Iron Deficiency in Young Women: Causes, Consequences and Solutions

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Abstract

Background

Iron deficiency is the most common nutritional deficiency worldwide and premenopausal women are at particular risk. Iron deficiency without anaemia is associated with a number of health consequences, including impaired work performance and possible impairments to self-perceived health and well-being, and increased fatigue. Research into iron deficiency and possible causes, consequences and solutions could help to improve the quality of life for many premenopausal women.

Objectives

This research aimed to investigate the causes, some of the consequences and a possible solution to iron deficiency in premenopausal women. Objectives were to determine the relative validity and reproducibility of an iron food frequency questionnaire (FeFFQ) developed to identify iron-related dietary patterns; to identify the most important determinants of suboptimal iron status and investigate the relative importance of dietary patterns among these determinants; to determine the relationship between iron status and self-perceived health, well-being and fatigue; and to investigate the effectiveness of a dietary intervention using an iron-fortified breakfast cereal and milk consumed with either high or low ascorbic acid, lutein and zeaxanthin-rich fruit to improve iron status in women with low iron stores.

Method

In a validation study, premenopausal women (n=115) completed the FeFFQ twice, one month apart to assess reproducibility and a four-day weighed diet record (4DDR) to assess validity. Dietary patterns from both FeFFQs and the 4DDR were identified using factor analysis and agreement between diet pattern scores were compared using correlation coefficients, Bland and Altman analysis, cross-classification and the weighted κ- statistic. In a cross-sectional study, 375 premenopausal women completed the FeFFQ (from which dietary patterns were identified) and a dietary practices questionnaire. They also completed a health and demographic questionnaire including questions regarding possible determinants of iron status, as well as a validated blood loss questionnaire. In a second cross-sectional study, 233 female university students completed the SF-36v2 General Health Survey and Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) questionnaire to investigate self-perceived health, well-being and fatigue. In

both cross-sectional studies, a blood sample was taken to determine iron status (serum ferritin (SF), haemoglobin (Hb), C-reactive protein (CRP)). In a randomised controlled trial (RCT), 69 women with low iron stores (SF≤25µg/L, Hb≥115g/L) received an iron-fortified breakfast cereal (16 mg iron as ferrous sulphate) meal and either kiwifruit (intervention) or banana (control) every day for 16 weeks. Iron status (SF, Hb, CRP, and soluble transferrin receptor) was assessed at baseline and end.

Results

Two dietary patterns ('healthy'; 'sandwich & drinks') were identified from the FeFFQs and 4DDR. Correlation coefficients between the FeFFQ and 4DDR diet pattern scores (validity) were 0.34 ('healthy'), and 0.62 ('sandwich & drinks'), both $P<0.001$. Correlation coefficients between the two FeFFQs (reproducibility) were 0.76 for both dietary patterns ($P<0.001$). Determinants of suboptimal iron status (SF<20µg/L) included blood donation in the past year (odds ratio (OR) 6.7, [95% confidence interval (CI) 3.1, 14.7]; $P<0.001$), being Asian (5.2 [2.4, 11.2]; $P<0.001$), having children (2.7 [1.4, 5.3]; $P=0.003$), previous iron deficiency (2.1 [1.1, 3.9]; $P=0.027$), longer duration of menstrual period (1.3 [1.1, 1.6]; $P=0.01$), and following either a 'milk & yoghurt' (1.4 [1.1, 1.9]; $P=0.014$), or a 'meat & vegetable' (0.6 [0.4, 0.8]; $P=0.002$) dietary pattern. Current iron status was not a determinant of self-perceived health, well-being or fatigue after controlling for other variables. In the RCT, iron status improved significantly ($P<0.001$) in the kiwifruit group (SF from baseline to end (median [25th, 75th percentile]) (17.0 [10.5, 22.0]µg/L to 25.0 [20.0, 32.0]µg/L; $P<0.001$)) compared to the banana group (16.5 [10.0, 20.8]µg/L to 17.5 [12.3, 22.8)]µg/L; $P=0.086$).

Conclusions

The FeFFQ was found to be a reproducible and reasonably valid tool for identifying iron-related dietary patterns. Following a 'meat & vegetable' dietary pattern reduced the risk, while following a 'milk & yoghurt' dietary pattern increased the risk of suboptimal iron status. The strongest predictors of suboptimal iron status were blood donation and Asian ethnicity, followed by parity and previous iron deficiency. Both dietary patterns were stronger predictors of suboptimal iron status than duration of menstrual period. Iron status had no effect on self-perceived health, well-being or fatigue. Consumption of an iron-fortified breakfast cereal with kiwifruit compared to banana improved iron status in women with low iron stores. Modification of dietary patterns and blood donation practices, as well

as the consumption of an iron-fortified breakfast cereal with an ascorbic acid, lutein, zeaxanthin-rich fruit may contribute to improved iron status in women with low iron stores.

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Approval has been obtained from the Massey University Human Ethics Committee (Southern A) for all research described in the thesis.

