS systems for Windows (ver 6.12)[125].

The dietary component of the statistical analysis of this validation study consisted of three components :-

1. Testing for the reliability of the food frequency questionnaire

2. Determining the correlation and ranking between the food frequency questionnaire and the weighed food records.
3. Measuring the correlation between the food frequency questionnaire and the biochemical iron and vitamin A status of the subjects.

A non-parametric statistical test - Spearman's rank correlation was used to determine the correlation between the food frequency questionnaires whilst the Limits of agreement test was also used to determine the relationship between food frequency and weighed food records.

Altman and Bland proposed the method of limits of agreement to determine absolute reliability by determining the error interval [130]. A correlation value does not reveal any detail on the range of the data set, and is thus unable to detect systemic bias. In comparison, the limits of agreement method provide information on the range of values of the data, which can then be used to plot a Bland-Altman plot. A Bland-Altman plot is the plot of the individual subject differences between the tests plotted against the individual means. From the plot, it can be said that should there be another observation, it has a 95% probability that the difference between any 2 tests will lie within the limits of agreement. This method has a greater potential of detecting systemic bias in a method than a correlation. (See Appendix J)

4.42 Determining the Availability of Dietary Iron

There is a need to distinguish between the iron that is available in foods and the iron that is actually absorbed in the small intestine for the body's use. Food Works [127] provides the food nutrient composition information for the meals but it does not consider the absorption and bioavailability of iron from meals. Monsen et.al (1978) [131] developed a model to estimate the amount of available iron from the meals that were eaten. This model is based on a semi-logarithmic transformation on the amount of iron present in the body. With the availability of non-haem iron plotted against the absorption of non-haem iron, the percentage of absorbable iron can be determined. Using the percentage of absorption, the approximate amount of iron can be estimated by grading them into three meal categories – low, medium and high availability meals (see appendix) for non-haem iron. A child with no iron stores is expected to absorb approximately 35% of haem iron, when ingesting meat, approximately 5% from a low availability meal, 10% from a medium availability meal and 20% from a high availability meal.

Factors taken into consideration when using this equation are the amount of meat eaten, amount of ascorbic acid (Vitamin C) and the iron stores. The calculation for the absorbable iron was carried out by identifying the amount of iron in each food in a meal. The meal was then classified into a high, medium or low iron availability meal and the iron value for each individual food was

obtained from the Food Works 2.0 [127] programme. The absorbable iron was calculated by using the percentages from the availability meal in Microsoft Excel 97 [124]. An example of the calculation can be seen in the Appendix I.

4.43 Regression Analysis

Multiple regression analysis of variance was undertaken to determine the sources of variance of Iron and vitamin A deficiency, taking into account ethnicity and mean daily iron and vitamin A intake. Further multiple regression analysis of variance with the estimated bioavailable iron, and a transformation of the log of mean daily iron intake was undertaken.

Logistic regression was then carried out to determine the odds ration and the 95% Confidence interval for the dependent variable being iron deficiency and iron deficiency anaemia, and the confounding variables that were included were mean daily iron intake and ethnic group of the child.

All statistical analysis was carried out in SAS for Windows system (ver 6.12)[125].

Results

5.1 Characteristics of Subjects

A total of 30 children participated and completed this validation study. The response rate was 56% (30 from 52), with the remaining children falling into two categories. One group of children were ineligible to be involved due to the differential sampling that was applied to the sample in order to obtain a stratified sample of equal numbers of children from the main ethnic groups in Auckland. The other group of children not enrolled were ones in which the care-givers declined to be involved. The table below (Table 5.1) summarises the characteristics of the subjects of this study.

Table 5.1 Characteristics of Subjects

Characteristics		N (%)
Age		14 months sd. 4.95
Ethnic Group		
	European	7 (23.3%)
	Maori	6 (20%)
	Pacific Island	11 (36.7%)
	Other	6 (20%)
Gender		
	Male	12 (40%)
	Female	17 (60%)

Forty percent (12) of the sample were males, and the remaining 60% females (18). The age of these children ranged from 6 months – 24 months (inclusive). The mean age of the children was 14 months (S.D. 4.95). The children's ethnicity was categorised into four groups, with the care –giver specifying the preferred ethnic group. These four groups are European, Maori, Pacific Island and Other ethnic group.

The mean combined income per annum of the households in this study sample was between $15, 001 to $20, 000, with the largest percentage (39%) of families (n=11) in the $10,001 - $15,000 per annum category. Despite random sampling, the statistics show that this study sample mainly consisted of low to middle class income families.

Table 5.2 Anthropometric Characteristics of the Study Sample

Characteristics	Mean for Boys n=10 (Std Dev)	Z Scores	Mean for Girls n=17 (Std Dev.)	Z scores
Age (months)	15.36 (6.10)		12.89(3.37)	
Birth Weight (kg)	3.40 (0.39)		3.30 (0.33)	
Height (cm)	77.28 (5.66)	- 0.486	73.9 (4.14)	-0.266
Weight (kg)	10.18 (1.94)	- 0.397	10.47 (1.32)	0.508
Triceps (mm)	6.72(2.33)		9.39 (1.76)	
Biceps (mm)	8.41 (1.14)		8.65 (1.88)	
Subscapular (mm)	7.19 (1.20)		7.95(1.63)	
Arm Circumference (cm)	16 (1.27)		15.44 (1.57)	

Table 5.2 describes the anthropometric measurements taken from the children. A comparison of the mean height and weight measurements with the Z scores show that the boys' height and weight and the girl's height in this study lie between –0.5 and 0 standard deviations of the mean in New Zealand children. In comparison, the girl's weight was 0.508 standard deviations from the mean the same population.

5.2 Iron Status of Subjects

The iron statuses of the children were obtained from blood samples that were taken during the interviews. The table below describes the biochemical status of the iron parameters measured by ethnicity.

Table 5.3 Biochemical Iron Status by Ethnicity

Parameter (Cut off Values)	n	European (Range)	n	Maori (Range)	N	Pacific Island (Range)	n	Other (Range)
Hb g/L (<110)	7	123.43 (112-137)	6	113.17 (100-123)	10	105 (77-120)	5	122 (106-130)
SF µg/L (<10)	7	22.71 (13-32)	5	18.4 (13-27)	9	22 (2 –50)	6	35.33 (5-111)
MCV fl (<72)	7	78.43	5	75.6	10	72.1	6	76
MCH pg (<24)	7	26.57	6	24	11	23.27	6	25
Iron Binding µmol/L	7	63.71	6	73.83	10	71.6	6	69.83
PCV	7	0.36	5	0.358	10	0.328	6	0.363

Hb = Haemoglobin, MCV = Mean corpuscular volume, MCH = mean corpuscular haemoglobin, SF = Serum Ferritin, PCV = Packed Cell Volume.

The means for Hb in all the ethnic groups were above the cut off values, with the exception of the Pacific Island ethnic group. This indicates that half of the Pacific Island children in this study had Hb levels below the cut-off values. Of the Haemoglobin values for the Pacific Island children, Hb values ranged from 77 - 120g/L. In contrast, European children had haemoglobin values above the cut-off values, indicating a healthy iron status within this ethnic group.

The mean serum ferritin levels were also above the 10µg/L cut off in all ethnic groups. However, the range of serum ferritin levels of the Pacific Island and Other children are below the cut –off values, indicating that iron deficiency anaemia is present in these two groups. The MCV means of all the ethnic

groups were above the cut off value of 72fl, although, the mean for Pacific Island children at 72.1fl just made the cut off values.

Table 5.3 below summarises the incidence of iron deficiency (%) in this study population sample in the four different ethnic groups. The highest incidence of both iron deficiency and iron deficiency anaemia was found in the Pacific Island children with iron deficiency at 40% and iron deficiency anaemia at 30%. Of the Maori children, 33% were iron deficient and 16.67% were iron deficient with anaemia.

Table 5.4 .Incidence of Iron Deficiency and Iron Deficiency Anaemia by Ethnicity

Ethnic Group (n)	Iron deficiency n (%)	Iron deficiency anaemia n (%)
European (7)	0 (0)	0 (0)
Maori (6)	2 (33.33%)	1 (16.67%)
Pacific Island (10)	4 (40%)	3 (30%)
Other (6)	1 (16.67%)	1 (16.67%)

5.3 Vitamin A Status of Auckland Infants

Fourteen percent (4 children) were found to have a vitamin A deficiency. The following table shows the biochemical status of vitamin A values obtained from the blood samples, and the range of values of these parameters.

Table 5.5 Biochemical Vitamin A Status by Ethnicity

Parameter (Cutoff value)	n	European (range)	n	Maori (range)	n	Pacific Island (range)	n	Other (range)
β-Carotene (μmol/l)	6	1.67	6	0.58	10	0.54	6	1.42
Vitamin A (μmol/l) (0.7 μmol/l)	6	1.37	6	1.017	9	1	6	1.467

Vitamin E (μmol/l)	3	46.77	3	29.5	7	29.86	2	23.5

Table 5.6 shows the incidence of vitamin A deficiency (%) by ethnicity and highlights that this deficiency was found to be present in Maori (18%) and Pacific Island infants (29%).

Table 5.6 Incidence Vitamin A Deficiency by Ethnicity

Ethnic Group (n)	Vitamin A deficiency n (%)
European (6)	0 (0)
Maori (5)	(18%)
Pacific Island (11)	(29%)
Other (6)	(0)

5.4 Food and Nutrient Intake Estimations

5.41 Food frequency Questionnaire

The food frequency questionnaire was completed by the care-givers of 28 children (93%). Most of the questionnaires were fully completed.

The first part of the food frequency questionnaire consisted of questions regarding the general eating patterns of the child. From these questions, 92.9% (26) children ate a variety of foods. Of the two remaining children, one child avoided eggs, but ate everything else, and the other child did not eat meat and meat products. Sixteen children (57.2%) ate more than two servings of fruit a day, and the same number of children ate 3-4 servings of vegetables per day. Eleven children (39.3%) had one or two servings of fruit a day, and one child (3.6%) did not eat any fruits. Most of the fruits that were eaten were mainly eaten with the morning meal or afternoon meal, and as snacks. Nineteen children (67.9%) had more than 2 servings of cereals a day whilst eight children

ate only one serving of cereal, and one child did not eat cereals at all. Only 50% of the children had 1 or more serving of bread a day, with two children not eating bread at all. Most of the bread given to the children was eaten as snacks, whilst about 25% of the bread was eaten as the morning or afternoon meal.

The mean food frequency patterns of this sample can be seen in Table 5.4. The study population was ranked and grouped into four groups of frequency consumers (Less frequently = LF, St/M = several times per month, St/W = several times per week, O/Std = One or more times a day).

Table 5.7 The Food Frequency Patterns per percent of Children.

Food	L/F (%)	St/M (%)	St/W (%)	O/Std (%)
Cow's Milk	24.07	3.70	9.26	62.96
Breast	75.00	0.00	0.00	23.21
Infant Formula	73.21	0.00	0.00	25.00
Baby food	67.86	5.36	14.26	12.50
Bread	17.86	10.71	12.50	58.93
Cereal	17.86	5.36	17.86	58.93
Red Meat	3.57	17.86	66.07	12.50
Liver	85.71	5.36	8.93	0.00
Chicken	14.29	17.86	66.07	1.79
Fish	26.79	39.29	33.93	0.00
Spinach	46.43	28.57	21.43	1.79
Carrot	14.29	21.43	58.93	3.57
Citrus Fruits	16.07	17.86	58.93	7.14
Apricot	91.07	1.79	3.57	1.79
Banana	12.50	10.71	66.07	10.71
Citrus Juices	42.86	16.07	23.21	16.07
Noncitrus Juices	44.44	20.37	12.96	22.22
Tea	75.00	3.57	16.07	5.36
Margarine	46.43	3.57	10.71	39.29

This table shows that the children in this sample are fairly high consumers of cow's milk, bread, and cereals; moderate consumers of meat, chicken, carrot and fruits; and low consumers of baby food, infant formula, breast milk, liver, apricots and tea. In general, the frequency of consumption of vegetables and fruits were significantly lower, with the exception of bananas.

Information from the food frequency questionnaire was then compared to the 4 day weighed food records in order to determine the validity of this dietary assessment tool. This specific food frequency questionnaire correctly placed an average of 25% (7 of 29) of the children. The sample was split into quartiles of high, medium, low and very low consumers of foods high in iron and vitamin A. This was then ranked against the quartiles of high iron and vitamin A intake from the weighed food records.

The validation of the food frequency questionnaire correctly placed between 0% and 66% in the respective quartiles. The ranking high consumers of red meat from the food frequency questionnaire and consumers of foods high in iron from the weighed food records correctly placed 50% (3 of 6) in the lowest quartile, 33% (2 of 6) in the second lowest quartile, and 33% in the highest quartile. For breakfast cereals, 66% of the lowest quartile along with 17% of the remaining three quartiles was correctly placed.

There was difficulty in obtaining accurate results of the quantitative aspect of the food frequency questionnaire. In general, this section of the questionnaire was not completed by 89.7% (26) care-givers. The main reason was the difficulty in remembering the average portion sizes of the foods the child was given to eat. Due to insufficient results in this section, there was no analysis of this section.

5.42 Weighed Food Records

Four day weighed food records were obtained from 29 of the children. Five diaries were incomplete, with care-givers providing one, two, or three day weighed food records, and others where the child was sick, providing data that was not representative of the child's normal eating patterns.

Table 5.6 shows the mean daily nutrient intakes as obtained by the four day weighed food records. Because there is considerable differences in younger and older infant's dietary habits, the group was split into two groups. The separation of the age groups will provide a more meaningful review of the mean nutrient intakes of this sample. Children under the age of 14 months were grouped as Age group 1 and the remaining half of the study population were grouped as Age group 2, where the children's ages ranged from 15 months to 24 months.

Table 5.8 Mean Daily Nutrient Intake of Children.

Nutrient	Age Group 1 Mean 9.33mths	RDI values	Age Group 2 Mean 18 mths	RDI
Energy Intake (kJ)	3882.30(1498.65)	415 kJ/kg 4531.8 kJ	4528.48 (1962.93)	4800-5000kJ
Mean Weight (kg)	10.92 (1.39)		9.81(1.5)	
Protein (g)	33.08 (17.81)	14	85.90 (125.35)	14-18
Protein(%Total energy)	15.94 (3.61)		17.48 (3.17)	
Fat (% Total energy)	31.26 (5.86)		34.40 (6.37)	
Carbohydrate (% Total energy)	47.35 (8.31)		45.06 (8.80)	
Iron (mg)	13.44 (18.98)	9	7.11(4.39)	6-8
vitamin A (μg)	435.71 (224.31)	300	1109.43 (1994.0)	300
Vitamin C (mg)	96.29 (120.44)	30	70.02 (46.41)	30

The average values of the main nutrient obtained from the Weighed food records were higher for the second age group (Age group 2). The amount of protein that the children in Age group 2 (85.90g) recorded to have eaten was two times greater than that of the children in age group 1 (33.08g). Children in age group 2 (over 14mths) were eating more solid food. The older children had a greater intake of vitamin A, whilst the younger children had a greater intake of foods high in iron and vitamin C. The standard deviations in each of these values are very high (greater than the means), indicating a large variation between the children of each group as well as within the children of the particular age group.

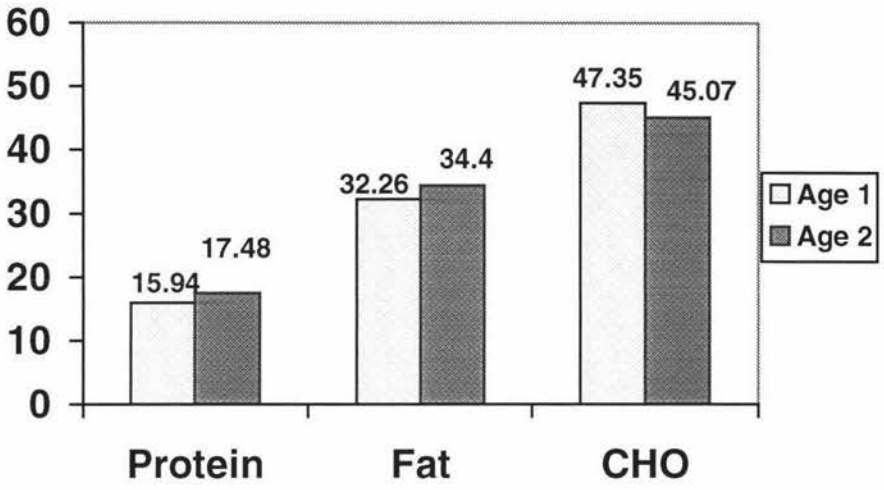

Figure 5.1 The Percentage Energy Obtained from the 4 day Food Records

The proportions of percentage energy obtained from the three macro-nutrients were consistent with the RDIs. The children in Age group 2 had a fractionally higher proportion of their energy obtained from protein and fat, and a slightly lower proportion of energy from carbohydrate. Comparing the values above with the RDI's for children in this age group, it is clear that these children were receiving adequate amounts of nutrients, with the exception of energy intake, which was significantly below the RDI's for this age group.

5.5 Availability of Iron

The values of iron intake from the 4 day weighed food records are consistent with the RDI's for children of this age group. As mentioned earlier in the Literature Review (See section 2.13), the bioavailability of iron differs between haem iron and non-haem iron. The values presented in the table above do not provide an accurate representation of how much iron is actually absorbed and utilised. It is misleading to assume that children from this study obtained an average of 9.64mg to 11.35mg of iron from their daily diet.

The calculation of the amount of available iron in a meal was calculated by separating the haem and non-haem iron sources, and taking in to account known enhancing substances Vitamin C (See section 2.13). Individual iron stores were also taken into account, since absorption increases as iron stores are depleted. This method for determining the available iron has been used in

adult men and women, but not in children. From the ferritin stores in table 5.4, it can be seen that 60% of the children have ferritin levels below the cutoff mark. The Monsen equation for calculating the availability of iron from food assumes a logarithmic relationship, with iron stores at 0, 250mg and 500mg. In this study, the subjects are children, and with 25% being iron deficient it has therefore been assumed iron stores are negligible. With this assumption, the absorption of haem iron is 35% and the absorption of non-haem iron is 5% (Low availability meal), 10% (Medium availability meal) and 20% (high availability meal) respectively. The factors for estimating the percentage absorption for dietary iron, as well as a sample of a day's calculation of absorbable iron can be seen in Appendix I.

From the calculation of the available iron from the weighed food records, the values that were presented in table 5.9 for iron intake are significantly higher. The calculations that were undertaken to determine the available iron from Monsen's equation are illustrated in Table 5.9 and in Appendix I. The mean available iron for this study population was 0.736 with a standard deviation of 0.44. Values ranged from 0.126 to 1.79.