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Abbreviations

aa	Ascorbic acid
AGP	α_1-acid glycoprotein
B	Baseline
BFI	Brief Fatigue Inventory
BLU	Blood Loss Unit
BMI	Body Mass Index
CI	Confidence Interval
CRP	C reactive protein
DcytB	Duodenal cytochrome B reductase
4DDR	4-Day Diet Record
df	Degrees of freedom
DHQ	Diet History Questionnaire
DMT1	Divalent Metal Transporter 1
DP	Dietary Pattern
DR	Diet Record
DRI	Dietary Reference Intake
E	End
EDTA	Ethylene Diamine Tetraacetic Acid
FAO	Food and Agriculture Organization of the United Nations
FeFFQ	Iron Food Frequency Questionnaire
FFQ	Food Frequency Questionnaire
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
FR	Food Record
FSS	Fatigue Severity Scale
GHQ	General Health Questionnaire
Hb	Haemoglobin
HCP1	Haem Carrier Protein 1
HNRU	Human Nutrition Research Unit
IBC	Iron Binding Capacity
ID	Iron Deficiency

IDA	Iron Deficiency Anaemia
IFNHH	Institute of Food Nutrition and Human Health
IUD	Intra-Uterine Device
KMO	Kaiser-Meyer-Olkin
LOA	Limits Of Agreement
MBL	Menstrual Blood Loss
MCS	Mental Component Summary
MFSI-SF	Multidimensional Fatigue Symptom Inventory – Short Form
MFP	Meat/fish/poultry
n	number
n/a	not applicable
NaFeEDTA	Sodium iron ethylenediaminetetraacetate
nd	Not detectable
NHANES	National Health and Examination Nutrition Survey
NS	Non Significant
NZ	New Zealand
OCP	Oral Contraceptive Pill
OR	Odds Ratio
PASW	Predictive Analytics SoftWare
PCDB	Placebo Controlled Double Blind
PCS	Physical Component Summary
PFS	Piper Fatigue Scale
POMS	Profile of Mood States
Psa-V	*Psuedomona's Syringae* pv. *Actinidiae*
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
RNI	Recommended Nutrient Intake
RPC	Randomised Placebo Controlled
RPCDB	Randomised Placebo Controlled Double Blind
SD	Standard Deviation
SF	Serum Ferritin

SF-36	SF-36v2 Health Survey
SLS-Hb	Sodium lauryl sulphate-Hb
SNP	Single Nucleotide Polymorphism
SPI	Short Performance Inventory
SPSS	Statistical Package for the Social Sciences
SST	Serum Separator Tube
sTfR	Soluble Transferrin Receptor
t	t-statistic
TfR	Transferrin Receptor
TIBC	Total Iron Binding Capacity
TS	Transferrin Saturation
UK	United Kingdom
USA or US	United States of America
VAS	Visual Analogue Scale
VT	Vitality
WHO	World Health Organization
WISE	Women's Iron Status and Education
y	years

CHAPTER 1

Introduction

1 Introduction

Iron deficiency is the most common nutritional deficiency worldwide (Food and Agricultural Organization of the United Nations/World Health Organization 2004). The World Health Organization has ranked anaemia caused by iron deficiency as one of the top ten most important factors contributing to the global burden of disease (McLean et al 2008). It is estimated that 42% of all women in developing countries are anaemic (World Health Organization 2001) (half of which is due to iron deficiency) (Zimmermann and Hurrell 2007), with 2.5 times the number of women with iron deficiency anaemia suffering from non-anaemic iron deficiency (World Health Organization 2001). Iron deficiency is also common in developed countries such as New Zealand, with the 2008/2009 National Adult Nutrition Survey showing 12.1% of women aged 31-50 years had iron deficiency and 6.3% had iron deficiency anaemia (University of Otago and Ministry of Health 2011).

Iron deficiency falls on a continuum ranging from low iron stores (reduced serum ferritin concentration) to iron deficiency anaemia (reduced serum ferritin and haemoglobin concentrations). The assessment of an individual's iron status prior to the development of iron deficiency anaemia is challenging due to a number of factors. These include the range of biochemical indices available to assess iron status, varying terminology and cut-off values for these biochemical indices at each stage of iron deficiency, and confounding factors which affect some of these indices (e.g. day-to-day variability and infection).

While the effects of iron deficiency anaemia on health are well known, less is known about the effects of non-anaemic iron deficiency. Further research is needed in this area due to the large number of women who are affected by non-anaemic iron deficiency. As these women are particularly vulnerable to iron deficiency anaemia, it is important that causes and possible solutions to iron deficiency are also investigated.

2 Causes of iron deficiency

A number of factors contribute to iron deficiency and iron deficiency anaemia, including genetics, infections (e.g. malaria), increased requirements (e.g. growth and pregnancy), inadequate intake or absorption of dietary iron, and iron losses via desquamated skin,

gastrointestinal cells, sweat, urine and blood loss (Coad and Conlon 2011). In women living in developed countries, dietary intake and blood loss through menstruation and the donation of blood are thought to be major contributing factors (Heath et al 2001b).

2.1 Dietary intake and iron status

A number of cross-sectional studies have investigated associations between dietary intake and iron status. However, the results of these studies have been inconsistent. For example, in young women a negative association between calcium intake and iron status has been observed in some (Galan et al 1998, Rangan et al 1997), but not all cross-sectional studies (Brussard et al 1997, Heath et al 2001b, Pynaert et al 2009).

Most studies investigating dietary determinants of iron status have focussed on individual foods and nutrients, which has several limitations relevant to iron nutrition. In most cases, people do not eat foods (e.g. meat) alone, but as meals consisting of a range of foods and nutrients that may interact (Hu 2002, Newby and Tucker 2004). For example, iron absorption is enhanced by ascorbic acid (Diaz et al 2003) and an unidentified factor in meat, fish and poultry (Hurrell et al 2006), while phytic acid (Hurrell et al 2003), polyphenols (Hurrell et al 1999) and calcium (Benkhedda et al 2010) all inhibit iron absorption. Due to the number of foods and nutrients affecting iron absorption it makes sense to consider the whole diet when assessing associations between dietary intake and iron status. Other reasons for considering the whole diet are the possibility that the small effects on iron status of individual foods and nutrients may not be detected (Hu 2002, Newby and Tucker 2004), the high levels of inter-correlation between some nutrients (e.g. calcium and phosphorus) making it difficult to investigate these nutrients separately (Hu 2002, Newby and Tucker 2004), and statistically significant associations that may occur by chance when a number of foods and nutrients are analysed independently (Farchi et al 1989, Hu 2002). Single nutrient analysis may also be confounded by the effect of dietary patterns (Hu 2002). For example, the inhibiting effect of phytic acid (in whole grain breads and cereals) on iron absorption (Siegenberg et al 1991), may be reduced if consumed with ascorbic acid-rich fruit and vegetables as part of a ‘healthy’ dietary pattern.

2.2 Dietary patterns, practices and iron status

The limitations described for single foods and nutrients when investigating associations between dietary intake and iron status may be overcome by the use of dietary pattern analyses. Dietary patterns derived empirically (e.g. using factor or cluster analysis) are an emerging area of research (Hu 2002, Newby and Tucker 2004), and consider how foods and beverages are consumed in the diet as a whole. Public health messages emphasising dietary patterns as opposed to individual foods and nutrients may be easier for the general public to understand (Hu 2002, Newby and Tucker 2004). Surprisingly, very little research has been undertaken to investigate associations between dietary patterns and iron status. One study in China found anaemia was positively associated with 'traditional' and 'sweet tooth' dietary patterns, and negatively associated with a 'healthy' dietary pattern (Shi et al 2006). However, the cause of anaemia was not determined, so it was not known if and how many participants were also iron deficient. In Norway, a 'reindeer meat' dietary pattern was associated with higher serum ferritin concentrations (Broderstad et al 2011). In this study, haemoglobin concentrations were not determined, meaning the severity of iron deficiency was not known. One of the limitations of both of these studies was that in addition to diet, only a limited range of factors that might impact on iron status were considered. Blood loss (e.g. through menstruation and blood donation) as a contributing factor to iron status, was not investigated.

While dietary pattern analysis describes combinations of foods consumed in the diet as a whole, it is also important to investigate dietary practices such as the consumption of foods and beverages in the same meal, as iron absorption is affected by the simultaneous consumption of other foods (Gleerup et al 1993, Gleerup et al 1995). Only a few studies have considered the association between iron status and combinations of foods and beverages eaten at mealtimes (Mennen et al 2007, Razagui et al 1991, van de Vijver et al 1999). In one study of 15 institutionalised women, those with a serum ferritin <12µg/L had a higher tea and lower ascorbic acid intake at meals (Razagui et al 1991). However, other studies have found no association between iron status and timing of calcium intake (van de Vijver et al 1999), or between iron status and whether tea was consumed with or in between meals (Mennen et al 2007).

As a number of components in food affect iron absorption, the investigation of dietary patterns and practices offers a novel and potentially powerful methodology of assessing associations between dietary intake and iron status in premenopausal women. However, it is also necessary to consider the relative importance of dietary patterns and practices in the context of other determinants of iron deficiency such as blood loss and ethnicity. By identifying determinants of iron status, they will be able to be targeted in the prevention and treatment of iron deficiency.

2.3 Assessment of dietary intake

In order to determine dietary patterns in premenopausal women, accurate dietary intake data are needed. Traditional gold standard methods of dietary analysis, such as food records, involve considerable participant and researcher burden (Heath et al 2000). Food frequency questionnaires (FFQs) are commonly used to identify dietary patterns (Broderstad et al 2011, Shi et al 2006). As well as lower participant and researcher burden, FFQs can summarise dietary intake over a longer period of time and therefore may be better able to describe habitual dietary intake (Crozier et al 2008).

While a number of FFQs have been developed to assess dietary intake of iron-rich food sources and or factors affecting iron bioavailability (Heath et al 2000, Heath et al 2005, Matthys et al 2004, Zhou et al 2005), no FFQs have been developed to investigate iron-related dietary patterns. An iron FFQ (FeFFQ) which groups foods according to their iron content, and their potential impact on iron absorption would be of benefit in identifying dietary patterns associated with iron status.