Table 5.9 Mean Iron Intake and Available Iron by Ethnicity

Ethnic Group (n)	Mean Iron Intake from WFR (SD)	Mean Iron Available † (SD)	Percentage Absorbed
European (6)	6.962 (4.70)	0.669 (0.432)	9.61%
Maori (4)	8.524 (2.54)	0.715 (0.214)	8.39%
Pacific Island (10)	13.748 (21.40)	0.880 (0.492)	6.4%
Other(6)	10.007(11.874)	0.554 (0.463)	5.54%

† Monsen et.al (1978)[131]

Table 5.9 demonstrates that there is a significant difference between the mean daily iron intake form the weighed food records and the available amount of iron from those foods that is absorbed. The differences in the percentage of estimated absorbed iron varies with the different ethnic groups.

5.5 Statistical Relationships

5.51 Correlation between the Food Frequency Questionnaires

In order to determine the reproducibility and reliability of the food frequency questionnaire, Spearman's rank correlation was used. Of the 54 foods that were contained in the questionnaire, 44 of the foods had a correlation of 0.5 or higher (81.5%). Correlation values ranged from 0.152 to 1. Maori bread, Paw Paw, Tammarillos, Soya milk, Cod Liver oil, Vitamin C, iron and general supplements had the highest correlation value of 1, while the lowest correlation values were 0.152 for chicken, 0.282 for mixed vegetables, and 0.236 for mangoes (Table 5.10).

Table 5.10 Correlation Values of Some Foods from the Questionnaire

Food	Spearman's Correlation Value (r)
Red Meat	0.7087
Liver	0.413
Chicken	0.152
Breakfast Cereals	0.583
Spinach	0.637
Babyfood	0.629
Carrots	0.505
Cow's Milk	0.683
Margarine	0.7095
Breastmilk	0.996
Infant Formula	0.891

5.53 Limits of Agreement [130]

To determine the validity of the food frequency questionnaire, comparison was made with the four day weighed food records. Quartiles were used to rank the various consumers of the four day weighed food records against the consumers of the food frequency. However, this did not provide sufficient proof of the validity of the food frequency questionnaire. Comparing food groups against nutrients resulted in fairly poor correlation except for red meat, breast milk, and

infant formula. This indicates that the food frequency questionnaire may have a degree of specificity.

The Limits of Agreement method by Bland and Altman tests for the reliability of the method by comparing the differences of the individual means against the averages of each method. A Bland-Altman plot or a scattergram of the differences against the mean provides a better illustration of the difference that may exist between the two methods.

The Limits of Agreement method was used to test for the reliability of the food frequency questionnaire, by comparing the differences between the first food frequency questionnaire and the second food frequency questionnaire. This method was also used to test for the validity of the food frequency questionnaire by comparing the differences between the food frequency questionnaire and the weighed food records.

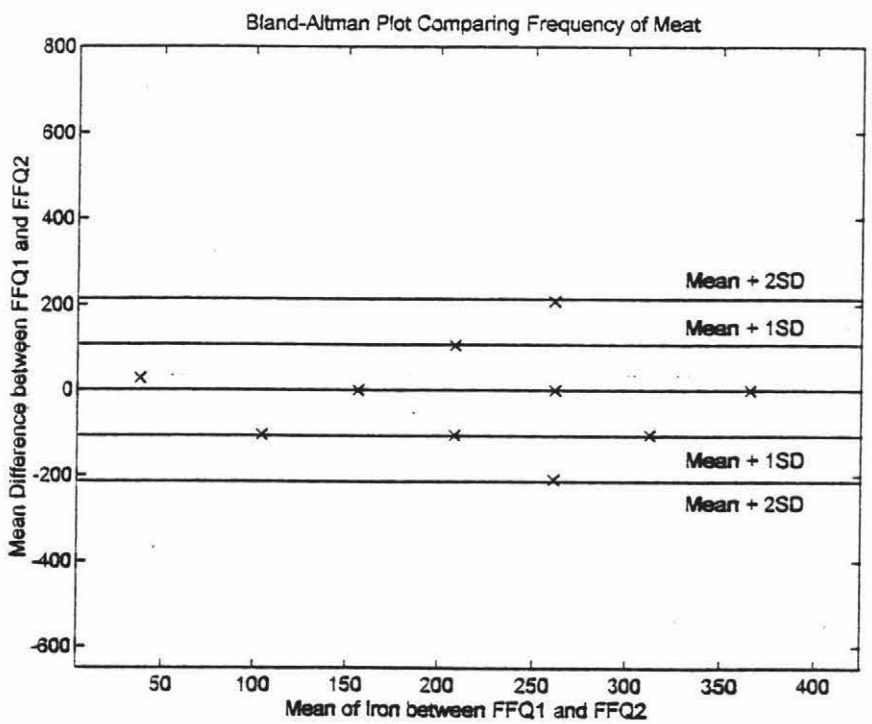

Figure 5.2 Bland-Altman Plot of the Frequency of Red Meat Consumption

The previous scatter gram in the previous page is the plot of the differences in the frequency of meat between the first and second food frequency questionnaire. From this scatter plot, all but three of the values fall between one standard deviation of the mean (107.05).

A similar scatter gram can be plotted of the differences between iron intake of the four day weighed food records and the frequency of meat in the first food frequency questionnaire.

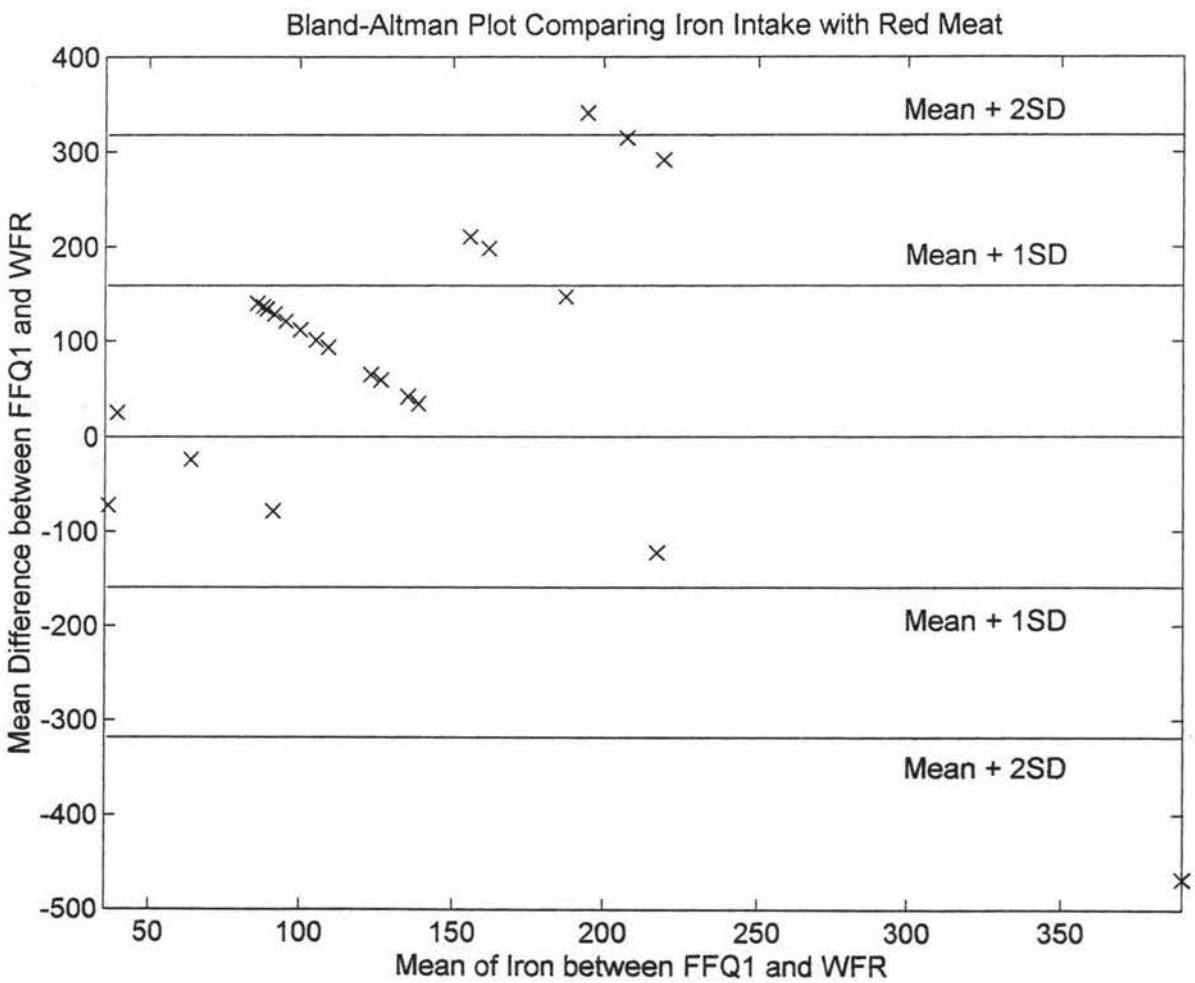

Figure 5.3 Bland-Altman Plot of Iron Intake and the Frequency of Red Meat Consumption

Most of the points in the plot lie within one standard deviation (168.05) of the mean, with no outliers that lie outside 2 standard deviations of the mean. This test was done for foods of specific interest because of their high iron, Vitamin C and vitamin A content. Other plots can be seen in the Appendix I.

5.54 Regression Analysis of Dietary Data and Biochemical Data

5.541 Multiple Regression Analysis of Variance

Multiple regression analysis was used to determine the sources of variance on this data set, with the primary aim of predicting the sources of variance that contribute to the child being iron deficient, or iron deficient with anaemia. Variables that were taken into consideration were the mean nutrient intake, biochemical iron status and ethnicity. In the regression analysis that was undertaken, iron deficiency and iron deficiency anaemia was defined by biochemical status (See section 4.341).

The F value (3.73) of the regression analysis, with iron deficiency as the dependant variable demonstrated that there was an association between iron deficiency, mean iron intake and being a Pacific Island child. No association was seen with the other ethnic groups. The probability values demonstrated that being Pacific Island contributed significantly ($p<0.05$) to the variance in iron deficiency, taking into account mean iron intake. There was no significance in the probability values in the remaining three ethnic groups. This regression accounted for 25.3% of the total variation in Pacific Island children, in comparison to the 13%-18.3% range in the other three ethnic groups.

There was no significant association in the regression analysis of iron deficiency anaemia, mean iron intake and ethnicity. The F values of all four groups were marginally greater than 1 indicating a very weak association. Probability values of all four ethnic groups were not significant at all levels. This regression accounted for 14.2% - 20.7% of the total variation in each of the different ethnic group.

A similar regression analysis of variance was also undertaken with the dependent variable being iron deficiency. The two variables taken into consideration were the mean available iron derived from Monsen et.al (1979)[131] and ethnicity. There was also a significant association between iron deficiency, estimated available iron and ethnicity, especially in the Pacific Island ethnic group. This was seen in the significant difference in F values as well as the Pr> |T| values for Pacific Island children. The probability of being Pacific Island is significant ($p < 0.01$) in contributing to the variance in iron deficiency, taking into account the estimated mean daily iron intake. This regression accounted for 20.9% of the source of variation in the Pacific Island children and between 0.7 and 7.9% of the variation in the remaining 3 ethnic groups.

The results of a multiple regression analysis of variance with the dependent variable being iron deficiency anaemia, with the estimated available iron from Monsen et.al (1979), and ethnicity revealed a significant association in Pacific Island children ($p<0.01$) contributing to iron deficiency anaemia. This association was not found in the remaining 3 ethnic groups. The regression accounted for 26.3% of the total variation in Pacific Island children, whilst the range was between 8.9% to 15.3% in the remaining three ethnic groups.

Table 5.11 Multiple Regression of Haemoglobin with Mean Iron Intake and Ethnicity

Ethnic Group	European	Maori	Pacific Island	Other
F Value	7.62	4.4	14.02	5.98
Estimate	11.255	-0.088	14.684	-8.733
Pr > |T|	0.04	0.9896	0.0012	0.146
Total variation (R_2)	0.409	0.2855	0.5604	0.352

Table 5.11 illustrates the multiple regression analysis of variance undertaken with haemoglobin being the dependent variable, and mean iron intake and ethnicity being the other confounding variables. The F values for the analysis of variance demonstrated a strong association between haemoglobin values and iron intake and being Pacific Island. Although the values for the European children show that there was a strong association between the variables

described above, there is a protective effect on these children as they have the lowest risk of being iron deficient. The probability values of Pacific Island children in this regression demonstrated that being Pacific Island contributes significantly to the variance in haemoglobin levels ($p<0.001$). The R_2 for Pacific Island children demonstrated that ethnicity, especially being Pacific Island contributed 56% of the source of variation with haemoglobin being the dependent variable in this analysis. The R_2 values for the remaining three ethnic groups was not significant, accounting for 28.6% - 40.9% of the source of variation in this regression.

Further regression analysis undertaken with haemoglobin as the dependent variable, and with confounding variables being estimated iron calculated from Monsen's model, and ethnicity, showed that there was a significant association only present in Pacific Island children. Being Pacific Island contributes significantly at the 0.001 level to the variance in haemoglobin levels.

Table 5.12 Multiple Regression of Haemoglobin with the Log of Mean Iron Intake and Ethnicity

Ethnic Group	European	Maori	Pacific Island	Other
F Value	4.54	1.92	10.21	2.71
Estimate	12.282	-2.749	16.081	-7.896
Pr > |T|	0.428	0.709	0.0010	0.2347
Total variation (R_2)	0.292	0.149	0.481	0.198

The mean iron intake results were transformed to the natural log. This confirmed the same result that was found in the multiple regression analysis of variance of haemoglobin, the log of mean iron intake and ethnicity. Table 5.17 demonstrates the F values of the different ethnic groups, with the Pacific Island subjects having the most significant association of 10.21. The probability values demonstrate that being a Pacific Islander demonstrates significantly ($p <0.001$) to the variance in haemoglobin levels, taking into account the log of mean daily iron intake. The total variation in the ethnic groups ranged from 14.9% to 48.11%, with Pacific Island children having the significant R_2 value.

Data analysis was also undertaken to determine the association between vitamin A intake and serum retinol levels and ethnicity. However, there was no association between vitamin A intake, vitamin A status and ethnicity. The F-values of all the groups did not demonstrate any significant associations. The highest F-values was for Pacific Island children, which was 2.39, demonstrating a low association between the variables. The probability values of this regression were not significant at all levels for the three remaining ethnic groups, with a range between 0.1848-0.903. Being Pacific Island contributed significantly ($p<0.05$) to the variance in vitamin A deficiency. The total variation value accounted for in this regression ranged from 6.7% (European) to 44.8% (Maori) A logarithmic transformation did not alter the level of significance in both the F-values and the probability values.

5.542 Logistic Regression

A logistic regression was carried out to determine the confounding variables that may have influenced the results of this study. The other variables included in this multiple logistic regression were mean daily iron intake and ethnicity. There was no significant association between these variables in the logistic regression in all ethnic groups. The probability values of the logistic regression analysis ranged from 0.146 to 0.265.

Table 5.12 95% Confidence Interval for the Odds Ratio for Iron Deficiency with Ethnicity and Mean Iron Intake

Ethnic Group	European	Maori	Pacific Island	Other
Parameter Estimate	0.0462	0.0495	0.0522	0.0531
Standard Error	0.0462	0.0380	0.0525	0.0393
95% Confidence Interval	0.903 –1.37	0.944 - 1.33	0.890 - 1.43	0.946 - 1.350
Odds ratio	1.046	1.049	1.047	1.052

The odds ratio demonstrated the probability of having iron deficiency taking into account the different ethnic groups and the mean iron intake in this sample

of children. For example, from the odds ratios in table 5.18, the odds of being Pacific Island, and having iron deficiency is 1.047, mean iron intake is taken into consideration. The odds ratios for all four ethnic groups are approximately one, indicating that ethnicity and mean iron intake are not significant confounding variables in predicting iron deficiency. The 95% confidence intervals for the four ethnic groups range from 0.89 to 1.43.

Table 5.13 95% Confidence Interval for Iron Deficiency Anaemia with Ethnicity and Mean Iron Intake

Ethnic Group	European	Maori	Pacific Island	Other
Parameter Estimate	0.0453	0.0480	0.0458	0.0509
Standard Error	0.0351	0.0353	0.0411	0.035
95% Confidence Interval	0.947 - 1.30	0.952 – 1.310	0.923 - 1.34	0.965 - 1.310
Odds ratio	1.046	1.049	1.047	1.052

Table 5.19 demonstrates the odds ratio for iron deficiency anaemia, taking into consideration ethnicity and mean iron intake from the weighed food records. The results are similar to that for the odds ratio for iron deficiency in this sample group. For all four ethnic groups, the odds ratio was approximately one, indicating that ethnicity and mean iron intake are not significant confounding variables in predicting iron deficiency anaemia. The 95% confidence interval for predicting iron deficiency anaemia ranges between 0.923 to 1.34 in the four ethnic groups.

Discussion

Vitamin A and iron deficiency are two of the most prevalent micro-nutrient deficiencies in children of the developed and developing countries. Deficiencies in either of these two micro-nutrients have serious consequences, particularly for children in their early developmental years. There is increasing evidence to show that IDA can lead to delays in motor development, dexterity and lower IQ levels [38]. Vitamin A has been shown to reduce the severity of measles in young children, and also prevent mortality from infection [58]. These consequences from insufficient intakes of these two micro-nutrients highlights the need to determine dietary intakes of young children in order to determine the extent of these deficiencies in the New Zealand population.

The use of accurate dietary assessment methods to determine the intake of these two micro-nutrients is crucial in identifying the extent of these deficiencies because the main source of Iron and vitamin A comes from the food children eat. Undertaking dietary studies in children poses several difficulties, particularly in young children. This pilot study addressed some of those difficulties, by testing and validating a food frequency questionnaire specific to these two micro-nutrients. However, internationally validation studies to determine the effectiveness and usefulness of a dietary method in this age group are limited, thus giving little opportunity to compare this study with other studies of similar aims. Therefore, the findings of this study are limited to that of the findings of this study, and can be classified into three groups – methodological and sampling issues, dietary aspects of the study subjects and research implications.

6.1 METHODOLOGICAL ISSUES

6.11 Recruitment and Interviewing Issues

The difficulty of using the cluster sampling technique lies in finding the sample within a population. The time and cost of this method needs to be taken into consideration, although the research benefit of using this method is clear. In this study, it was hoped that a child from each start point would be enrolled in the study. However, this was not the case, as it took more than 3 start points to enrol one child into the study. The start points included the industrial areas, and suburbs that did not have a high percentage of residential dwellings. Towards the end of the study, there was difficulty in identifying and enrolling adequate numbers of Maori children. Attempts to continue with this sampling technique did not bring any further Maori children into the study within the fixed timeline. In comparison, the response rate of the National Survey of Diet and Nutrition in the UK was 88% for the interviews and 81% for undertaking the weighed food records. The lower response rate in this study could be the result of several factors – firstly the age group of the children was at the lower end of the scale of the UK study. This limited age group would have meant a smaller pool of subjects to recruit for the study. Secondly, the random sampling of the study population may have restricted the response rate. Finally, the response burden of completing a four day weighed food record diary, two food frequency questionnaire, anthropometric measurements and blood samples and a general questionnaire may have been a key factor in the low response rates received.

The percentage of care-givers that chose not to participate in this study (44%) highlights the high respondent burden. Reasons given for not wanting to participate in the study included lack of time, being too busy and also uneasiness with the blood test. There was a greater hesitation in Maori and Pacific Island mothers to enrol their children in the study. A possible explanation of this is the reluctance and embarrassment of disclosing the low income that they have which impacts on the food availability for their family.

The mean combined income of the families involved range from $15 001-$20 000 illustrating that this study sample consisted of mainly low to moderate income families. Although the cluster sampling was applied in order to obtain a true random sample, this method failed to obtain a randomly distributed sample of all ethnic groups and also from all income levels of the population. Two possible conclusions can be made. One conclusion is that in small numbers, this method of obtaining a true random sample is difficult and very inaccurate. The other conclusion is that most of the families in the Central Auckland District come from low to moderate income families, earning between $15 001 - $20 000 of combined income.

The home visit by the Phlebotomist, combined with the play therapy and the EMLA cream that was applied, proved invaluable. The blood test that was taken was undertaken with no great difficulty, with the child being more distressed at being held down than with the needle prick. There were some difficulties in finding the veins for some of the children, but overall, blood samples were obtained for all the children in the study. Another difficulty that was encountered was the volume of blood that was required was difficult to obtain. It required more than one source, which was very difficult to obtain from young children as it meant using the veins of both arms of the child. Obtaining the co-operation of the child on the second hand to collect the remaining blood was much harder.

Although it has been reported that 7 day food records provide the best estimates of an individual's nutrient intake, the practicalities of carrying this out in children has meant that this was not possible. A compromise was made, based on the findings of the National survey in UK, that four-day dietary records provided acceptable and accurate results on the nutrient intake of a population of children[110].

This study was part of the pilot study undertaken to validate the food frequency questionnaire for use in Auckland children. The small numbers of

this sample needs to be taken into consideration when analysing and interpreting the data of this study.

6.21 DIETARY ASPECTS

The main aim of this study was to validate the use of the food frequency questionnaire for specific use in determining the nutrient intake, which in turn will be used to determine the causes of iron deficiency and vitamin A deficiency in Auckland children. From the results of the data analysis, it is clear that iron deficiency, iron deficiency anaemia and subclinical vitamin A deficiency exists in the population.

The average nutrient values were higher for the older children in age group two. This is consistent with the fact that the older children have greater energy and nutrient needs because of their size and activity. The mean daily nutrient intake of the children in this study sample is consistent with the Recommended Daily Intakes (RDI) for children in this age group, with the exception of the energy intake. In both age groups, the energy intake (3882kJ for Age group 1, and 4528.48 kJ for Age group 2) was significantly lower than the RDI's (4531.8kJ for Age group 1 and 4800-5000kJ for age group 2). The National Diet and Nutrition survey in UK had similar findings of energy intakes lower than the EAR's (similar to RDI in New Zealand) and significantly higher protein intakes.

The protein intake that was reported in the NDNS survey reported that only 1% of the children in the respected age groups were under the RNI/RDI for their age groups. Children in age group 2 had a fractionally greater amount of energy from protein, accounted for by the solid food that these children were eating.

The difference between the estimated energy intake and the RDI could be due to the fact that these children are still consuming a significant amount of breast milk or infant formula rather than solids, or due to the low consumption of "high" energy food. The significantly high amount of protein in both age-groups supports the latter conclusion. There is almost a three-fold increase in the difference between the protein intake that was obtained from the weighed

food records with the RDI's. A possible explanation for such a high protein intake could be the high consumption of milk – cow's milk, and infant formula as a drink. A significant proportion of the weighed food diaries reported that the child drank up to 4 bottles (250ml) of cow's milk diluted with water a day.

The graph (figure 5.1) shows that the main source energy for children in age group 1 was carbohydrate (47.85%). Similarly, children in age group two had a fractionally lower proportion of carbohydrate as energy at 45%. This can be expected in children, particularly young children in this study as a major part of their diets is milk – breast milk, infant formula or cow's milk. The carbohydrate found in milk primarily consists of lactose. Similar proportions of the macro-nutrients were reported in the National Diet and Nutrition Survey in the UK. Protein accounted for 13.6% of the total energy, fat with 36.4%, whilst carbohydrate accounted for 49.9% of the total energy in children aged 1 ½ - 2 ½ years. These proportion values are closer to the children in age group 1 of this validation study, although the ages of children from the UK are closer to the children in age group 2[110].

The variation between individuals in dietary intake from the weighed food records is very large. Standard deviations in both age groups confirm this large variation, with some standard deviations greater than the means. This is comparable with other studies that have demonstrated large variation between individuals as well. In the National survey of Diet and Nutrition[110], the average daily energy intake ranged from 2634kJ (lower 2.5 percentile) to 6596kJ (upper 2.5 percentile) for children aged between 1 ½ -2 ½ years. The range of average daily energy intake in this sample was 1342kJ to 8921kJ in children aged between 6 months and 24 months. The larger range in the study sample may be due to the small numbers of the study sample.

Despite the heavy respondent burden, 29 weighed food records were returned. This is a high compliance rate, considering the difficulties that these care-givers would have faced. Similar response rates were found in the National Diet and Nutrition survey in the UK where 81% completed four-day weighed food

records. A possible explanation for the slightly higher co-operation in this study can be attributed to the telephone calls the interviewer made to remind the care-givers and to sort out difficulties and misunderstandings. The eating out diary was not used by most of the care-givers. Only 2 eating out diaries were completed and returned for analysis, indicating that either these children obtained majority of their meals at home, or the specific days that were given to the care-giver to weigh the children's foods were days that the children remained at home.

The difficulties in the use of the 4 day weighed food records as a method were primarily in the accuracy of the weighing. Possible error sources include incorrect use of the weighing scales, and especially in the amount of food that was actually consumed by the child. The difficulty that children of this age group pose is that they eat so little, but frequently, so weighing is burdensome. Toddlers are also very mobile at this age and a comment made by several mothers was that they were not certain that the child ate the piece of fruit or biscuit or if the child might have put it down. The tare mechanism in the weighing scale was understood and used by the majority of care-givers, with only one diary that had large amounts of food, consistent with failing to press the tare button. However, the results from this study indicate that this method can be used in children of this age group.

From the record diary, there was a column given for the weight of the leftovers to be recorded. This column was not filled out correctly by most of the care-givers, if it was filled out at all. Instead of indicating amounts of food leftover, care-givers described what was left over in an abstract way, leaving no opportunity to quantify what remained. Several mothers commented that it was too difficult to weigh the leftovers, with more food on the child and the floor itself, rather than on the plate.

From the food frequency questionnaire, it could be seen that foods most popular among this age group were bananas, infant foods (custard and instant desserts), and cow's milk as a drink, as would be expected.

Results from the food frequency questionnaire demonstrate that this can be an effective tool to rank the quartiles in consumption of foods with the nutrients of interest. The correlation values between the two questionnaires were lowest for foods that were consumed frequently, like chicken (r = 0.152), whilst high for foods that were not consumed or eaten very rarely – like soy milk (r = 1). A possible explanation could be that unfamiliarity is associated with memory, meaning that care-givers are more likely to remember what they have not given their child, than what the child regularly eats and the frequency of eating that particular food.

Although the ranking of quartiles of consumption between nutrient intake and high consumers of foods high in that specific nutrient intake was unacceptable in this study, the numbers in this study may have limited the effectiveness of this method of analysis. Further larger studies can confirm if this is still an effective tool for ranking the consumers of high nutrient intake with foods high in the specific nutrient. Undertaking this same validation study in different seasons of the year can provide different correlation values due to the availability of the foods in the questionnaire.

Comparing the correlation values from similar validation studies undertaken in adults has shown that the variation in correlation values between different foods is consistent with the other studies. Willett et. al [132] validated a semi-quantitative food frequency questionnaire in women against four one week diet records over a period of a year. In this study, the correlation values ranged for 0.49 for vitamin A to 0.79 for Vitamin B6. In a validation study undertaken by Bingham et. al (1994), correlation of vitamin A intake from the food frequency questionnaire and 16-day food records was 0.23 [133]. Howarth validated a food frequency questionnaire in elderly subjects with correlation values ranging from 0.34 – 0.76[134].

Another recognised validation study was undertaken in adult men by Pietinen et.al.[135,136] that measured the intakes of total fat, saturated fats,

polyunsaturated fats, Vitamins A, C, E, Selenium and dietary fibre. Reproducibility studies showed an intra-class correlation between 0.52 (vitamin A) and 0.85 (Polyunsaturated Fats). A comparison of the food frequency questionnaire was made with twelve 2-day food consumption records for this validation study. Correlation coefficients ranged between vitamin A and Vitamin C were 0.52 and 0.74 respectively.

From these studies, it is clear that obtaining values that are accurate estimates for vitamin A intake is a difficult task. These low correlation values for vitamin A could be due to the large day to day variation.

Although the Spearman's ranked correlation values between the two food frequency questionnaires did not indicate a significant correlation for a number of foods, the Bland-Altman Limits of Agreement method demonstrated that the results were acceptable both at one and two standard deviations from the mean. A correlation value above 0.9 does not indicate that a method is accurate, as it does not provide information on the range of values. Although lower correlation values have been obtained in this study, the ranges of values from the Bland-Altman plots indicate good agreement between the questionnaires. Any possible errors may come from the variation in the length of time between the administration of the questionnaires.

Comparison between the food frequency questionnaire and the weighed food records were not acceptable or adequate at ranking the high, medium, low and infrequent consumers of specific foods high in iron, vitamin A and Vitamin C. The ranking correctly placed 25% of children in the correct quartiles. A possible explanation for the poor agreement between the two methods is that one method is measuring solely the foods that the child is eating, whilst the other is providing measurement of nutrient intake. To correlate the difference may produce a large margin of error that such a small sample group may not fall into the bounds of acceptable agreement.

However, the use of the Limits of Agreement method provided further information on the results, demonstrating the range of values that the measurements provided. The range of values from the comparison between the food frequency questionnaire and the four-day weighed food records show good agreement from the Bland-Altman Plots.

6.22 Data Analysis

There were several key issues in the data analysis component of this study that needed to be addressed. Food Works provided the nutrient data for most of the foods found in the children's weighed food records, however several key foods were missing. Suitable alternatives with similar nutrient values were found.

Another issue was that of analysing the weighed food records is that the children's meals were often composite. The different components of the meal were not measured separately, rather as a whole. In order to determine the approximate proportions of foods, estimations were made to the specific proportions of food from the weight of the food on the plate. Because the amounts of food these children eat are so small, this is likely to increase the margin of error when the approximations were made.

Several questionnaires were returned with a tick in the middle of the two boxes. Possible reasons for this indecisiveness may have been that the care-givers could not remember the frequency of the actual food given. This was for the minority of care-givers as most managed to complete the questionnaires correctly without any difficulty.

6.23 Availability of Iron in Food

Increasing evidence have revealed that not all iron from food is absorbed. Monsen et.al (1979) [131] developed a model of determining the actual amount of iron that is absorbed from the diet. For the purposes of this study, the Monsen model provided further information about the actual amount of iron that these children in the study were getting from the food that they were eating. The

difference between the iron that was contained in the foods that the children ate and the actual amount that the children absorbed revealed a disturbing picture.

The mean estimated actual iron intake into the body ranged from 0.554 – 0.880mg per day while the iron in the food ranged from 6.962mg (European) to 13.748mg (Pacific Island) per day in the various ethnic groups. These range of values have thus demonstrated different percentages of iron absorbed from foods eaten. There is a large difference between the percentage absorbed in Pacific Island children (5.545) to European children (9.61%). Although it is not stated if the main source iron is haem or non-haem iron, it is clear that the lower absorption percentage in the Pacific Island children indicate that the main source of iron for these children comes from non-haem iron. The high daily intake of iron and also the highest absorption of iron according to Monsen's model didn't result in highly absorbed ratios. This is consistent with the analysis undertaken with the biochemical status, and also nutrient intake, where the Pacific Island children are most at risk of iron deficiency and iron deficiency anaemia.

Multiple regression analysis of variance shows that there is a significant association between Pacific Island children and iron deficiency and iron deficiency anaemia and mean daily iron intake. The remaining three ethnic groups did not show significant association between iron deficiency and iron deficiency anaemia, and mean daily iron intake. The R_2 values in the regression analysis of iron deficiency anaemia, ethnicity and mean daily iron intake contributed under 20% of the total source of variance. Similarly, the R_2 values contributed under 20% of the total source of variance with the dependent variable being iron deficiency or iron deficiency anaemia, with estimated absorbed iron intake and ethnicity taken into consideration.

From the regression analysis of variance with haemoglobin being the dependant variable, with mean iron intake and ethnicity variables taken into consideration, R_2 values in the Pacific Island children accounted for 56.8% of the total variance for this regression. This accounts for over 50% of the variance found in Pacific Island children, when looking at the mean daily iron intake and

haemoglobin values. The R_2 values for the remaining three ethnic groups were below 25%.

The R_2 values from the multiple regression analysis of variance demonstrate that although ethnicity, mean iron intake, iron deficiency, iron deficiency anaemia contribute to the total variance of the regression analysis, the percentage of the R_2 values indicate that this is not the main source of variance in predicting iron deficiency and iron deficiency anaemia. Similar findings were also demonstrated in the multiple regression analysis with vitamin A deficiency being the dependent variable. Due to the sample size, other possible variables that may predict iron deficiency and iron deficiency anaemia could not be taken into consideration in the regression analysis. Further regression analysis of a larger sample size may be able to take into account income, age, mother's iron status to predict iron deficiency and iron deficiency anaemia.

The findings of this analysis indicate that there are several issues underlying the nutrition of Pacific Island children in New Zealand that need to be addressed.

6.3 RESEARCH IMPLICATIONS

The research implications of this study have been identified as follows: -
Undertaking dietary studies to measure nutrient intake in this age group is possible. The validation of a method is a vital step in ensuring that the method used to measure nutrient intake in children is compatible and can provide accurate results for the study. In this study, the validation of a food frequency questionnaire against four-day weighed food records and biochemical parameters to measure iron and vitamin A intake in children, obtained accurate and acceptable results for using the food frequency questionnaire in further research.

It is however recommended that should this questionnaire be used in any further studies that continued validation should take place in order to ensure that the results are monitored and screened for systemic bias and error in the

sample. Further studies during the different seasons of the year may alter the results of the correlation values.

The use of the Limits of Agreement method as a statistical analysis tool to determine the reliability of a method is recommended for further use in dietary assessment studies. The ability of the method to detect systemic bias provides a more accurate account of the data set that correlation values can provide. Although it has not been reportedly used in any dietary assessment and nutrient intake studies, the findings of this study show that this method of analysing data is possible in nutrition research, and is recommended.

The estimation of available iron that was undertaken to analyse the mean daily iron intake in the children's four day food records demonstrated an alarming situation, where the iron that is available in food is significantly higher than the actual iron that is absorbed. This raises the question about the foods that are being eaten and the need to identify and promote high "haem" sources of iron, in order to address the issue of iron deficiency and iron deficiency anaemia in young children. From the findings of this part of the analysis, the RDI for iron in children of this age group is clearly too low. Although these RDI's have been set to take into account that 30% of iron is absorbed from the food eaten, the results demonstrate that the percentage absorbed is much lower. In order to protect children from becoming deficient in iron, it is recommended that the RDI's for iron be reviewed.

The prevalence of vitamin A deficiency in this study population is of concern. As there is no prevalence data on vitamin A deficiency in children in New Zealand to compare these values with, the need for further studies to confirm the findings of this study is urgently needed. Classification of the severity of vitamin A deficiency as a public health problem is dependent on the proportion of individuals in the sample who have a low serum retinol level. It is considered a mild public health problem if 2%-10% of the sample have serum retinol levels of less than or equal to 0.70 μmols/L, while it is a moderate problem if between 10-20% of the sample have low serum retinol levels. Finally

it is a severe problem if greater than 20% of the sample have a low serum retinol level [11]. On the basis of these recommendations, the children in this sample, 14% of the sample were reported to be vitamin A deficient. This indicates that it is a moderate problem amongst this sample. However, the percentage of vitamin A deficient children in the different populations demonstrate that vitamin A deficiency is a serious public health problem in the Pacific Island children of this sample, and a highly moderate public health problem in the Maori children. Should this sample be a true representative of the population of young children in Auckland, there is every reason for concern at such alarming rates of sub-clinical vitamin A deficiency present in Auckland.

The findings from the biochemical data detecting the presence of vitamin A deficiency is consistent with the results food frequency questionnaire and the four day weighed food records. Although the care-givers specified in the eating patterns section of the questionnaire that 45% of children had more than 3 servings of vegetables a day, the frequency tables of vegetables and fruits consumed does not confirm this. In fact, the frequency of fruits and vegetables in these children reveal that the consumption of fruits and vegetables is significantly lower than expected. The results from the weighed food diaries confirm this finding. This is a significant indication that these children are not consuming a major component of a well balanced diet – fruits and vegetables. If this trend is prevalent in the population of New Zealand children, there is serious cause for concern. Public education, further research in the identifying the effects of consuming a diet low in fruits and vegetables as well as further research into identifying how widespread this problem is for this population of children is recommended.

Conclusion

Iron and vitamin A nutrition is essential for the health and well being of growing children. Sufficient amounts of these two key micro-nutrients in the crucial years of growth and development will protect against impaired motor development and dexterity, and severity of measles. The effects of being deficient in these micro-nutrients are known to be long term. Further research will demonstrate the strength of the causal link between Iron and impaired motor development, and the severity of measles in a vitamin A deficient state.

The main source of nutrients, including Iron and vitamin A comes from food. Therefore, dietary assessment provides an invaluable tool to determine the mean nutrient intake of the sample population.

Validation of a dietary assessment method is necessary to determine the accuracy and the reliability of a dietary instrument. It is recognised that validation is an important and necessary part of the development of a method to measure dietary intake in a population. The value of undertaking validation studies lies in the opportunity it gives to test the method to determine its suitability and usefulness. Validation studies are able to identify sources of error, systemic bias and expected outcomes of the study.

From the results of this study, it indicates that the food frequency questionnaire is reliable and valid in ranking individuals within the population sample studied. Several conclusions can be made in the case of this validation study. Firstly, dietary assessment in young children is possible, with methodology that is suitable for children, and care-giver's full co-operation. The results from this

study have provided very useful information not only on validation itself, but also dietary data that can be expected in the main study to be carried out in Auckland infants. This study highlighted the difficulties and problems of having very young children as subjects. It is recommended that two dietary methods be used to obtain dietary intake information from these children. The use of both a prospective and retrospective method, particularly in this age group will provide information that is more useful, and takes into account the large differences in the variation of food that a subject eats from day to day as well as the differences between subjects.