It is important that FFQs are validated against more accurate methods of dietary assessment such as food records to ensure they measure what they are intended to measure (Cade et al 2004). However, only a handful of studies have investigated the validity of an FFQ used to identify dietary patterns using factor analysis (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Nanri et al 2012, Okubo et al 2010, Togo et al 2003), and only three of these studies have considered the reproducibility of dietary patterns by comparing the FFQ with the same FFQ completed at a later date (Hu et al 1999, Khani et al 2004, Nanri et al 2012). The FFQs used in studies investigating dietary patterns and iron status (Broderstad et al 2011, Shi et al 2006) do not

appear to have been validated for foods, nutrients or dietary patterns. If validity and reproducibility can be demonstrated, an FeFFQ would be of particular use for determining how dietary patterns impact on iron status.

3 Consequences of iron deficiency

Iron deficiency is associated with a number of consequences including anaemia. Anaemia is associated with weakness, reduced work tolerance, impaired cognitive development and eventual heart failure (Food and Nutrition Board: Institute of Medicine 2001, MacPhail 2007), and in pregnancy with prematurity, low birth weight and increased maternal and infant mortality (Food and Nutrition Board: Institute of Medicine 2001). Iron deficiency without anaemia is likely to be associated with decreased physical work capacity (Zhu and Haas 1997) and deficits in cognitive function (Murray-Kolb and Beard 2007), although this remains controversial. The research is not clear regarding the effects of non-anaemic iron deficiency on self-perceived health, well-being and fatigue (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Mansson et al 2005, Patterson et al 2000, Patterson et al 2001a, Rangan et al 1998, Verdon et al 2003).

An individual's perception of their own health and well-being is increasingly being recognised as an important indicator of health status. Fatigue is a common concern of women visiting their general practitioner (Ridsdale et al 1993) and is often attributed to iron deficiency (Patterson et al 2000). One group who may be at risk of both iron deficiency and fatigue are students due to diets low in energy (Hendricks et al 2004), and possibly limited finances, time pressures and making the transition from home to independent living.

In previous studies investigating the relationship between iron status and quality of life, iron status has been determined using a range of biochemical indices and cut-off points (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Mansson et al 2005, Patterson et al 2001a, Rangan et al 1998, Verdon et al 2003), or through self-reporting of previous iron deficiency (Patterson et al 2000). In some studies, participants have known their iron status prior to completing questionnaires on health, well-being, and fatigue (Patterson et al 2000, Patterson et al 2001a), which may have influenced their responses. Various tools have been used to measure self-perceived health, well-being and fatigue.

For example, the SF-36v2 Health Survey (SF-36 questionnaire) is one of the most extensively validated and used generic tools for measuring quality of life (Contopoulos-Ioannidis et al 2009) and has previously been used to measure associations between iron status and quality of life (Grondin et al 2008, Patterson et al 2000, Patterson et al 2001a). The Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) is able to measure several dimensions of fatigue (Lim et al 2005), has good psychometric properties (Stein et al 2004, Whitehead 2009), contains no reference to disease (Dittner et al 2004), and does not assume the presence of fatigue (Stein et al 2004). However, the MFSI-SF has not previously been used to investigate the relationship between iron deficiency and fatigue. As female students are likely to be vulnerable to both iron deficiency and fatigue, it is important that this relationship is further investigated using tools such as the SF-36 questionnaire and the MFSI-SF while participants remain unaware of their iron status.

4 Solutions to iron deficiency

Iron deficiency is often treated through dietary intervention, including the addition of fortified foods to the diet (Hurrell et al 2004) or by improving the bioavailability of iron within meals (Rossander-Hulthen and Hallberg 1996). However, only a few studies have investigated the long-term effect of diet on iron status (Heath et al 2001a, Patterson et al 2001b). As well as improving iron status, dietary interventions should be realistic and able to be incorporated into daily life. This is not always easy to achieve. For example, in a dietary intervention study where women received intensive counselling to improve the iron bioavailabilty of their diet, they were only able to increase their intake of meat by 31g per day (Heath et al 2001a). In another intervention study, despite participants being given detailed advice on how to improve the iron bioavailability of their diet, they were unable to significantly change their intake of iron, ascorbic acid or phytate (Patterson et al 2001b).

Ascorbic acid is the best known enhancer of iron absorption (Cook and Monsen 1977, Diaz et al 2003). Paradoxically, studies investigating the effect of consuming ascorbic acid with meals have shown little or no effect on iron status (Cook et al 1984, Garcia et al 2003, Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986). However, in these studies ascorbic acid has been added to meals containing limited amounts of iron, meaning ascorbic acid may not be able to exert its effect on iron absorption. Further

limitations of these studies have included the use of participants with normal iron stores (who absorb less iron), small sample sizes, and studies of short duration.

As breakfast is a relatively easy meal to make changes to (i.e. involves replacing one breakfast item for another), consuming an iron-fortified breakfast cereal with gold kiwifruit (high in ascorbic acid) may be a practical and realistic dietary intervention to incorporate into everyday life. Kiwifruit are also high in the carotenoids, lutein and zeaxanthin. These carotenoids have recently been shown to enhance iron absorption when added to a wheat-based breakfast meal (Garcia-Casal 2006). A food-based approach offers a potentially sustainable method of addressing iron deficiency (World Health Organization 2001), and may provide other nutritional benefits compared with nutrient supplementation alone.

For these reasons, it would be beneficial to determine whether consuming an iron-fortified breakfast cereal with gold kiwifruit improves iron status in healthy women with low iron stores.

5 Study aim and objectives

The aim of this research is to investigate the causes, some of the consequences and a possible solution to iron deficiency in premenopausal women.

The objectives of this research are to:

- Investigate the relative validity and reproducibility of a FeFFQ specifically designed to identify iron-related dietary patterns in premenopausal women.

- Investigate the dietary patterns and practices of premenopausal women in relation to risk of suboptimal iron status.

- Investigate the relative importance of dietary patterns and non-dietary factors associated with suboptimal iron status in premenopausal women.

- Determine the relationship between iron depletion and self-perceived health, well-being and fatigue in a female university student population.

- Investigate whether consuming a high versus low ascorbic acid, lutein, zeaxanthin-rich fruit (gold kiwifruit versus banana) with an iron-fortified breakfast cereal and milk every day for 16 weeks improves iron status in women with low iron stores.

6 Study hypotheses

Hypothesis 1: The FeFFQ will be a valid and reliable tool for the assessment of food groups and iron-related dietary patterns.

Hypothesis 2: Certain dietary patterns will increase or decrease the risk of suboptimal iron status in premenopausal women.

Hypothesis 3: These dietary patterns will still be significant predictors of suboptimal iron status when other potential determinants of iron status (e.g. blood loss) are controlled for.

Hypothesis 4: The self-perceived health and well-being will be lower and fatigue will be higher in iron depleted compared with iron sufficient female university students.

Hypothesis 5: The consumption of an iron-fortified breakfast cereal and gold kiwifruit (high in ascorbic acid, lutein and zeaxanthin) every day for 16 weeks will improve iron status in women with low iron stores.

7 Structure of thesis

This thesis begins with a review of the literature focusing on the causes, consequences and possible solutions to iron deficiency in young women. This is followed by five manuscripts presenting the results of this research. As each study is presented in the form of a manuscript suitable for publication, there may be some repetition throughout the thesis. The first study investigated the relative validity and reproducibility of a FeFFQ

specifically designed to identify iron-related dietary patterns in premenopausal women (chapter three). The second study explored the dietary patterns and practices of premenopausal women in relation to risk of suboptimal iron status (chapter four). This was followed by a third study which investigated the relative importance of dietary patterns and non-dietary factors associated with suboptimal iron status in premenopausal women (chapter five). The fourth study considered the relationship between iron depletion and self-perceived health, well-being and fatigue in a female university student population (chapter six). The final study investigated whether consuming high versus low ascorbic acid, lutein, zeaxanthin-rich fruit (gold kiwifruit versus banana) with an iron-fortified breakfast cereal and milk every day for 16 weeks improved iron status in women with low iron stores (chapter seven). The source of participants for the various studies are shown in figure 1.1. The thesis is concluded with a discussion which brings together the main results observed including their significance and relevance, and methodological strengths and limitations. Final conclusions are drawn bringing the thesis to a close.