Although the response rate was significantly lower than was expected, (56%) it may be potentially attributed to the high respondent burden that was required of the care-givers of the child. From this perspective, the response rate may have been low, but the co-operation of the care-givers that were involved was a significant contribution. This can be seen in the number of completed weighed food records for the children. Response rates for undertaking the four-day weighed food records in this study was 93% of the enrolled subjects. This is significantly higher that the response rates for the four day food records in the National Diet and Nutrition survey undertaken in children, which only had a response rate of 88%. The difference between these two response rates may vary with the large difference in sample sizes.

Secondly, this study has tested a dietary method that was able to determine the iron and vitamin A intake in Auckland children. Data from this study was analysed using Spearman's rank correlation coefficients, Bland-Altman's Limits of Agreement and regression analysis. The use of Spearman's ranked correlation coefficient and regression analysis are commonly used in nutrition research, but currently, the Limits of Agreement method has not been reported as used in any validation of a dietary method to assess nutrient intake in a population. Another significant finding of this study is the use of the Limits of Agreement method to determine the reliability of the food frequency questionnaire. The strength of this method is that it is able to describe absolute reliability rather than relative reliability, thus able to detect any systemic bias in

the method. The Bland-Altman plot shows the measurement error, allowing a simple comparison of reliability of two different measurement tools. Findings from this study support the view that this method provides a more accurate assessment of the measure of how effective a dietary assessment method is in comparison to the "gold standard".

The correlation values of this study ranged from 0.132 to 1 for the two food frequency questionnaires. The large range in correlation is also reported in other validation studies of food frequency questionnaires. In a validation study of a food frequency questionnaire for elderly subjects in Australia, the correlation values ranged from 0.34 –0.76 [134]. Willett et.al. (1985) [132] validated a semi-quantitative food frequency questionnaire in adult women that compared it against four one week diet records over a period of a year. The correlation values ranged for 0.49 for vitamin A to 0.79 for Vitamin B6. Bingham et. al (1994) validated a study of a food frequency questionnaire against 16 day food records, and found the range of correlation values from 0.21 to 0.6 [133]. Blom (1989) reported correlation values of 0.52-0.76 in a validation study in children. From these validation studies, it can be concluded that in undertaking validation studies and comparing between dietary methods, a range of correlation values can be expected. This does not confirm that the method being validated is inaccurate or unreliable, but that there is variation in the results of the method.

The limits of Agreement method provided further information on the differences between the two methods, whilst the correlation values provided a number indicating the strength of the relationship. Visually, it can be seen that 95% of the values lie within two standard deviations of the mean. From the Bland-Altman plots, the next value can be predicted to lie within 2 standard deviations of the mean.

Multiple regression analysis with the independent variable being iron deficiency or iron deficiency anaemia, with ethnicity and mean daily iron intake as additional variables, demonstrated a significant relationship between being Pacific Island, and iron deficient. No significant relationship was seen in the three other ethnic groups.

A further finding of this study is the actual iron that is available for absorption from the foods that the young children are eating. Using the data from the four-day weighed food records and Monsen's et.al. (1979)[130] model, the approximate values of iron that was absorbed from the foods eaten was determined. The difference between the iron that was absorbed and the actual amount available in foods is significantly large. Thus, the children may not have been eating enough food containing high iron availability, and that the food eaten was primarily from a non-haem iron source. Given that the mean iron intake from all the children were within the RDI's set, it can be concluded that the foods the children were eating were primarily from a non-haem iron source. The implications of this study demonstrate that, although sufficient iron is present in the foods that the children are eating, this still fails to meet the actual amount of iron that is required for the body's demands and needs. Recommendations that iron high in haem iron is promoted as the best source of iron are currently in place. A further recommendation that the RDI's for iron at this age may need to be reviewed in order to accommodate for the high consumption of non-haem iron sources of food that children of this age group may be eating.

The results of this study demonstrate that the validation of this study has generated results that are acceptable in this sample size. However, it is recommended that repeated validation of the food frequency questionnaire will provide improved results, and also test if this validation study is significant when the subject numbers are greater than thirty.

From these findings and recommendations, the need for further research in this area of paediatric nutrition is enormous. The lack of research in this area

cannot be justified by difficulties of having children as subjects, but should be the incentive to undertake further research into the nutritional issues that this future generation of the population is facing.

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Appendix A

Information Sheet

... And if you have any questions?

If you have any questions about our project, either now or in the future, please feel free to call us at the Department of Paediatrics, University of Auckland or at the Nutrition Department at Massey University. If you need an interpreter, one can be provided.

Contact numbers

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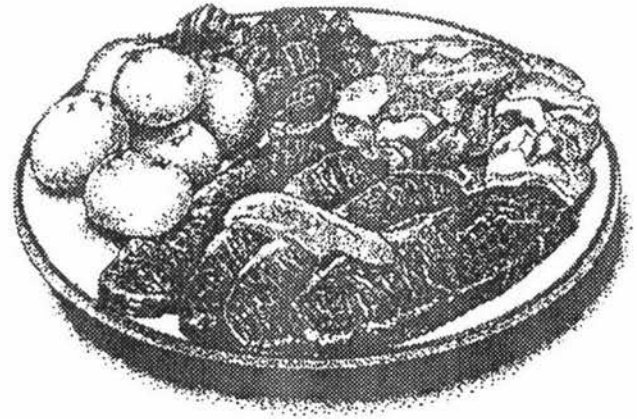
If you have any queries or concerns about your rights as a participant in this study you may wish to contact a Health and Disability Services Consumer Advocate, telephone 637 5799.

This study has received ethical approval from the North Health Ethics Committee.

The Iron and Vitamin A Status of Auckland Infants

The Iron and Vitamin A Status of Auckland Infants



The University of Auckland

Principal Investigator:

Dr Cameron Grant
Department of Paediatrics
The University of Auckland
Telephone: 373-7599 ext 6192

Why are we doing this survey of Auckland children?

Iron and vitamin A are both needed by young children to help then grow and learn. Children who do not have enough iron do not learn properly. Children who do not have enough vitamin A become more sick if they get measles or diarrhoea. By participating in this study you will be able to find out if your child has enough iron and vitamin A. You will help us learn why some children do not have enough iron and vitamin A and what could be done to stop this from happening.

Who is in this study?

This study is being done in all of Auckland during 1998, and 1999. Your child has been chosen at random from all children aged 6 to 24 months living in Auckland. This study is being carried out by the Auckland University School of Medicine and Massey University.

What is involved?

Should you agree to take part in this project, you will be interviewed by a trained interviewer and will be shown how to measure and record what your child eats and drinks for four days. The interviewer will measure and weigh your child. An experienced nurse will take a blood sample (about 2 teaspoons) from your child. We will use an anaesthetic cream on the skin, and some toys to make the blood test more pleasant for your child. We will not take blood from a distressed child. Occasionally, some children may have a small amount of bruising in the area where the blood sample was taken, but this normally disappears in a few days. The visit will take between one and one and a half hours.

The interviewer would need to visit your house again once you have finished measuring what your child eats.

Do I have to take part in the survey?

Your participation and the participation of your child is **entirely voluntary**. If you agree to take part in the study, you are free to withdraw at any time, and this will not disadvantage you in any way or affect the future health care of you or your child. You do not have to answer all the questions, and you may stop the interview at any time. It will not cost you anything to take part in this study. You will be told if your child's blood test show low iron or vitamin A and whether s/he needs to see a doctor.

What will happen to the results?

The information about your child and family is completely confidential. No information which could identify you will be used in any reports on this study. The results will be stored by a code number on a computer in the Department of Paediatrics at the University of Auckland. The questionnaires will be stored in a locked room. They will be stored for 10 years then destroyed. You will receive a copy of your child's blood test results and will be told what to do about them. You will receive newsletters about the study to tell you how it is going and what the study found.

Appendix B

Consent Form

The Iron and Vitamin A Nutrition of Young Children in Auckland
Consent form

English	I wish to have an interpreter.	Yes	No
Maori	E hiahia ana ahau ki tetahi tangata hei korero Maori ki ahau.	Ae	Kao
Samoan	Oute mana'o e iai se fa'amatala upu.	Ioe	Leai
Tongan	'Oku fiema'u ha fakatonulea.	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo.	Ae	Kare
Niuean	Fia manako au ke fakaaoga e tagata fakahokohoko vagahau.	E	Nakai

I have read and I understand the information sheet dated ..... for volunteers taking part in the study designed to find out my child has low iron and/or vitamin A levels and if s/he has enough iron in his/her diet. I have had the opportunity to discuss this study . I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect the future health care of myself or my child.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study. I understand the compensation provisions for this study.

I have had time to consider whether to take part. I know who to contact if I have any side effects to the study. I know who to contact if I have any questions about the medication or the study.

I ..... (full name) hereby consent to take part in this study. Where the participant is a child the consent of the child in addition to the signature of the parent or guardian should be obtained.

If you have any concerns about the study, you may contact:
Mrs. Mavis Roberts, Department of Paediatrics, School of Medicine, phone 3737599 ext 6434

Date
Signature:

Full names of Researchers: Cameron Grant, Colin Tukuitonga, Sue Crengle, Clare Wall
Contact Phone Number for researchers 3737599 ext 6192
(Note: A copy of the consent form to be retained by participant and (in the case of patients) a copy to be placed in the medical file.)

Appendix C

Four day Weigh Food Records

Home Record Diary

ID : _____

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health,
Massey University

HOME RECORD DIARY

Background :

This diary is designed to detail your child's food intake over the period of allocated days. Three out of the four days are to be weekdays, and the other a weekend day.

Name : _____

Age: _____ M / F

Days to record food eaten **by your Child**

Mon Tues Wed Thurs Fri Sat Sun

INSTRUCTIONS

Please record all food and drink as shown on the tables in the following **pages just before they eat or drink** not from memory at the end of the day.

Use a new line for each food or drink. You can use more than one line for a food or drink.

Weigh the plate or container first. Foods that come in containers should be weighed before and after the contents are eaten.

Start each food or drink on a separate line. Give as much detail as possible.
For example, how the food was cooked, added foods, recipes, and snack wrappers.
If possible, please provide a full description on the food that your child has eaten.

REMEMBER TO RECORD

- all drinks even tap water
- Vitamin Supplements
- Medicines

It is important that the information that you record in the tables represent **exactly what your child** has actually eaten rather than what you think he/she should have eaten.

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

Thank you for participating in this research study.
We really appreciate the time you are giving.

PLEASE START A NEW PAGE FOR EACH DAY EVEN IF ONLY SOME OF THIS PAGE IS USED

Day : _____ Date: _____

Time Am/pm	Food Eaten (Tick) **Home**	**Away**	**Weighed by (Tick) Mother**	**Other**	**Brand Name of Item in Full (except fresh produce)**

TICK A BOX TO SHOW WHICH DAY THIS IS 1 2 3 4

☐ ☐ ☐ ☐

Full Description Dried/frozen/fresh Sweetened or not, cooking method, fat type	Weight of Plate	Weight of Food	Weight of leftovers	Comments Problems , Difficulties

Appendix D

Four Day Weighed Food Records

Eating out Diary

ID : _____

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland,
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health,
Massey University

EATING OUT RECORD DIARY

Mothers/carers: Please use this notebook to write down any food or drink the child has while away from home, even if the food was brought from home.

Carers : Please hand this notebook back to the mother at the end of the day

Name : _____

Age: _____ M / F

Days to record food eaten **by your Child**

Mon Tues Wed Thurs Fri Sat Sun

INSTRUCTIONS

When you are out of home, please take this diary with you and record everything the child eats and drinks in this diary, even if the food has been made at home.

Write down the day and date on each page.
Where possible, write down each day and brand name of food/drink.
Write down a full description of food and drink, including the price and place of purchase if bought.

It is important that the information that you record in the tables represent **exactly what your child** has actually eaten rather than what you think he/she should have eaten.

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

Thank you for participating in this research study.
We really appreciate the time you are giving.

ID. _____

PLEASE START A NEW PAGE FOR EACH DAY EVEN IF ONLY SOME OF THIS PAGE IS USED.

Day _____ Date _____

Time eaten am/pm	Place where it was eaten	Brand name in full unless fresh	Description including price, place it was bought, quantity	Any leftovers?

Appendix E

Food Frequency Questionnaire

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland,
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health, Massey University

FOOD FREQUENCY QUESTIONNAIRE

BACKGROUND

This questionnaire is designed to estimate **your child's** *usual* pattern of food intake by providing information on how often on average, your child consumes certain food and beverages.

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

Thank you for participating in this research study. We really appreciate the time you are giving.

Instructions (PLEASE READ THIS BEFORE COMPLETING THE QUESTIONNARE)

For each food item listed, tick the column that best represents **your child's** average pattern of consumption that food.

Please use the comments column to fill in anything that may be helpful for us to know. For example brand names, exact or estimated quantities that your child may usually consumed, cooking methods.

It is important that the information you give represents **what your child actually eats** rather than what you think he/she should be eating.

I.D. .....

First of all, we have some questions about your child's general eating habits

1. How would you describe your child's eating pattern? *(Please mark only one)*
   - ❑ Eats a variety of all foods, including animal products
   - ❑ Eats eggs, dairy products, fish and chicken but avoids red meat
   - ❑ Eats eggs, dairy products, but avoids all meats and fish
   - ❑ Eats eggs, but avoids dairy products, all meats and fish
   - ❑ Eats dairy products, but avoids eggs, all meats and fish
   - ❑ Eats no animal products
   - ❑ Other *(please specify)*_____

2. On average, how many servings of fruit (fresh, frozen, canned or stewed) does your child eat per day? Do not include fruit juice *(Please mark only one)*

One serving of fruit = 1 medium piece **or** 2 small pieces of fruit **or** 1/2 cup of stewed
e.g. 1 apple = 1 serving

Per Day
- ❑ Does not eat fruit
- ❑ Less than 1 per day
- ❑ 1 serving
- ❑ 2 servings
- ❑ 3 or more servings

I.D. .....

3. Does your child eat fruit with the main meals in the day? *(Please mark all those which applies to your child's usual dietary habits)*

   - ❑ Does not eat fruit
   - ❑ Usually eats fruit as a snack between main meals
   - ❑ Usually eats fruit with the main morning meal
   - ❑ Usually eats fruit with the main afternoon meal
   - ❑ Usually eats fruit with the main evening meal
   - ❑ Usually has fruit juice with the main meal

4. On average, how many servings of vegetables (fresh, frozen, canned) does your child eat per day? Do not include vegetable juices *(Please mark only one)*

> One serving of vegetables = 1 medium piece **or** 1 cup of salad **or** 1/2 cup of cooked
> *e.g. 1/2 cup peas + 1 medium potato = 2 servings*

Per Day

- ❑ Does not eat vegetables
- ❑ Less than 1 per day
- ❑ 1 serving
- ❑ 2 servings
- ❑ 3 servings
- ❑ 4 or more servings

I.D. .....

5. On average, how many servings of cereals (eg, pasta, rice, breakfast cereal) does your child eat per day and per week? Do not include bread *(Please mark only one)*

One serving of cereals = 1 cup cooked rice/pasta/porridge **or** 1/2 cup muesli **or** 1 cup of other comercial breakfast cereals **or** 2 weetbix)

e.g. 1/4 cup muesli 2 times per week + 1 weetbix 6 times per week = 4 servings per week and less than 1 per day

Per Day

- ❑ Does not eat cereals
- ❑ Less than 1 per day
- ❑ 1-2 servings per day
- ❑ 3-4 servings per day
- ❑ 5-6 servings per day
- ❑ 7 or more servings per day

Per Week

- ❑ Does not eat cereals
- ❑ 1 or more per day
- ❑ Less than 1 serving per week
- ❑ 1-2 servings per week
- ❑ 3-4 servings per week
- ❑ 4-6 servings per week

6. On average, how many servings of breads (e.g., fresh, toast, rolls, pita) does your child eat per day? *(Please mark only one)*

Per Day

- ❑ Does not eat breads
- ❑ Less than 1 per day
- ❑ 1-2 servings per day
- ❑ 3-4 servings per day
- ❑ 5-6 servings per day
- ❑ 7 or more servings per day

7. What type(s) of bread does your child eat most often?

- ❑ White
- ❑ White - high fibre
- ❑ Wholemeal or wholegrain
- ❑ Other *(please specify)* _____

I.D. .....

8. Does your child eat bread with the main meals in the day? *(Please mark all those which apply to your child's usual dietary habits)*

- ❑ Does not eat bread
- ❑ Usually eats bread as a snack
- ❑ Usually eats bread with the main morning meal
- ❑ Usually eats bread with the main afternoon meal
- ❑ Usually eats bread with the main evening meal

We would like to know more detail about the foods your child usually eats

9. How often does your child eat the following types of foods or drinks?

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
DAIRY FOODS Unflavoured milk as a drink Cow's Milk as a drink										Please specify type of Milk
Flavoured milk as a drink Milo/Ovaltine/Nesquick										Please specify brand
Milk added to cereals										
Cheese										Please specify brand and type of cheese
Yoghurt										Please specify brand, or if its homemade
MILK & BABY FOODS Breast Milk										
Infant Formula										Please specify Brand Name and Type of Infant Formula
Commercial Baby Food										Please specify brand name and type of Baby food

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
CEREALS Breads, rolls, pita										Please specify the brand and type of bread
Savoury baked products (meat base)										Please specify the type of baked products
Maori bread										
Paraoa Parai (frybread)										
Rice -										
Pasta (Egg Based) Spaghetti, Lagsane, Pizza										Please specify the type of Pasta
Noodles – Chop Suey Rice Vermecilli										
Breakfast cereals										Please specify the Brand name

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
MEAT, FISH & EGGS Red meat Corn Beef Roast Beef/Pork Mince										Please give an example of how much is eaten, and how it is cooked.
Liver Cooked/Fried Pate										
Chicken										Please specify the amount of chicken eaten and how its Cooked
Fish - Fresh Fish Tin Fish										
Shell fish Mussels Pipis Oysters										Please specify what sort is eaten and amounts
Eggs										

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
VEGETABLES & ROOTS Leafy vegetables										Please specify amount and how it was cooked
Lettuce										
Spinach & Silverbeet										
Watercress & Puha										
Potatoes – Mashed or boiled, Baked or fried.										
Tomatoes										
Taro Leaves										
Carrots										
Pumpkin – Boiled, or mashed										
Sweet Potatoes										
Frozen Mixed Vegetables										
Other Vegetables										

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
FRUITS Citrus fruits (Oranges, lemons, mandarins)										Please specify type and amount
Apricots Dried Apricots										
Paw Paw										
Nectarines and Peaches										
Feijoas										
Mangoes										
Tamarillos										
Bananas										

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
LEGUMES & NUTS Beans & lentils										
Nuts										
OTHER BEVERAGES Citrus juices										
Non-citrus juices										
Soya milk										
Soft drinks										
Tea										
Coffee										

	Never	< once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day	Amount	Comments
FATS Butter										Please specify brand, and type of butter
Margarine										Please specify brand and type of margarine
MINERAL & VITAMIN SUPPLEMENTS Supplement with iron										Please specify brand and type of supplements
Supplement with Vitamin C										
General Vitamin and Mineral Supplements										
Cod Liver Oil										

Once again, THANK YOU FOR YOUR TIME.