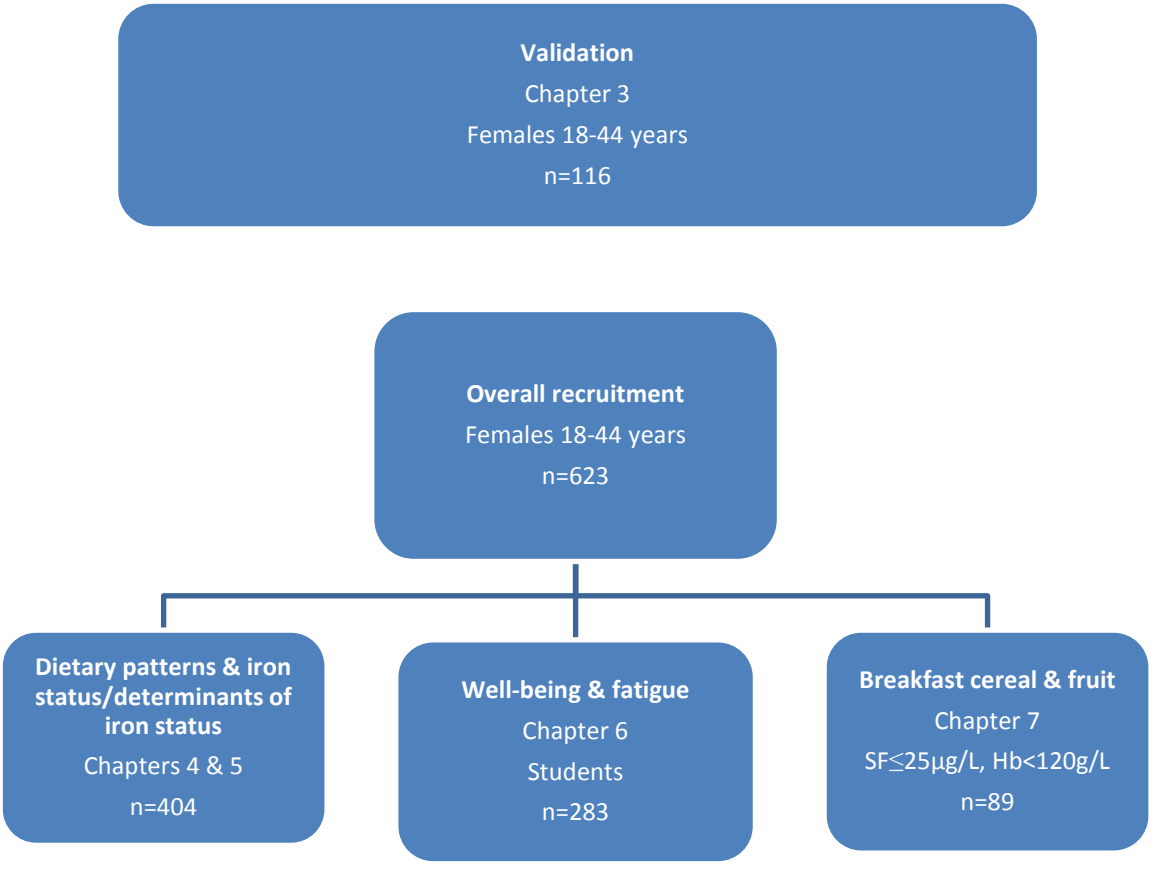

Figure 1.1. Source of participants for the various studies

8 References

Ambrosini GL, O'Sullivan TA, de Klerk NH, Mori TA, Beilin LJ, Oddy WH (2011). Relative validity of adolescent dietary patterns: a comparison of a FFQ and 3 d food record. *Br J Nutr* **105:** 625-633.

Benkhedda K, L'Abbe MR, Cockell KA (2010). Effect of calcium on iron absorption in women with marginal iron status. *Br J Nutr* **103:** 742-748.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Brussard JH, Brants HAM, Bouman M, Lowik MRH (1997). Iron intake and iron status among adults in the Netherlands. *Eur J Clin Nutr* **51:** S51-S58.

Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM (2004). Food-frequency questionnaires: a review of their design, validation and utilisation. *Nutr Res Rev* **17:** 5-22.

Coad J, Conlon C (2011). Iron deficiency in women: assessment, causes and consequences *Curr Opin Clin Nutr Metab Care* **14:** 625-634.

Contopoulos-Ioannidis DG, Karvouni A, Kouri I, Ioannidis JPA (2009). Reporting and interpretation of SF-36 outcomes in randomised trials: systematic review. *Br Med J* **338:** 1-6.

Cook JD, Monsen ER (1977). Vitamin C, the common cold and iron absorption. *Am J Clin Nutr* **30:** 235-241.

Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS (1984). The effect of high ascorbic acid supplementation on body iron stores. *Blood* **64:** 721-726.

Crozier SR, Inskip HM, Godfrey KM, Robinson SM (2008). Dietary patterns in pregnant women: a comparison of food-frequency questionnaires and 4d prospective diaries. *Br J Nutr* **99:** 869-875.

Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP (2003). The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78:** 436-440.

Dittner AJ, Wessely SC, Brown RG (2004). The assessment of fatigue - A practical guide for clinicians and researchers. *J Psychosom Res* **56:** 157-170.

Duport N, Preziosi P, Boutron-Ruault MC, Bertrais S, Galan P, Favier A *et al* (2003). Consequences of iron depletion on health in menstruating women. *Eur J Clin Nutr* **57:** 1169-1175.

Farchi G, Mariotti S, Menotti A, Seccareccia F, Torsello S, Fidanza F (1989). Diet and 20-y mortality in two rural population groups of middle-aged men in Italy *Am J Clin Nutr* **50:** 1095-1103.

Food and Agricultural Organization of the United Nations/World Health Organization (2004). Iron. *Vitamin and Mineral Requirements in Human Nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand*, 2nd edn. World Health Organization: Geneva. pp 246-278.

Food and Nutrition Board: Institute of Medicine (2001). Iron. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. National Academy Press: Washington, DC. pp 290-393.

Fordy J, Benton D (1994). Does iron status influence psychological functioning? *J Hum Nutr Diet* **7:** 127-133.

Galan P, Yoon HC, Preziosi P, Viteri F, Valeix P, Fieux B *et al* (1998). Determining factors in the iron status of adult women in the SU.VI.MAX study. *Eur J Clin Nutr* **52:** 383-388.

Garcia-Casal MN (2006). Carotenoids increase iron absorption from cereal-based food in the human. *Nutr Res* **26:** 340-344.

Garcia OP, Diaz M, Rosado JL, Allen LH (2003). Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. *Am J Clin Nutr* **78:** 267-273.

Gleerup A, Rossander-Hulten L, Hallberg L (1993). Duration of the inhibitory effect of calcium on non-haem iron absorption in man. *Eur J Clin Nutr* **47:** 875-879.

Gleerup A, Rossander-Hulten L, Gramatkovski E, Hallberg L (1995). Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* **61:** 97-104.

Grondin M-A, Ruivard M, Perreve A, Derumeaux-Burel H, Perthus I, Roblin J *et al* (2008). Prevalence of iron deficiency and health-related quality of life among female students. *J Am Coll Nutr* **27:** 337-341.

Heath ALM, Skeaff CM, Gibson RS (2000). The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. *Eur J Clin Nutr* **54:** 592-599.

Heath ALM, Skeaff CM, O'Brien SM, Williams SM, Gibson RS (2001a). Can dietary treatment of non-anemic iron deficiency improve iron status? *J Am Coll Nutr* **20:** 477-484.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001b). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Heath ALM, Roe MA, Oyston SL, Fairweather-Tait SJ (2005). Meal-based intake assessment tool: relative validity when determining dietary intake of Fe and Zn and selected absorption modifiers in UK men. *Br J Nutr* **93:** 403-416.

Hendricks KM, Herbold N, Fung T (2004). Diet and other lifestyle behaviors in young college women. *Nutr Res* **24:** 981-991.

Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al* (1999). Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* **69:** 243-249.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH (1990). Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *Am J Clin Nutr* **51:** 649-655.

Hunt JR, Gallagher SK, Johnson LK (1994). Effect of ascorbic acid on apparent iron absorption by women with low iron stores. *Am J Clin Nutr* **59:** 1381-1385.

Hurrell RF, Reddy M, Cook JD (1999). Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br J Nutr* **81:** 289-295.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* **77:** 1213-1219.

Hurrell RF, Lynch S, Bothwell T, Cori H, Glahn RP, Hertrampf E *et al* (2004). Enhancing the absorption of fortification iron: A SUSTAIN task force report. *Int J Vitam Nutr Res* **74:** 387-401.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2006). Meat protein fractions enhance nonheme iron absorption in humans. *J Nutr* **136:** 2808-2812.

Kandiah J (2002). Impact of tofu or tofu + orange juice on hematological indices of lacto-ovo vegetarian females. *Plant Foods Hum Nutr* **57:** 197-204.

Khani BR, Ye WM, Terry P, Wolk A (2004). Reproducibility and validity of major dietary patterns among Swedish women assessed with a food-frequency questionnaire. *J Nutr* **134:** 1541-1545.

Lim WJ, Hong S, Nelesen R, Dimsdale JE (2005). The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. *Arch Intern Med* **165:** 910-915.

MacPhail AP (2007). Iron. In: Mann J, Truswell AS (eds). *Essentials of human nutrition*, 3rd edn. Oxford University Press: Oxford. pp 125-137.

Malone H, Kevany J, Scott J, O'Broin S, O'Connor G (1986). Ascorbic acid supplementation: it's effects on body iron stores and white blood cells. *Irish J Med Sci* **155:** 74-79.

Mansson J, Johansson G, Wiklund M, Baigi A, Marklund B (2005). Symptom panorama in upper secondary school students and symptoms related to iron deficiency - Screening with laboratory tests, questionnaire and interventional treatment with iron. *Scand J Prim Health Care* **23:** 28-33.

Matthys C, Pynaert I, Roe M, Fairweather-Tait SJ, Heath ALM, De Henauw S (2004). Validity and reproducibility of a computerised tool for assessing the iron, calcium and vitamin C intake of Belgian women. *Eur J Clin Nutr* **58:** 1297-1305.

McLean E, Cogswell M, Egli I, Woidyla D, de Benoist B (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* **12:** 444-454.

Mennen L, Hirvonen T, Arnault N, Bertrais S, Galan P, Hercberg S (2007). Consumption of black, green and herbal tea and iron status in French adults. *Eur J Clin Nutr* **61:** 1174-1179.

Murray-Kolb LE, Beard JL (2007). Iron treatment normalizes cognitive functioning in young women. *Am J Clin Nutr* **85:** 778-787.

Nanri A, Shimazu T, Ishihara J, Takachi R, Mizoue T, Inoue M *et al* (2012). Reproducibility and validity of dietary patterns assessed by a food frequency questionnaire used in the 5-

year follow-up survey of the Japan public health center-based prospective study. *J Epidemiol* **22:** 205-215.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Okubo H, Murakami K, Sasaki S, Kim MK, Hirota N, Notsu A *et al* (2010). Relative validity of dietary patterns derived from a self-administered diet history questionnaire using factor analysis among Japanese adults. *Public Health Nutr* **13:** 1080-1089.

Patterson A, Brown W, Powers J, Roberts D (2000). Iron deficiency, general health and fatigue: Results from the Australian longitudinal study on women's health. *Qual Life Res* **9:** 491-497.

Patterson AJ, Brown WJ, Roberts DCK (2001a). Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. *J Am Coll Nutr* **20:** 337-342.

Patterson AJ, Brown WJ, Roberts DCK, Seldon MR (2001b). Dietary treatment of iron deficiency in women of childbearing age. *Am J Clin Nutr* **74:** 650-656.

Pynaert I, De Bacquer D, Matthys C, Delanphe J, Temmerman M, De Backer G *et al* (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutr* **12:** 1775-1782.

Rangan AM, Aitkin I, Blight GD, Binns CW (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pac J Clin Nutr* **6:** 291-295.

Rangan AM, Blight GD, Winns CW (1998). Iron status and non-specific symptoms of female students. *J Am Coll Nutr* **17:** 351-355.

Razagui IB, Barlow PJ, Izmeth MGA, Taylor KDA (1991). Iron status in a group of long-stay mentally handicapped menstruating women: some dietary considerations. *Eur J Clin Nutr* **45:** 331-340.

Ridsdale L, Evans A, Jerrett W, Mandalia S, Osler K, Vora H (1993). Patients with fatigue in general practice - a prospective study. *Br Med J* **307:** 103-106.