Appendix F

General Questionnaire

and

Show Cards for General Questionnaire

(Questions 17, 25 and 40)

ID : _____

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health,
Massey University

GENERAL QUESTIONNAIRE

The aim of this questionnaire is to collect some general information for statistical purposes only.

All information you give is strictly confidential.
No one outside of the study will have access to this information.

Thank you for participating in this research study.
We appreciate the time you are giving .

ID. _____
OFFICE USE ONLY

1. The child who is participating in the study is a
(Please tick the appropriate box)
- BOY ☐
- GIRL ☐

☐ 1

2. He/she was born on

_____/_____/_____ *(Day/Month/Year)*. ☐☐☐☐☐☐

3. Is your child one of twins or triplets (or more!)?

- NO ☐
- YES ☐

☐

4. Which ethnic group or groups does your child belong to?
(*Tick all that apply)*

NZ European/Pakeha ..... ☐
Other European ..... ☐
NZ Maori..... ☐
Samoan ..... ☐
Cook Island Maori ..... ☐
Tongan ..... ☐
Niuean ..... ☐
Tokelauan ..... ☐
Fijian ..... ☐
Other Pacific Island groups ..... ☐
Southeast Asian ..... ☐
Chinese ..... ☐
Indian ..... ☐
Other Asian ..... ☐
Other ethnic groups ..... ☐

If ticked more than one,
Is there a group that your child belongs to most ? _____

☐ 4

ID. _____
OFFICE USE ONLY

5. Does your baby have any brothers or sisters?

- NO
- YES

☐ ☐ ☐ 5

If yes, how many brothers and sisters? ☐ ☐

(b) Where does ……rank in the family?
(Oldest/Third/Youngest etc)

_____ ☐

6. How many weeks long was your pregnancy?

- DON'T KNOW
- Normal Term / 9 months / 40 weeks

☐ ☐

Less than 36 weeks ☐
36 weeks (4 wks early) ☐
37 weeks (3 wks early) ☐
38 weeks (2 wks early) ☐
39 weeks (1 wk early) ☐
40 weeks (on time) ☐
41 weeks (1 wk late) ☐
42 weeks (2 wks late) ☐
Other _____ ☐

☐ ☐

7. How much did your baby weigh when he/she was born? (Look in Health Book)

_____Kilograms or _____lbs

DON'T KNOW ☐ ☐ 7

ID. _____
OFFICE USE ONLY

8. What was the length of your baby when he/she was born?

_____centimetres_____inches

DON'T KNOW ☐ ☐ 8

9. What is the age of the child's mother?

_____ ☐

10. What is the height of the child's mother?

_____feet and inches/centimetres

DON'T KNOW ☐ ☐

11. What is the age of the child's biological father?

_____ ☐

12. What is the height of the child's biological father?

_____feet and inches/centimetres

DON'T KNOW ☐ ☐

HISTORY OF WHEN YOUR CHILD WAS A BABY

13. Did you breastfeed your baby at any time?
- NO *(Please go to Question 12)* ☐
- YES ☐ ☐

14. How long was your baby breastfed (i.e. not given formula or other food)?

_____days/weeks/months ☐

15. How long was your baby exclusively breastfed (i.e. not given formula or other food)?

_____days/weeks/months ☐ 15

ID. _____
OFFICE USE ONLY

16. How old was your baby when he/she stopped having breast milk even at bed time?

_____ days/weeks/months

☐ 16

17. What kinds of milk other than breast milk have you given your baby?

Standard infant formula ☐
Standard follow-on formula ☐
Cow's milk ☐
Soya formula ☐
Goats milk formula ☐
Other (please specify) _____ ☐

☐

(b) Can you tell us the brand(s) name of the formula most often used?

SHOW CARD ☐☐☐

☐

Don't know

For each milk, can you tell us at what age did you start to give this milk to your baby and when did you stop giving this milk even at bed time.

Milk Brand	Age Began	Age Stopped

☐☐☐ 17(b)

Why did you use this milk with your baby?

_____

_____

☐

ID. _____
OFFICE USE ONLY

18. How old was your baby when he/she stopped having any infant formula of follow-on formula even at bed time?

_____months

☐ 18

19. How old was your baby when he/she first started taking cows milk as a drink?

_____months

☐

20. Please tell us all other types of drinks your baby drank before he/she was 1year old. *(You can tick more than one)*

Fruit juices ..... ☐
Fruit drinks ..... ☐
Soft fizzy drinks ..... ☐
Soya milk ..... ☐
Coffee ..... ☐
Tea ..... ☐
Flavoured Milk (Milo, Nesquik, Ovaltine) ..... ☐

☐☐

21. How old was your baby when he/she first ate solid food?

_____

☐

22. What type of solid food did he/she first eat?
Commercial (in Tins and Jars)
Homemade
Commercial and Homemade

☐
☐

☐

23. How old was your baby when he/she first ate real meat that you prepared yourself (NOT commercial baby dinners)?

_____

☐ 23

ID. _____
OFFICE USE ONLY

24. Are there any foods that you avoid giving to your baby?

- NO ☐
- YES ☐ ☐ 24

If yes, what sort and why?

_____

_____

_____ ☐

_____

Low Fat ☐
Low Sugar ☐
Low Salt ☐
High Fibre ☐
No Additives ☐ ☐

25. How would you describe your baby's eating pattern? *(Please mark only one) (SHOW CARD)* ☐ ☐

26. a) Does any of your family have food allergies?

- YES ☐
- NO ☐ ☐

b) Does your baby have food allergies?

- YES ☐
- NO ☐ ☐

27. Did your baby take an iron supplement/syrup as a baby (<1 year)?

- YES ☐
- NO ☐
- SOMETIMES ☐ ☐ 27

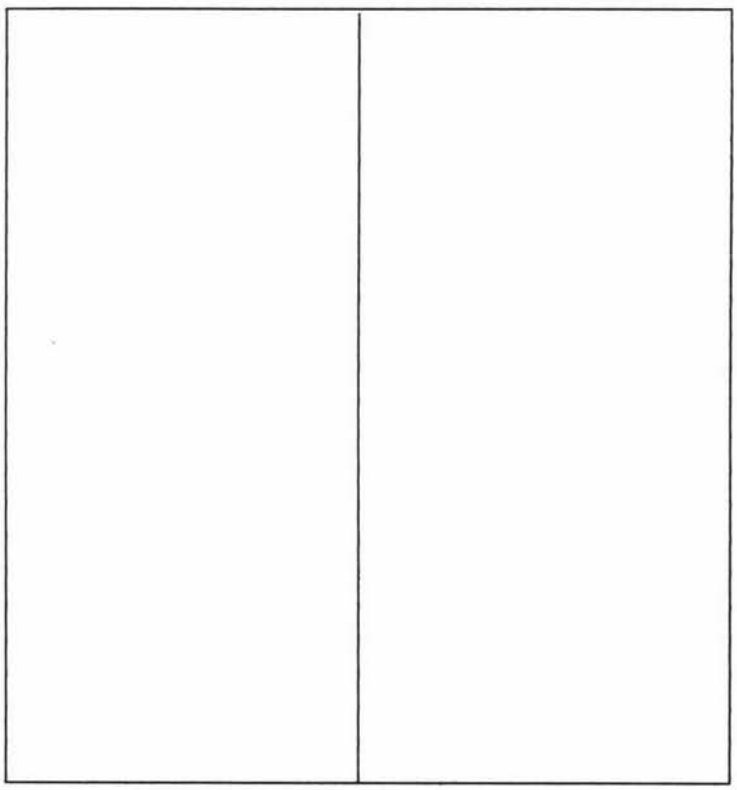
ID. _____
OFFICE USE ONLY

28. Does your baby/child currently take vitamin or mineral Supplements?

- NO*(Please go to Question 28)* ☐
- YES ☐
- SOMETIMES ☐

☐☐ 28

What sort? (Ask to see it if possible)



☐

ID. _____
OFFICE USE ONLY

29. Who looks after your baby/baby during the day?

		Approximately how long?
• Mother	☐	_____ hours/week
• Father	☐	_____ hours/week
• Grandmother/grandfather	☐	_____ hours/week
• Auntie/Uncle	☐	_____ hours/week
• Baby sitter	☐	_____ hours/week
• Nanny	☐	_____ hours/week
• Older brother/sister	☐	_____ hours/week
• Early Childhood Centre/ Kohanga Reo/language Nests	☐	_____ hours/week
• Other	☐	_____ hours/week

Please specify _____

☐☐ 29

30. Has your baby been immunised within the last month?

- NO ☐
- YES ☐

When? _____ days ago ☐

☐ 30

ID. _____

OFFICE USE ONLY

31. Has your child been sick in the past 3 months? (including colds, infections etc).

	YES	NO	No. of Times	
Diarrhoea and/or vomitting	☐	☐	_____	☐
Infections – Ear, Chest				
Skin, Throat	☐	☐	_____	☐
Cold /cough	☐	☐	_____	☐
Flu	☐	☐	_____	☐
Measles	☐	☐	_____	☐
Mumps	☐	☐	_____	☐
Chicken pox	☐	☐	_____	☐
Asthma	☐	☐	_____	☐
Eczema	☐	☐	_____	☐
Anaemia	☐	☐	_____	☐
Rash	☐	☐	_____	☐☐ 31

Other, please specify _____

_____

32. In the last 12 months, has..... seen a doctor, or a doctor at an A & E centre or been visited by a doctor? By "doctor" I mean any GP or family doctor, but not a specialist.

- NO ☐
- YES ☐

What for? ☐☐

_____

_____

ID. _____

OFFICE USE ONLY

33. Does your child have any problems which affect his/her eating patterns?

- NO *(Please go to Question 29)*
- YES

What kind of problem is it?

_____

_____

33

34. Please tell us to what extent your child shows these behaviours

	Never	Rarely	Sometimes	Often
Irritable				
Picky eater				
Listless				
Tired				
Sensitive to cold				
Pica				

(eating non-food items such as soil, ice)

Listless : eg. Low energy, lack of interest in food
Tired : Falling asleep during meals

35. Do you have any concerns about your child's eating?

- No
- Yes

If yes, what are these concerns? _____

_____

_____

Have you found anything that is helpful for your child's eating?

_____

_____

Thank you for your help – We appreciate the time you're giving.

Now we would like to ask some questions about your household.

ID. _____
OFFICE USE ONLY

36. Did the baby's mother complete……?

- Primary
- Intermediate
- Secondary

☐ 35

37. Did she go to….

- University
- Polytechnic
- Training College

☐

38. Does the mother or main care-giver have any other qualification, such as a trade certificate, a diploma or a degree, that they had to pass a course lasting at least 3 months full-time or the equivalent to get?

- YES
- NO
- DON'T KNOW

☐

39(a). Which best describes the mother's or main caregiver's position?

- Full time home maker
- Full time employment *(Please go to Question 51a)*
- Part time employment *(Please go to Question 51b)*
- Part time employment *(Please go to Question 51b)* and Benefit
- On a Benefit
- A Student
- Other

☐

Please describe_____

If employed what is her current or most recent job?

ID. _____
OFFICE USE ONLY

40. What would be the total income, for the household from all sources (*wages and benefits*), before tax or anything that was taken out of it, in **the last 12 months**?
SHOW CARD ☐ ☐ 40

41. Could you please estimate the amount in dollars , spent each week on :

Rent/Mortgage _____ ☐

Food _____ ☐

42. Could you please estimate the amount in dollars , spent each month on :

Household services (Water,power,phone) _____ ☐

Health Care (prescriptions and appointments) _____ ☐

43. Does anyone in your family have the following conditions?

	YES	NO	Relationship to child	
Thalassemia	☐	☐	_____	
Haemophilia	☐	☐	_____	
Haemochromotosis	☐	☐	_____	☐
Iron Deficiency Anaemia	☐	☐	_____	☐

44. Did the child's mother take an iron supplement during the pregnancy?

- Never ☐
- Rarely ☐
- Sometimes ☐
- Often ☐
- Don't Know ☐ ☐ 42

What was the name of the supplement?

_____ ☐

ID. _____
OFFICE USE ONLY

45. How many people living in the household smoke cigarettes Regularly ? (*If NO smokers go to question 40).*

_____ (number of people) [ ] 45

46. Are there any places in the house where smoking is not allowed? [ ]

- YES
- NO [ ]

47. Did the mother smoke during the pregnancy? [ ]

- YES
- NO [ ]

48. Can you tell me how many people live in the household and list the their ages (approximately)? [ ]

_____People

Ages : (Circle your children) [ ]

a) _____ b) _____ c) _____ d) _____

e) _____ f) _____ g) _____ h) _____

i) _____ j) _____ k) _____

49. How many of each of the following rooms are available in this Dwelling for your household's use? (*count open-plan rooms such as kitchen - lounge as **2** rooms. Don't count bathrooms, showers, toilets, laundries, spa rooms, pantries, halls;garages*). [ ]

Bedrooms - _____

Sleepout or caravan used as a bedroom _____

Kitchens? _____

Lounges or living rooms? _____

Dining rooms? _____

Other rooms such as rumpus rooms, family rooms, [ ]

Studies, hobby rooms etc? _____

ID. _____
OFFICE USE ONLY

Please answer the following questions only if the father or a male partner is currently part of the household *(ie. living at home or working away from the house)*

50. Did the father complete.....?

- Primary
- Intermediate
- Secondary

☐ 50

51. Did he go to....

- University
- Polytechnic
- Training College

☐

52. Does the father or male partner have any other qualification, such as a trade certificate, a diploma or a degree, that they had to pass a course lasting at least 3 months full-time or the equivalent to get?

- YES
- NO
- DON'T KNOW

☐

53(a). Which best describes the father's or male partner's position?

- Full time home maker
- Full time employment *(Please go to Question 51a)*
- Part time employment *(Please go to Question 51b)*
- Part time employment *(Please go to Question 51b)* and Benefit
- On a Benefit
- A Student
- Other

☐ 53(a)

Please describe_____

ID. _____
OFFICE USE ONLY

53(b). If employed what is his current or most recent job?

_____

_____

☐ 53(b)

THANK YOU VERY MUCH FOR YOUR TIME AND HELP


SMA
infant formula
suitable from birth
900 g net
GOLD CAP
SMA
infant formula
suitable from birth
900 g net
Nurture
Premium
Suitable for Infants
from Birth
425g NET
Nurture
Premium
Suitable for Infants
from Birth
900g NET
Nurture
Plus
Infant Formula
to Satisfy Hungry
Bottle Fed Babies
1kg NET
Nurture
Follow-On
Suitable for Infants
6 Months and Over
A nourishing milk which is richer in iron
and vitamins than cow's milk
900g NET
SMA
PROGRESS
infant formula suitable only for
infants aged over 6 months
900 g net
from the makers of S-26
INFASOY
Progress
FOLLOW-ON INFANT FORMULA
Suitable only for infants
aged over 6 months
milk free, lactose free
Wyeth
from the makers of S-26
Infant Formula
INFASOY
milk free, lactose free
infant formula
suitable from birth
Wyeth
900 g net
Karicare
First
Formula
birth to 4 months
NUTRICIA
Karicare
Infant
Formula
Suitable from birth
NUTRICIA
Karicare
Follow-On
aged over 6 months
NUTRICIA
Karicare
Goat Infant
Formula
Suitable from birth
NUTRICIA
Karicare
Goat
Follow-On
aged over 6 months
NUTRICIA
Karicare
Soya Infant
Formula
Suitable from birth
NUTRICIA
Karicare
Soya
Follow-On
aged over 6 months
NUTRICIA

Question 25

1. **Eats a variety of all foods, including animal products**
2. **Eats eggs, dairy products, fish and chicken but avoids red meat**
3. **Eats eggs, dairy products but avoids all meats and fish**
4. **Eats eggs but avoids dairy products, all meats and fish**
5. **Eats dairy products but avoids eggs, all meats and fish**
6. **Eats no animal products**
7. **Other – Please Specify_____**

Question 40

1. Loss/Zero
2. $1-$5000
3. $5001-$10,000
4. $10,001-$15,000
5. $15,001 -$20,000
6. $20,001 - $25,000
7. $25,001 - $30 000
8. $30,001 - $35,000
9. $35,001 - $40,000
10. $40,000- $50,000
11. $50,001- $70,000
12. $70,001 - $ 100,000
13. $100,000 or more
14. Don't know

Appendix G

Anthropometric Record Diary

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland,
Department of Paediatrics, University of Auckland,
and
Institute for Food, Nutrition and Human Health,
Massey University

ANTHROPOMETRIC RECORDING BOOK

INTERVIEWER/RECRUITER NAME :

_____

If Found, please return to:

Iron and Vitamin A Nutrition in Young Children in Auckland Study
C/- Mrs. Mavis Roberts
Department of Paediatrics,
School of Medicine,
University of Auckland,
Private Bag 92019,
Auckland

Name of Child: _____ ID._____

Age:_____

Date: _____

Weight _____ Height:_____

Arm Circumference : _____

Skinfolds:

Skinfolds	**Readings**	**Average**
Triceps #1		
#2		
#3		
Biceps #1		
#2		
#3		
Subscapular #1		
#2		
#3		

Appendix H

Training Manual

Iron and Vitamin A Nutrition of Young Children In Auckland

Department of Maori and Pacific Health, University of Auckland,
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health, Massey University

TRAINING MANUAL FOR RECRUITERS AND INTERVIEWERS

Recruiter and Interviewer Name : _____

If found please return to :

Iron and Vitamin A Nutrition in Young Auckland Children Study,
C/- Mrs. Mavis Roberts,
Department of Paediatrics,
School of Medicine,
University of Auckland,
Private Bag 92019,
Auckland.

TABLE OF CONTENTS

INTRODUCTION to the STUDY

The project aims to find out

1. what proportion of Auckland children aged between 6 months and 23 months have iron or vitamin A deficiency,
2. the causes of the iron or vitamin A deficiency, for example whether it is the child's diet, the mother's pregnancy.

This study will involve the different ethnic communities in Auckland, and will seek to gather information that is relevant to children from the Maori, Pacific Island and European/others communities.

Being the pilot study, there will only be approximately 40 subjects. We aim to test the methodology and also to validate the food frequency questionnaires for use in the main study.

This project is a collaborative project involving the Department's of Paediatrics and Maori and Pacific Health at the University of Auckland and the Institute of Food, Nutrition and Human Health at Massey University.

The data collection of these infants will involve :
Sociodemographic data from a questionnaire
Dietary data from a food frequency questionnaire and a weighed food record
Anthropometric Measurements
Biochemical from blood samples.

KEY PEOPLE INVOLVED IN THIS STUDY

Dr. Cameron Grant
Dr. Grant is the principal investigator for this study.
Department of Paediatrics, School of Medicine,
Phone: 373 7599 ext 6192
Or 3580825 then enter 4919 and leave a number or voice message at the tone.

Dr. Sue Crengle
Department of Maori and Pacific Health, School of Medicine,
Phone: 373 7599 ex 6470

Dr. Clare Wall
Institute of Food, Nutrition and Human Health, Massey University.
Phone: 443 9748

Dr. Colin Tukuitonga
Department of Maori and Pacific Health, School of Medicine,
Phone: 373 7599 ext 6951

Mrs. Mavis Roberts
Department of Paediatrics, School of Medicine
Phone : 373 7599 ext 6192

Ms. Binky Taua ,
Department of Paediatrics, School of Medicine,
Phone 3737599 ext 6434

Miss Shireen Chua
Institute of Food, Nutrition and Human Health, Massey University
Phone: 5374248 (H)

Kath Donovan
Ph: 527 7993

Ngahiriua Thompson
Ph: 818 7555 or 836 7714

Anna Bonai
Ph : 576 3223

Tali Royal
Ph: 620 4424

Diagnostics Lab
Ph: Lyn Pearce
(work) 357 4297 ext 245
(Mob) 025 956 239

TIMETABLE OF DATA COLLECTION

The timeline for enrolment for each enrolled infant from identification to completion of data collection is four weeks.

Week 1 : Identification of eligibility by recruiter, application of sampling ratio based on ethnicity.

Week 2 : Family invited to enrol in study

- Subject Information sheet handed out
- An appointment is made for interview

Week 3 : Interviewer visit #1

- Informed consent obtained
- Food Frequency Questionnaire (FFQ) completed
- Collection of anthropometric data and blood sample
- Caregiver instructed on how to complete four day weighed food record

Week 3-4 : Caregiver completes four day weighed food record and FFQ. Telephone follow-up by interviewer.

Week 4 : Interviewer visit #2.

- FFQ completed for a second time **before....**
- Demographic Questionnaire completed
- Completion of four day weighed food record checked by interviewer.
- Collection of scales.

SAMPLING PROTOCOL FOR INTERVIEWERS

SELECTION OF THE STUDY SAMPLE

Both the pilot and the main study will use the same sampling methodology. The sampling method that will be used is cluster sampling using random addresses as start points in Auckland. The sample will be stratified by ethnicity (Maori, Pacific Islander, Other). In Auckland equal numbers of each of the 3 ethnic groups will be enrolled. Approximately 40 children will be enrolled in the pilot study and 600 in the main study in Auckland.

Coding of ethnicity

Children will be assigned to an ethnic group based on the ethnicity identified for them by their caregiver. When caregivers indicate multiple ethnicities then ethnicity will be assigned based on the ethnicity that the parent states the child identifies with the most.

Stratification of sample

In the pilot we are aiming to enrol 42 children and assuming that only 80% of those who agree will participate. Therefore we will need to identify 51 children in order to enrol 42. As for the main study we will be enrolling equal numbers of Maori, Pacific Island and European/Other children so we will be aiming to enrol 17 children of each ethnic group with the expectation that only 14 of each ethnic group will complete the study.

Based on the 1991 census 20% of this age group are Maori, 21% are Pacific Islander and 59% are European/Other. Therefore a different proportion of each ethnic group identified will be enrolled. Based on these percentages each eligible Maori child identified will have a 100% chance of being enrolled, each eligible Pacific Island child will have a 95% chance of being enrolled and each eligible European/other child will have a 34% chance of being enrolled.

Therefore for the pilot we will need to identify 17 eligible Maori children to enrol 17, 18 Pacific Islander children to enrol 17 and 50 European/Other children to enrol 17. Therefore 85 eligible children need to be identified to enrol 51.

The process of identifying children to be enrolled is by door knocking using a series of random start points. Ten houses will be visited per start point. Each child identified as eligible from these houses must have a chance of being enrolled. ie it is not appropriate to simply enrol the first 17 children of each ethnic group that are identified. If we did this then we would enrol the European/other part of the sample before the other 2 ethnic groups and then would be approaching houses and indicating at each household that we are only interested in enrolling children from these households if the children were Maori or Pacific Islander.

All eligible children will be identified from each start point. To apply these proportions a table of random numbers is used. This will be done by Cameron and Mavis. The last 2 digits of the random number are selected and expressed as a proportion. This number is then compared to the proportion for the identified child's ethnic group. If the random number is less than or equal to the proportion, then the child is eligible for the study.

Cluster sampling using random addresses as start points was the sampling method used during a recent pilot study conducted in Auckland to identify risk factors for meningococcal disease[1]. In this study 118 children aged < 6 years of age were identified by visiting 62 start points and 10 houses from each start point. Approximately forty-six percent of the children identified by this process were aged 6 to 23 months. Therefore we expect it will require approximately 60 start points to identify our sample of 51 children.

DETAILS OF SAMPLING METHODOLOGY

Study subject identification from each start point

The study recruiter will always move in the same direction from each start point. This direction has been arbitrarily set at **Right**. After visiting the start point, the recruiter will return to the street. S/he will then move to the right, based on the direction when facing the dwelling. The recruiter will continue from dwelling to dwelling in this fashion until a full series of households has been visited.

Town houses, flats, retirement villages and caravan parks will be treated in the same way, except that the common drive will be treated as the street.

A slight variation will be required for apartment blocks and buildings that are arranged vertically. Here the recruiter will move through them in ascending order, based on their number or letter.

Should the starting point be a block of houses, or a set of apartments, the middle number of the houses or apartments will be the starting point to begin.

If the recruiter gets to the start or end of a street, then they will simply continue around the corner staying on the same side of the road. The same rule applies if the recruiter hits the end of a dead end street.

In rare circumstances this process could bring the recruiter back to a household that had already been visited. In this situation, the recruiter should go to the household immediately behind him/herself, when facing the last household visited.

Documentation of household visits

1. Timing of visits

The timing of visits is designed to maximise efficiency, by visiting at times when people are most likely to be at home. It is also designed to reach people who have a range of work and recreational routines.

The initial visits to each starting point should occur on a Saturday between 9.00 am and 6.00 pm or a Sunday between 1.00 pm and 6.00 pm (to avoid church time).

A second visit should occur during the day, Monday through Friday between 9.00 am and 6.00 pm. The ideal time during the week may be between 10.00 am and 12.00 pm when the caregivers are at home.

A third visit should occur on any day different to the previous two visits. If the third visit is on a Monday through Friday, it must be at a different time of day to the previous mid-week

visit. eg. if the previous weekday visit was in the morning, the next weekday visit should be either in the afternoon or early evening.

2. Making return visits

Households where there is no access or no one home MUST be visited again. This step is essential to avoid selecting a sample of children who spend more time than average at home. Up to two subsequent visits must be carried out for each of these households before the attempt is abandoned. All of these visits must be on different days and different times of the day than the initial visit. Households where the caregiver was not home also require a return visit. Ideally, the timing for the visit would have been arranged with someone else in the household.

3. Opportunity to discuss with household, resulting in one or more of the following outcomes:

- Record number of children in household 6-< 23 months of age
    - none → no need to return
    - one or more → attempt to recruit as potential subject
- child present but caregiver out → make appointment for return visit
- caregiver present and study discussed with them
    - agree → record, fill in details about child on Study Log form
    - decline → record, no need to return
- No English spoken by people in the household when visited → record language spoken and either treat as if out and return with person who speaks that language, or treat as decline if they are clearly not interested.

4. No opportunity to discuss with householder

The following strategies should be used when there is no opportunity to discuss with householder, because:

- No access → record reason, and return at a later visit
    - because of a refusal to communicate
    - because of a dog
    - because of another reason → record reason in comments field
- Out → return at a later visit

5. No one home at one address, so information obtained from people at next address

- no child at preceding address → record and return to address at later visit
- one or more children at preceding address → record and return to address at later visit

Recruiters should not take the word of neighbours regarding the question of whether an eligible child resides at a particular address. However if no one is home during a visit, then the next house visited should be asked if there are children living at the previous house. The house in question should still receive return visits regardless of the answer to this question. This information will be used to assess whether many potentially eligible children are being missed by the recruitment process because they are not at home.

6. Starting points in non-residential areas

A small number of starting points that are selected may be in mostly non-residential areas eg. commercial, retail, and industrial areas. These starting points should be rejected from the recruitment process after ensuring that there are no residential properties in the immediate area. To do this the recruiter should first establish that the property at the starting point is non-residential. The recruiter should then proceed from the starting point in the manner described above. If the next 10 addresses visited are also non-residential then this starting point can be rejected. However, if even one property among the 10 visited is residential, then the starting point should be used. Recruiters should make efforts to include households that are attached to commercial premises. eg. people living above shops.

INTERVIEW GUIDELINES (Recruitment Phase)

RECRUITMENT

A) 1) Interviewer introducing themselves
   2) Telling the caregivers what the study is about, give subject info letter
   3) Inquire if they are interested in being involved in the study
   4) If yes, explain the possibility that they may not be in the study but if they are

ENROLLING THE FAMILY

1) Invite family to participate in the study
2) Arrange a date and time that would suit the participant
3) Ask for the name, address, phone number or an alternative phone number.

DATA COLLECTION

B) 1) A Four day weighed food record is explained.
   2) A food frequency questionnaire is administered.
   3) Anthropometric measurements
   4) Analysis of a blood sample

FOLLOW-UP

C) Interviewer will telephone after the first day of the four day weighed food record is completed to support and encourage the participant and ask if they would like to be reminded later on the week to continue doing the weighed food intake. The participant can ask any questions and highlight problems at this stage.

SECOND VISIT

D) 1) The Weighed food records need to be reviewed by the interviewer to make sure that it has been completed correctly.
   2) FFQ administered for a second time **FIRST**, before the general questionnaire.
   3) General Questionnaire will be administered
   4) Collection of Scales
   5) Thank the family for participating in the study.

EQUIPMENT USE

A) TANITA WEIGHING SCALES FOR WEIGHING CHILDREN'S WEIGHT (FOR UPRIGHT CHILD)

To use scale :

Gently tap the start switch that is located on the side of the scale. (Fig 1) "HELO" appears on the screen.

When 0.0 appears after about 1 second, get the child to step on the scale and stand still until a stable reading is displayed.

Note: "0.0" disappears if no one steps on the scale within 5 seconds.
When you step off the scale, the reading returns to "0.0" and the scale will turn off automatically.
"OL" appears when the load weight is in excess of weighing capacity.

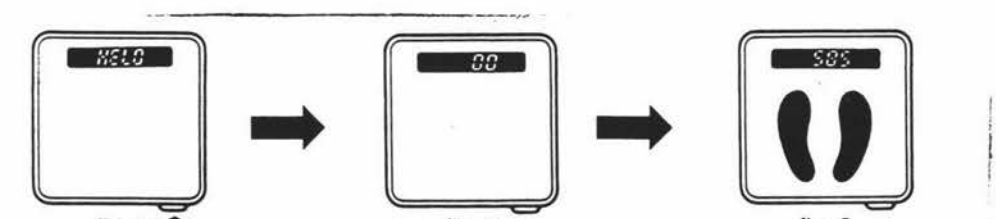


fig. 1 TAP START SWITCH

fig. 2 WAIT FOR 0.0

fig. 3 THEN STEP ON

FOR WEIGHING ACCURACY :
This scale is very sensitive to body movement.
For best accuracy, place scale on a hard surface, step on the scale gently, and stand still.

B) SECA 734 BABY WEIGHING SCALE

To weigh properly ....
Push the "Start" button to activate the scale. 18888 will appear on the digital display. Zero-point calibration occurs automatically. If another value appears, press the START button again until 0 appears. The scale is now ready to use.

After approximately 5 secs, the weight in grams will be indicated.

The scale will power down automatically after 30 – 40 seconds after the constant baby weight has been held in the display or if the scale is not being used.

Switching from Kg/lbs
To change Kg to lbs, press the "Start Key" for approximately 4 seconds.
To revert to Kg in the display, repeat this process.

Tare Calibration
Objects with a weight of 0-3 kg can be tared with this scale. To tare, place object on the scale and then press the start button.

To weigh small children, simply remove the tray. It is fastened to the weighing platform with two clips located right and left on the underside of the tray. Pull both clips out and remove the tray.

C) DIGITAL SCALES

ORDINARY MEASUREMENT
Press ON/TARE. Put object to be weighed on the unit. The display indicates the weight of the object.

NET MEASUREMENT
Press ON/TARE. Put a container on the unit. Press ON/TARE again. (Display reading reset to zero) Put the object to be weighed on the unit. The display indicates the weight of the object.

Press OFF to turn off the power.

OVERLOAD
If the total weight of a container and the object being weighed exceeds the units maximum capacity, the display indicates EEEE.

AUTO POWER OFF
If the same display figure is shown for display for over ten minutes, power is automatically turned off.

NOTE : The maximum capacity that these weighing scales can take is 1kg

FOOD FREQUENCY QUESTIONNAIRES

Food frequency questionnaires are designed to estimate a person's, in this case the child's usual pattern of food intake. The questionnaire aims to find out if the child has eaten certain foods and drunk certain drinks, and the frequency of consumption. From this we can gather the average intake of certain foods and drinks.

In this case, the food frequency questionnaire has been designed to identify foods that are a rich source of Iron and Vitamin A and foods that may have an effect on iron absorption.

This questionnaire will be administered in both interviews. It will be partially administered in that the partipicant's care-giver will fill out the form and if there are any problems or queries, your role as the interviewer is to try and answer the questions.

Note: It is important that your answers will not influence the way the care-giver answers the question. The main role you have is mainly to clarify the questions in a way that the participant will understand them in order to complete them.

It is also important that the food frequency questionnaire will be administered before the general questionnaire.

The instructions are found on the front page of the questionnaire. Please remember to place the child's ID on the questionnaire.

FOUR DAY WEIGHED FOOD RECORD

In order to determine if the food frequency questionnaire is valid, we will be comparing it with the four day weighed food record.

This is another way of measuring a person's food intake, however it requires a greater degree of motivation and compliance on the participant's or in this case the caregivers part.

In the first interview, the caregivers will be given some digital weighing scales and a set of cups (query). You will need to explain to them how to use the scales.

The digital scales are able to be tared, which makes it easier to weigh all the different types of food the infant will be eating.

INSTRUCTIONS

1. When the meal is ready to be dished up, place the plate on the digital scale, record the weight and press TARE/ON. This will zero the scale so that the weight of the plate is not included.
2. Once the scales are zeroed with the plate on it, the food can be added on to the plate as separate entities. For example the rice or potatoes can be weighed first, before the vegetables or meat are added on to it. *The tare or zero button needs to be pressed after each time* so that when the next food is added on, the amount represents only the particular food.
3. If there are any leftovers, the food and the plate are placed on the scales. It is also important to emphasise that they need to specify if they have subtracted the plate weight from the final value.

EATING OUT DIARY

This is provided for the care-giver to record the child's food intake if they are taken out for a meal, or when they are at daycare, Kohanga reo and language nests. It is important to record as accurately as possible the description of the food as well as the amount.

In this case, the care-giver doesn't need to take the weighing scales along.

Offering lots of encouragement and enthusiasm may increase the participant's motivation to do this correctly. Highlighting the benefits of the results of this study may also increase the participant's compliance.

ANTHROPOMETRIC MEASURMENTS

Selected anthropometric measurements will be used to assess overall nutritional status of the child.

The measurements that will be taken are :

1. Weight of the child
2. Height
3. Arm circumference
4. Triceps, biceps and subscapular skinfolds

A) Weight

The weight of the child will be measured with the appropriate weighing scales. If the child is able to stand, the general weighing scales will be used. Or else, the SECA scales where the child is laid on the scale.

B) Height

The height of the child will be measured in the supine position.

C) Arm Circumference

The arm circumference of the child is measured with a fibre glass insertion tape.

The child needs to have his/her arm hanging loosely by the side during the measurement, while you are comfortable with the level that the subject is at. Use the RIGHT arm to take the circumference measurement from.

Mark the mid point of the arm as the diagram below show and position the tape correctly.

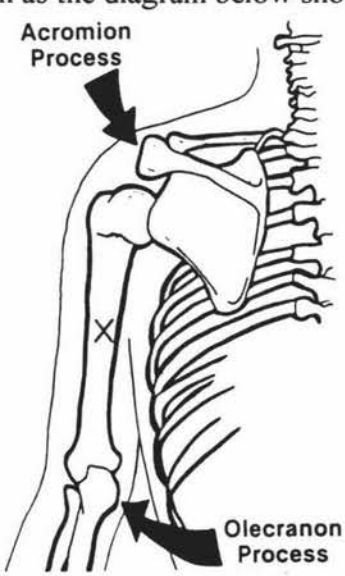


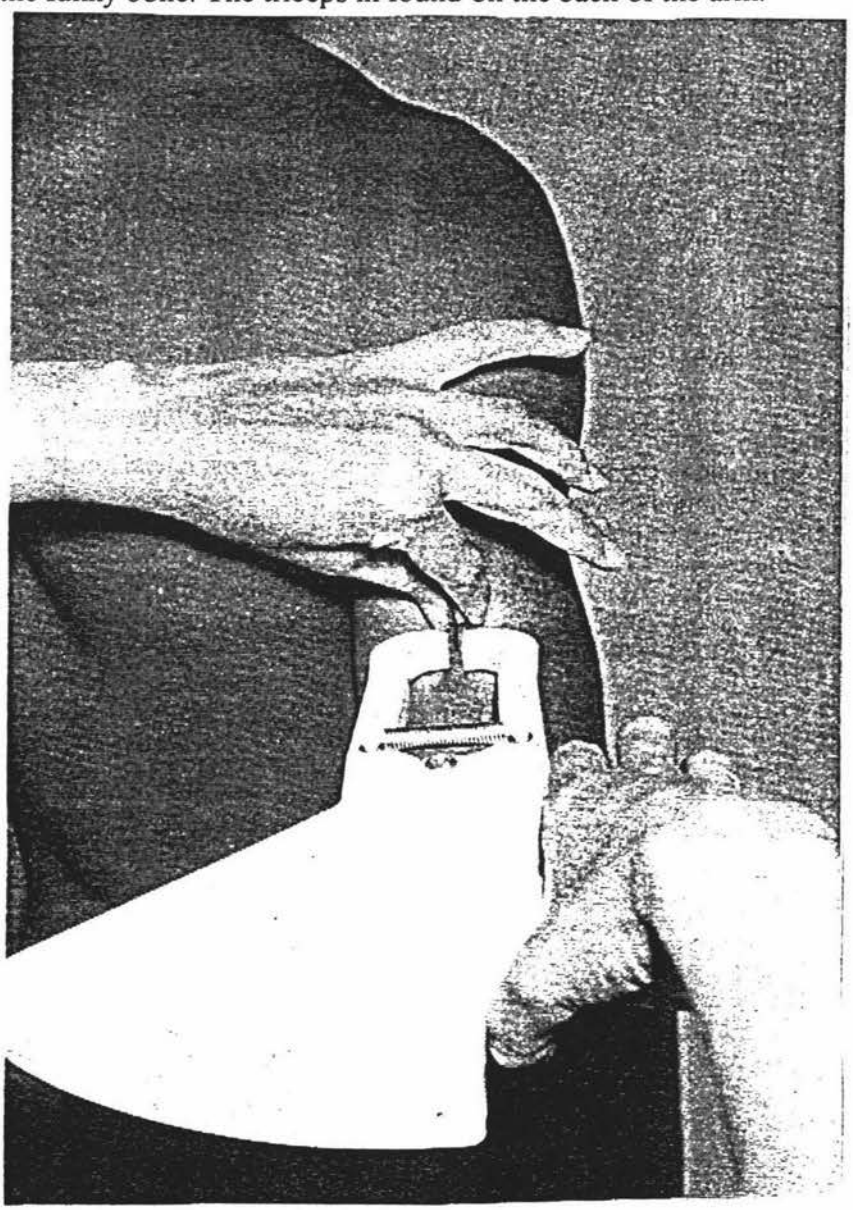
Figure 2.8. Take the triceps skinfold at the midpoint between the acromion process and the olecranon process.

D) SKINFOLDS

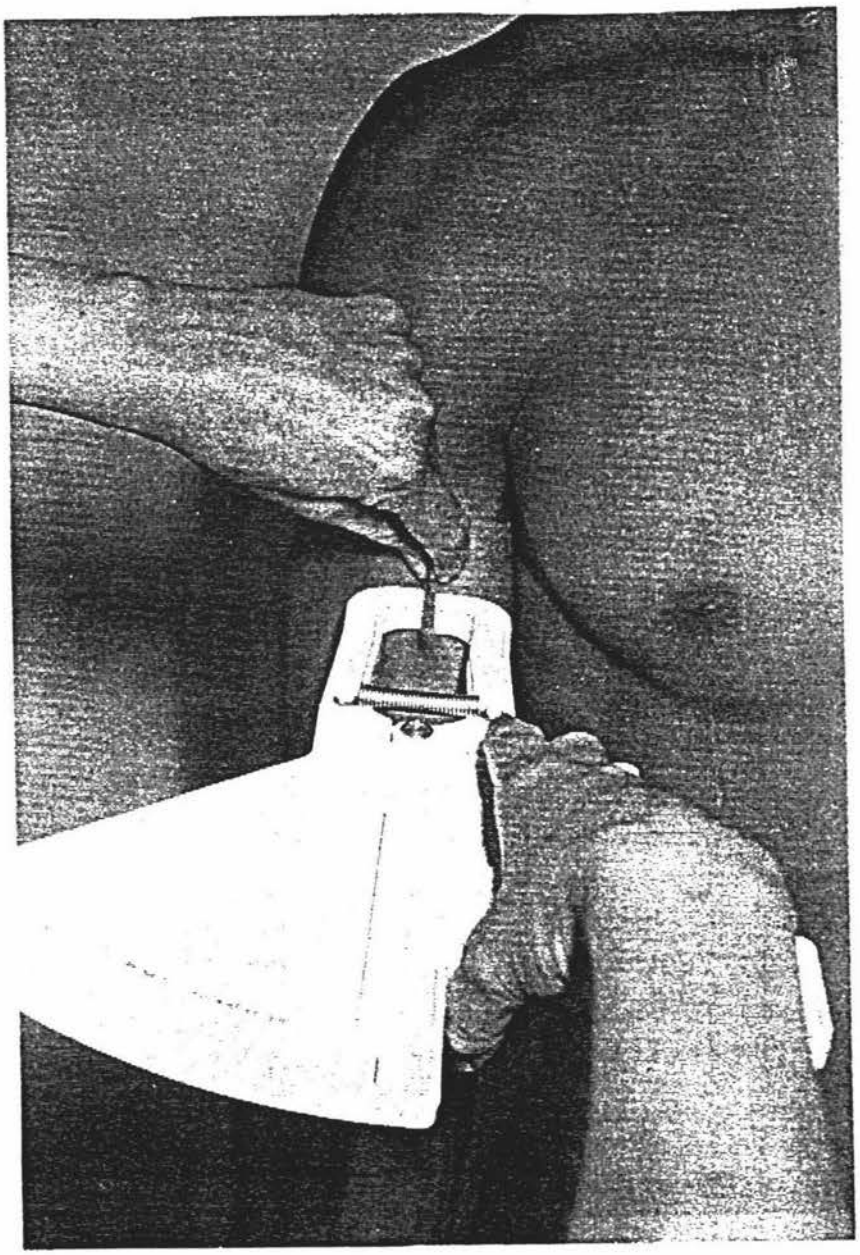
Guidelines :

1. All sites are measured on the RIGHT side of the body.
2. Grasp the skinfold at the designated area with the index finger and thumb of the left hand, with the back of the hand facing the measurer.
3. Hold the calliper on the right hand always, and apply it at RIGHT angles to the fold 1 cm below the thumb and index finger.
4. The fold is grasped firmly and held throughout the measurement.
5. Read after two seconds of applied pressure. This permits full spring pressure of the instrument to be applied by a complete release of the calliper trigger.
6. A complete sequence of all sites is taken before taking a second reading of the same sites. **Three** sets of measures will be taken for skinfolds.

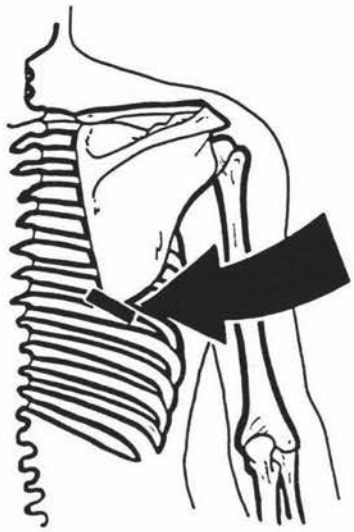
Triceps: Take the triceps skinfold at the midpoint between the end of the shoulder blade and the funny bone. The triceps in found on the back of the arm. (Figure 1)



Biceps: The biceps are found on the front of the arm at the same place where the triceps are. (Figure 2)



Subscapular : This is found at the bottom or the right shoulder blade. (see diagram). (Figure 3)



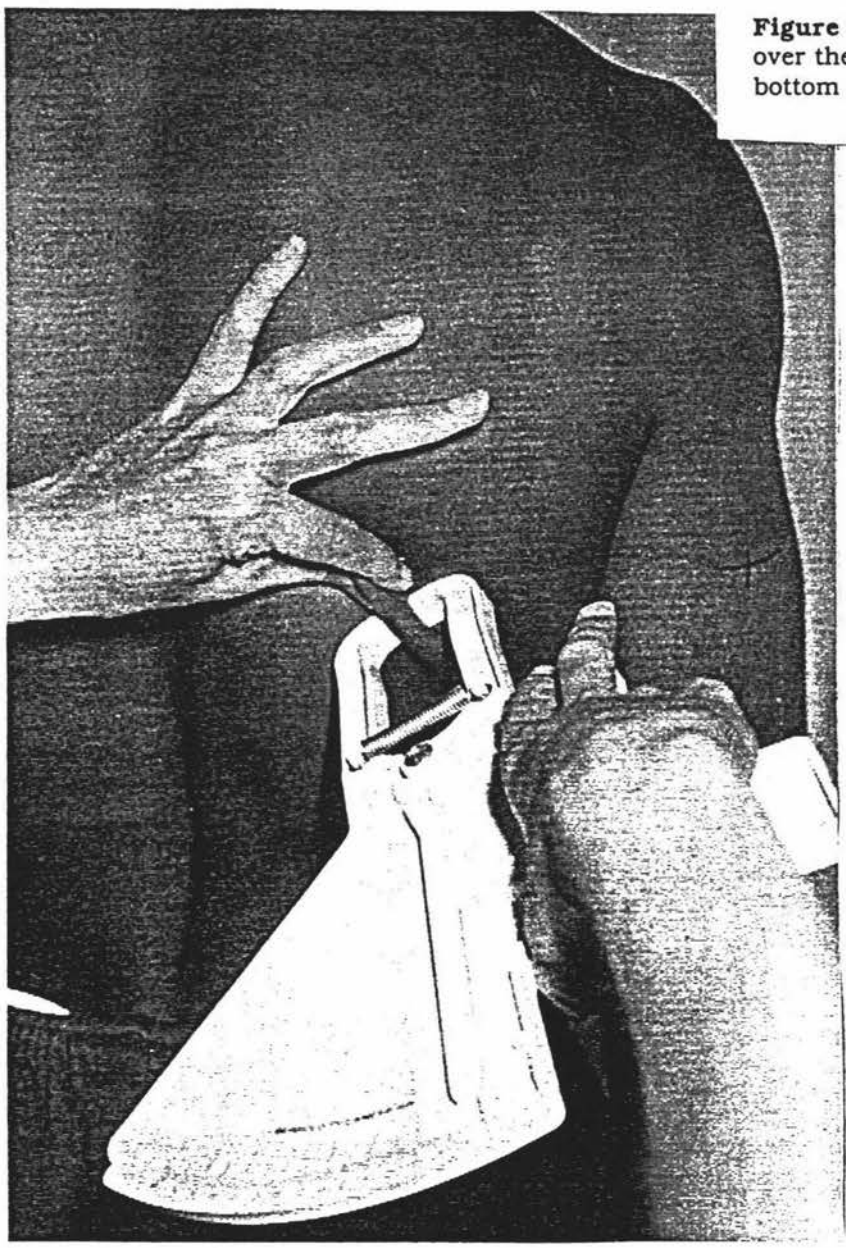


Figure 2.12. Take the subscapular skinfold over the inferior angle of the right scapula (the bottom right "shoulder blade").

PLAY THERAPY AND BLOOD SAMPLE

Play therapy will be used to make it easier for the child to have the blood test (and to make it more likely that the blood sample will be collected. The play therapy Department at the Starship will be providing instruction and play therapy kits. The kits consist of age appropriate toys that can be used to distract the child during the interview and during the blood sampling. Emla cream (a numbing cream) will also be used. This cream is applied to the skin. It numbs the skin when it has been on for 40 to 60 minutes.

When the interviewer arrives at the house she applies the Emla cream to the back of both hands of the child and covers each patch with one of the clear dressings. She then contacts the person at Diagnostic Laboratory to tell them the cream has been applied and the interview started.

When the FFQ, anthropometry and explanation of the 4 day weighed food intake are completed the blood sampling is performed. The phlebotomist from Diagnostic will come to the house to do the blood test. The interviewer assists by doing play therapy during the blood sampling.

At the second interview the play therapy kit is used again to distract the child. At the completion of the second interview the child can chose a toy from the 'treasure box' (this will also be provided by Play therapy)

Some helpful guidelines :

1. Use positive and direct language. Remember to eliminate "Can I" from your vocabulary. "I am going....."
2. Build a routine, therefore avoid using the bedroom or playroom to take the blood sample. The lounge room would be best.
3. Talk with the family, and give the parents the option of leaving the room.
4. Coping procedures : Environment, your attitudes, play, preparation, language, child's understanding . (See play therapy notes)
5. Warn the child that you are leaving, and that you are taking the toys with you to avoid any tantrums.
6. You may need to hold the bruise for at least 3 mins.

Sample of Subject Information Sheet and Consent Form

Iron and Vitamin A Nutrition of Young Children in Auckland

Department of Maori and Pacific Health, University of Auckland,
Department of Paediatrics, University of Auckland
and
Institute for Food, Nutrition and Human Health, Massey University

SUBJECT INFORMATION SHEET

Principal Investigator: Dr. Cameron Grant, Department of Paediatrics, University of Auckland. Ph 373 7599 ext 6192.

Thank-you for your interest in our project. You and your child are invited to take part in this study on iron and vitamin A.

Why are we doing this survey of Auckland children? Iron and vitamin A are both needed by young children to help then grow and learn. Children who do not have enough iron do not learn properly. Children who do not have enough vitamin A become more sick if they get measles or diarrhoea. By participating in this study you will be able to find out if your child has enough iron and vitamin A. You will help us learn why some children do not have enough iron and vitamin A and what could be done to stop this from happening.

Who is in this study?
This study is being done in all of Auckland during 1998, and 1999. Your child has been chosen at random from all children aged 6 to 24 months living in Auckland. This study is being carried out by the Auckland School of Medicine and Massey University.

What is involved? Should you agree to take part in this project, you will be interviewed by a trained interviewer and will be shown how to measure and record what your child eats and drinks for four days. The interviewer will measure and weigh your child. An experienced nurse will take a blood sample (about 2 teaspoons) from your child. We will use an anaesthetic cream on the skin, and some toys and/or a video to make the blood test more pleasant for your child. We will not take blood from your baby if he/she is too upset. Occasionally, some children may have a small amount of bruising in the area where the blood sample was taken, but this normally disappears in a few days. **The visit will take between one and one and a half-hours.**

The interviewer would need to visit your house again once you have finished ,measuring what your child eats.

Do I have to take part in the survey? Your participation and the participation of your child is **entirely voluntary**. If you agree to take part in the study, you are free to withdraw at any time, and this will not disadvantage you in any way or affect the future health care of you or your child. You do not have to answer all the questions, and you may stop the interview at any time. It will not cost you anything to take part in this study. You will be told if your child's blood test show low iron or vitamin A and whether s/he needs to see a doctor.

What will happen to the results? The information about your child and family is completely confidential. No information which could identify you will be used in any reports on this study. The results will be stored by a code number on a computer in the Department of Paediatrics at the University of Auckland. The questionnaires will be stored in a locked room. They will be stored for 10 years then destroyed. You will receive a copy of your child's blood test results and will be told what to do about them. You will receive newsletters about the study to tell you how it is going and what the study found.

. . . and if I have any questions? If you have any questions about our project, either now or in the future, please feel free to us at the Department of Paediatrics, University of Auckland or at the Nutrition Department at Massey University. If you need an interpreter, one can be provided.

Dr. Cameron Grant TEL 373-7599 ext 6192 or Dr Clare Wall TEL 443-9700
If you have any queries or concerns about your rights as a participant in this study you may wish to contact a Health and Disability Services Consumer Advocate, telephone 637 5799

This study has received ethical approval from the North Health Ethics Committee.

The Iron and Vitamin A Nutrition of Young Children in Auckland
Consent form

English	I wish to have an interpreter.	Yes	No
Maori	E hiahia ana ahau ki tetahi tangata hei korero Maori ki ahau.	Ae	Kao
Samoan	Oute mana'o e iai se fa'amatala upu.	Ioe	Leai
Tongan	'Oku fiema'u ha fakatonulea.	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo.	Ae	Kare
Niuean	Fia manako au ke fakaaoga e tagata fakahokohoko vagahau.	E	Nakai

I have read and I understand the information sheet dated _____ for volunteers taking part in the study designed to find out my child has low iron and/or vitamin A levels and if s/he has enough iron in his/her diet. I have had the opportunity to discuss this study . I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect the future health care of myself or my child.

I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study. I understand the compensation provisions for this study.

I have had time to consider whether to take part. I know whom to contact if I have any side effects to the study. I know whom to contact if I have any questions about the medication or the study.

I ..... (full name) hereby consent to take part in this study. Where the participant is a child the consent of the child in addition to the signature of the parent or guardian should be obtained.

If you have any concerns about the study, you may contact:
Mrs. Mavis Roberts, Department of Paediatrics, School of Medicine, ph:3737599 ext 6192

Date
Signature

Full names of Researchers: Cameron Grant, Colin Tukuitonga, Sue Crengle, Clare Wall
Contact Phone Number for researchers 3737599 ext 6192
(Note: A copy of the consent form to be retained by participant and (in the case of patients) a copy to be placed in the medical file.)

SAMPLE OF THE HOME RECORD DIARY

PLEASE START A NEW PAGE FOR EACH DAY EVEN IF ONLY SOME OF THIS PAGE IS USED

Day : _____ Date: _____

Time **Am/pm**	*Food Eaten (Tick)* **Home**	**Away**	**Weighed by (Tick)** **Mother**	**Other**	**Brand Name of Item in Full (except fresh produce)**

TICK A BOX TO SHOW WHICH DAY THIS IS

1 2 3 4

☐ ☐ ☐ ☐

Full Description Dried/frozen/fresh Sweetened or not, cooking method, fat type	Weight of Plate	Weight of Food	Weight of leftovers	Comments Problems , Difficulties

SAMPLE OF THE EATING OUT DIARY

PLEASE START A NEW PAGE FOR EACH DAY EVEN IF ONLY SOME OF THIS PAGE IS USED.

Day _____ Date _____

Time eaten am/pm	Place where it was eaten	Brand name in full unless fresh	Description including price, place it was bought, quantity	Any leftovers?

SAMPLE OF ANTHROPOMETRIC RECORD
SAMPLE OF ANTHROPOMETRIC DATA RECORD SHEET

Name of Child: _____ ID._____

Age:_____

Date: _____

Weight _____ Length:_____

Arm Circumference : _____

Skinfolds:

Skinfolds	**Readings**	**Average**
Triceps #1		
#2		
#3		
Biceps #1		
#2		
#3		
Subscapular #1		
#2		
#3		

CHECK LIST FOR INTERVIEWERS

Visit 1:

- ❑ Reintroduce yourself and the study
- ❑ Check that it is all right to spend 1 – 11/2 hour of their time
- ❑ Informed Consent obtained
- ❑ Emla cream applied to the child
- ❑ Diagnostics Lab Rung
- ❑ Blood sample procedure briefly explained, introduced to the child and mother
- ❑ Explanation of the Weighed food diary – Home record diary
  - Eating out diary
  - Use of Scales
- ❑ Anthropometric measurements taken
  - ❑ Height/Length
  - ❑ Weight
  - ❑ Arm Circumference
  - ❑ Skinfolds
- ❑ Food Frequency Questionnaire Administered
- ❑ Blood sample taken/ Coping procedures
- ❑ Let the child know that you are leaving soon, taking your toys with you.
- ❑ Check all paperwork has an ID number for the baby before leaving
- ❑ Arrange if you need to call them with regards to giving reminders
- ❑ Arrange for a suitable time for second interview.
- ❑ Questions that need answered?

Visit 2:

- ❑ Reintroduce yourself and the study
- ❑ Check that it is all right to spend about 1 – 11/2 hour of their time.
- ❑ Find out if there were any problems
- ❑ Collection of the Home record and Eating out diary – clarify anything that was not clear to you.
- ❑ Collection of the weighing scales
- ❑ Food Frequency Questionnaire administered
- ❑ General Questionnaire administered
- ❑ Explanation of Blood results
- ❑ Giving out of information with regards to nutrition
- ❑ Gift and toy

NOTES :

Appendix I

Estimation of the Bioavailability of Iron

Table 1 : Factors for estimating percent absorption of dietary Iron at increased levels of Iron status (indicated by the quantity of Iron stores)

	Women Iron Status Mg			Men
	0	250	500[a]	100
Haem Iron	35%	28[b]	23[ab]	15
Non-Haem Iron				
A. Low availability meal	5	4	3	2
1. Meat, poultry or fish < 30g (ie. <1 ounce) lean, raw wt				
OR				
2. Ascorbic acid <25 mg				
B. Medium availability meal	10	7	5[a]	3
1. Meat, poultry, or fish 30-90g (ie.1-3 ounce)lean raw wt.				
OR				
2. Ascorbic acid 25 –75 mg				
C. High availability meal	20	12	8[a]	4
1. Meat, poultry, or fish >90g(ie.>3ounce) lean raw wt.				
OR				
2. Ascorbic acid >75ml				
OR				
3. Meat, poultry or fish 30-90g plus ascorbic acid 25-75mg				

[a] The factors for 500mg Iron stores are suggested for most dietary calculations.
[b] These factors are approximate values based on a semi-logarithmic relationship between Iron stores(the linear function) and haem Iron absorption (the logarithmic function)

An Example of a day's calculation of absorbable Iron

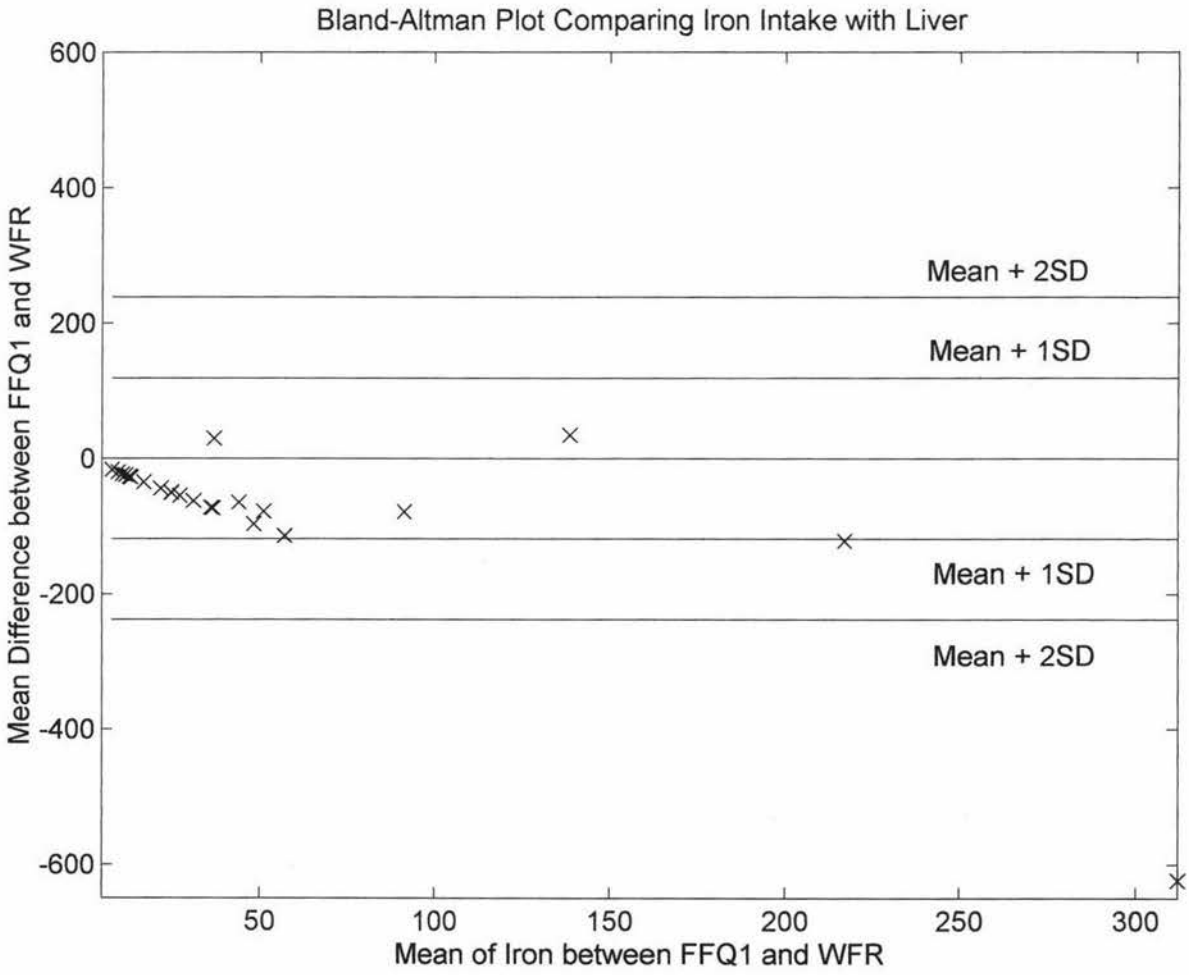
Food	Weight	Total Iron	Heme Factor	Heme Iron	Non Heme Iron	Ascbc Acid	meat	Level avai	%abs	Total
	g	mg			mg		oz			mg
Rice bubbles	28	2.32			2.32	0				
Milk whole fluid	92	0.092			0.092	0.92				
Bread wholemeal	13	0.35			0.35	0				
Butter	3	0			0	0				
Jam	6	0.02			0.02	0.06				
					2.782	*0.98*		Low	5	0.1391
Raisins	14	0.59			0.59	0				
Sultanas	26	0.52			0.52	0				
Ribena	107	0.11			0.11	69.13				
Sub Total					*1.22*	*69.13*		Medium	10	0.122
Sweet corn canned	67	0.34			0.34	1.34				
Cheese cheddar	7	0			0	0				
Bread whlml	4	0.15			0.15	0				
Butter	1	0			0	0				
Marmite	2	0.06			0.06	0				
Pear	50	0.15			0.15	3.04				
					0.7	*4.38*		low	5	0.035
Biscuit	17	0.17			0.17	0				
Muesli bar	19	0.32			0.32	0				
Prune	10	0.11			0.11	0.2				
Subtotal					*0.6*	*0.2*		low	5	0.03
Beef chuck steak	20	6.72	0.4	2.688	4.032	0				
Carrot	20	0.06			0.06	0.8				
Taro	20	0.06			0.06	2.4				
Peas	20	0.32			0.32	2.2				
Potato	20	0.1			0.1	4.2				
Pumpkin	20	0.1			0.1	2.2				
Cauliflower	20	0.12			0.12	11.2				
Ice cream	41	0.04			0.04	0				
Apricot	32	0.13			0.13	1.28				
Bread whlml	19	0.44			0.44	0				
Butter	3	0			0	0				
Subtotal				*2.688*	5.402	24.28		Low	5	0.2701
								Haem	35%	0.9408
Total Absorbable Iron										1.537

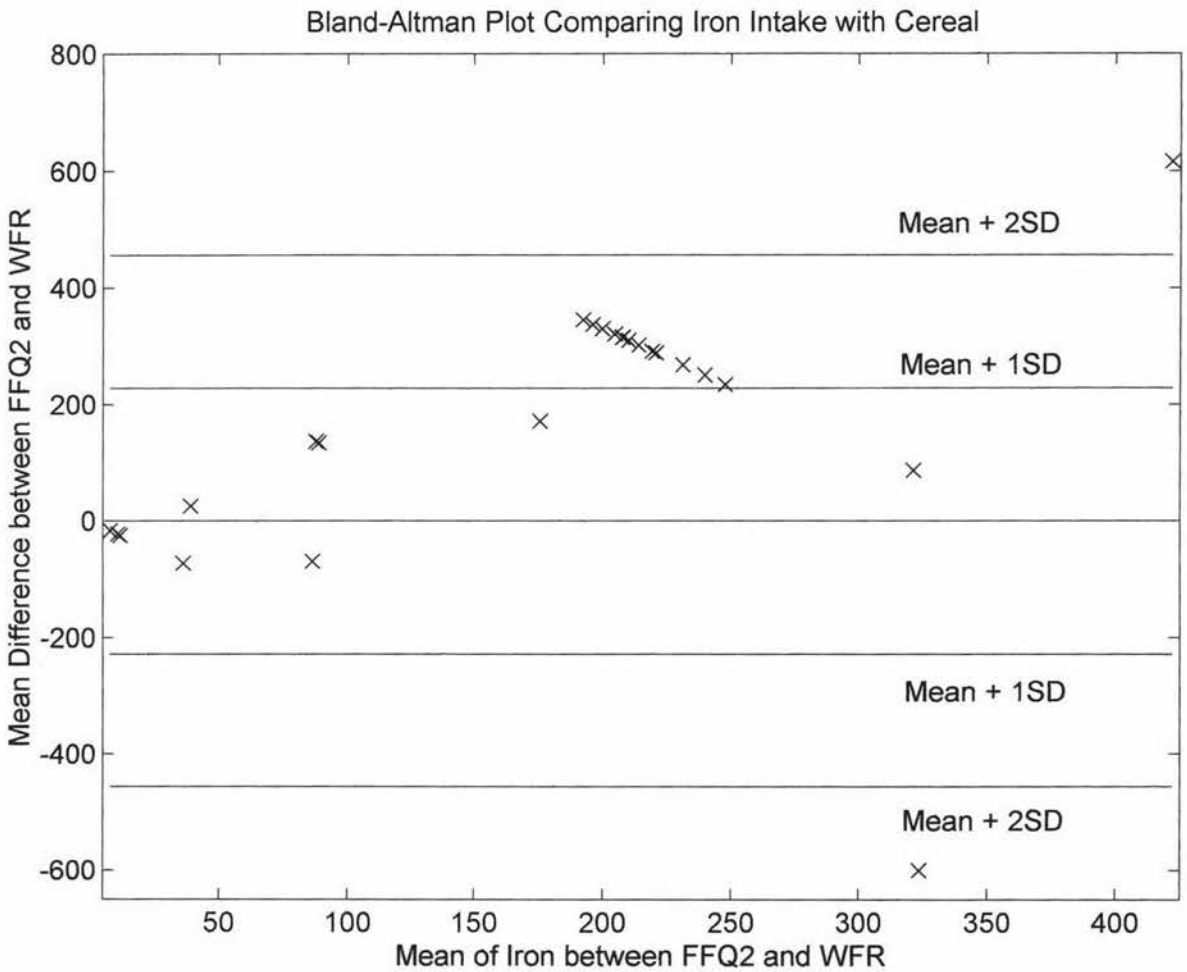
Appendix J

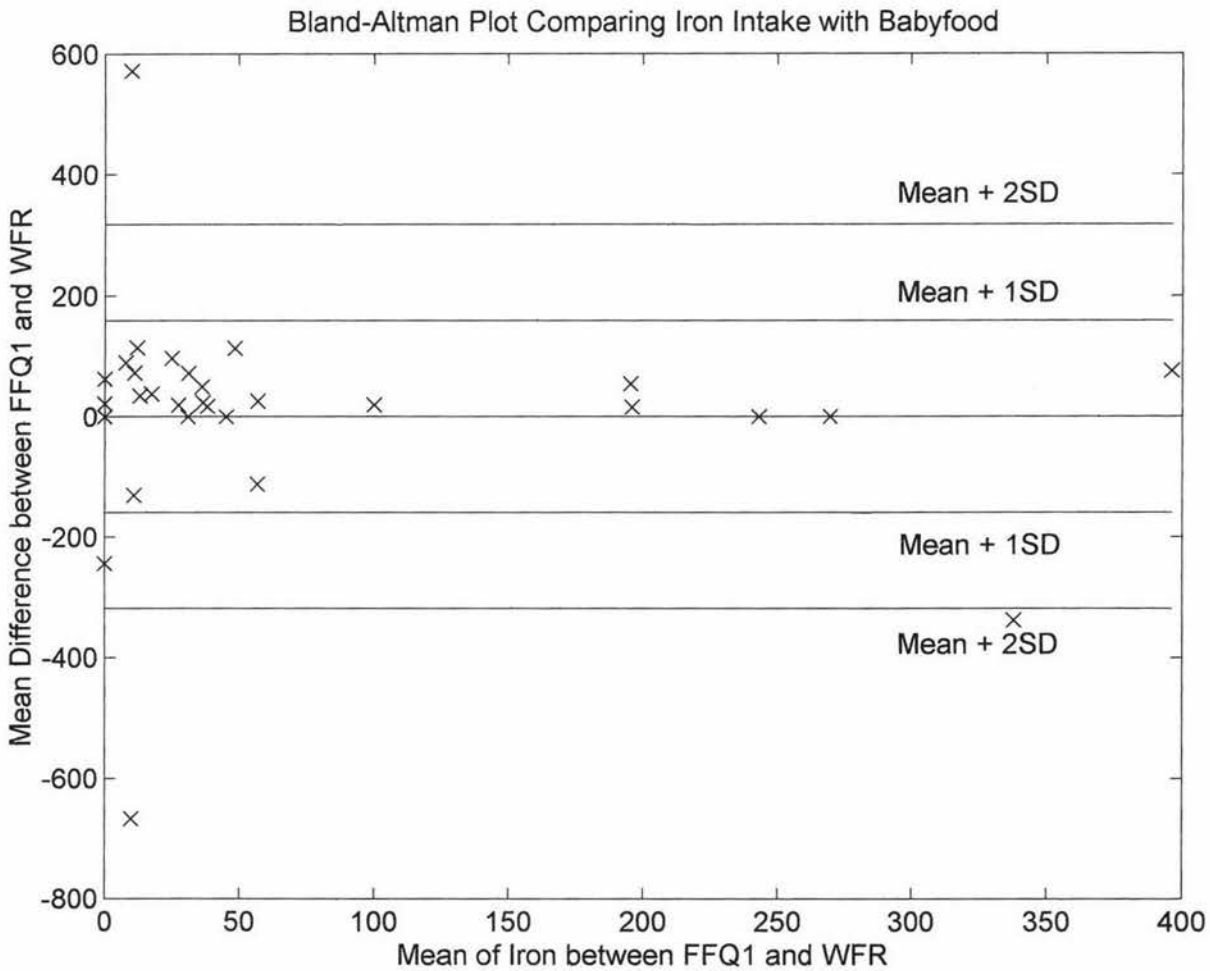
Bland-Altman Plots

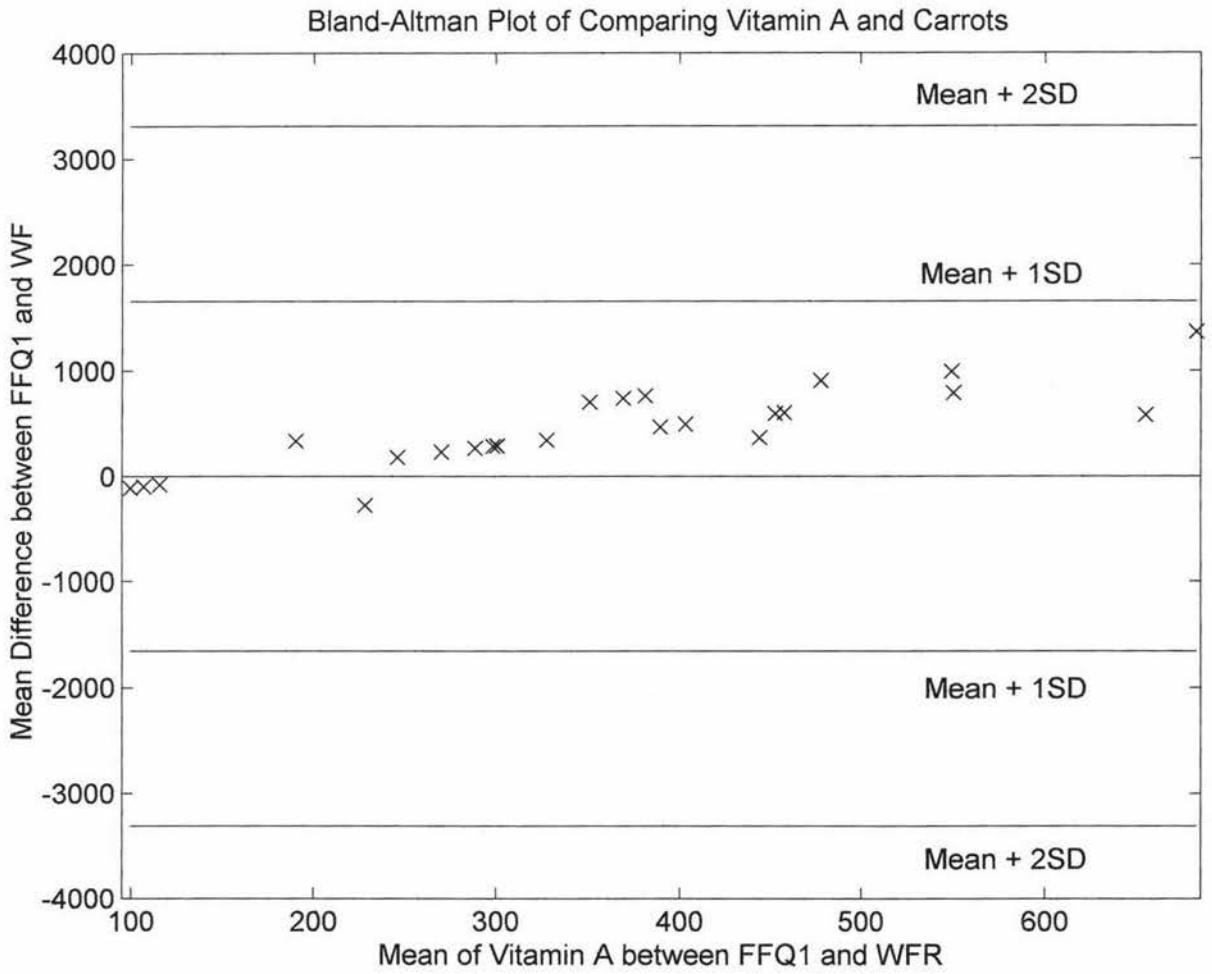
Comparing iron intake with
Liver
Cereals
Babyfood

Comparing vitamin A intake with
Carrots


Bland-Altman Plot Comparing Iron Intake with Liver
Mean Difference between FFQ1 and WFR
Mean of Iron between FFQ1 and WFR
600
400
200
0
-200
-400
-600
50
100
150
200
250
300
Mean + 2SD
Mean + 1SD
Mean + 1SD
Mean + 2SD


Bland-Altman Plot Comparing Iron Intake with Cereal
Mean Difference between FFQ2 and WFR
Mean of Iron between FFQ2 and WFR
Mean + 2SD
Mean + 1SD
Mean + 1SD
Mean + 2SD
800
600
400
200
0
-200
-400
-600
50
100
150
200
250
300
350
400


Bland-Altman Plot Comparing Iron Intake with Babyfood
Mean Difference between FFQ1 and WFR
Mean of Iron between FFQ1 and WFR
Mean + 2SD
Mean + 1SD
Mean + 1SD
Mean + 2SD
600
400
200
0
-200
-400
-600
-800
0
50
100
150
200
250
300
350
400


Bland-Altman Plot of Comparing Vitamin A and Carrots
Mean + 2SD
Mean + 1SD
Mean + 1SD
Mean + 2SD
Mean Difference between FFQ1 and WF
Mean of Vitamin A between FFQ1 and WFR
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
100
200
300
400
500
600