Rossander-Hulthen L, Hallberg L (1996). Dietary factors influencing iron absorption - an overview. In: Hallberg L, Asp N (eds). *Iron Nutrition in Health and Disease*. John Libbey and Company Ltd: London. pp 105-115.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG *et al* (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* **53:** 537-541.

Stein KD, Jacobsen PB, Blanchard CM, Thors C (2004). Further validation of the multidimensional fatigue symptom inventory-short form. *J Pain Symptom Manage* **27:** 14-24.

Togo P, Heitmann BL, Sorensen TIA, Osler M (2003). Consistency of food intake factors by different dietary assessment methods and population groups. *Br J Nutr* **90:** 667-678.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

van de Vijver LPL, Kardinaal AFM, Charzewska J, Rotily M, Charles P, Maggiolini M *et al* (1999). Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *J Nutr* **129:** 963-968.

Verdon F, Burnand B, Stubi CLF, Bonard C, Graff M, Michaud A *et al* (2003). Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *Br Med J* **326:** 1124-1127.

Whitehead L (2009). The Measurement of Fatigue in Chronic Illness: A Systematic Review of Unidimensional and Multidimensional Fatigue Measures. *J Pain Symptom Manage* **37:** 107-128.

World Health Organization (2001). *Iron Deficiency Anaemia: Assessment, Control and Prevention. A guide for programme managers*. World Health Organization: Geneva.

Zhou SJ, Schilling MJ, Makrides M (2005). Evaluation of an iron specific checklist for the assessment of dietary iron intake in pregnant and postpartum women. *Nutrition* **21:** 908-913.

Zhu YI, Haas JD (1997). Iron depletion without anemia and physical performance in young women. *Am J Clin Nutr* **66:** 334-341.

Zimmermann MB, Hurrell RF (2007). Nutritional iron deficiency. *Lancet* **370:** 511-520.

CHAPTER 2

Review of the literature

1 Introduction

Iron deficiency is the most common nutritional deficiency worldwide and is a major public health concern. There is a wealth of literature available on iron nutrition ranging from studies on the cellular mechanisms of iron absorption through to dietary interventions in humans aimed at addressing iron deficiency. However, controversy and questions remain regarding aspects related to the causes and consequences of, and possible solutions to iron deficiency.

Much of what we understand regarding the role and physiology of iron in the body comes from in vitro studies and experiments in animals. This work and findings from human studies, including national nutrition surveys have been used to inform Recommended Dietary Intakes (RDIs) for iron. These will be reviewed in both the New Zealand and global context.

An important step in investigating iron deficiency is the clarification of the stages and biochemical measures of iron deficiency. This will enable an in-depth review of the prevalence of iron deficiency in New Zealand and in specific population groups.

Although the consequences of iron deficiency anaemia have been comprehensively investigated, less is known about the consequences of non-anaemic iron deficiency, and the impact of non-anaemic iron deficiency on self-perceived health, well-being and fatigue is controversial. This will be examined.

Both dietary and non-dietary factors that may contribute to iron deficiency will be investigated. This will include the available evidence regarding the impact of dietary factors on iron absorption, and results from cross-sectional and intervention studies investigating factors associated with or affecting iron status.

The advantages and limitations of possible solutions to iron deficiency will be discussed. The focus will be on dietary solutions to iron deficiency, including the efficacy of iron fortification and ascorbic acid in improving iron status.

Finally, the literature review will investigate various methods of assessing dietary intake related to iron. This will include a review of dietary pattern analysis, the development of food frequency questionnaires (FFQs), and ways to assess the validity and reproducibility of FFQs. Previous work regarding the validity and reproducibility of FFQs designed to assess dietary patterns will be reviewed.

2 The role of iron in the body

Prior to investigating the causes, consequences and possible solutions to iron deficiency it is important to understand the role of iron in the body.

2.1 Iron distribution

The human body contains 2 to 4g of iron (Bothwell et al 1979). Women have less iron in the body than men due to smaller erythrocyte mass and iron stores (Food and Nutrition Board: Institute of Medicine 2001), and are more vulnerable to iron deficiency (McLean et al 2008). The majority of iron in the body (60-70%) is found in haemoglobin (Hb) in red blood cells. Approximately 10% is found in the myoglobin of muscles. The remaining iron is stored as ferritin and haemosiderin in the liver (20-30%), incorporated into iron containing enzymes (approximately 1%), and bound to transferrin, a transport protein in the blood (<0.2%) (Scientific Advisory Committee on Nutrition 2010).

2.2 Functions of iron

Iron has several vital roles within the body. Haemoglobin carries oxygen from the lungs to the body's tissues, while myoglobin is important for the storage and use of oxygen in muscles. Iron exists in two valency states: the reduced ferrous form (Fe^{2+}) and the oxidised ferric form (Fe^{3+}). Iron is able to accept or donate electrons, meaning iron is an efficient catalyst for electron transfer and free radical reactions (Scientific Advisory Committee on Nutrition 2010). The reactivity of iron also means iron is potentially toxic. Several iron-containing enzymes are involved in oxidative phosphorylation, while others are involved in the detoxification of foreign substances in the liver, synthesis of steroid

hormones and bile acids, and signaling by some neurotransmitters (Food and Agricultural Organization of the United Nations/World Health Organization 2004).

2.3 Iron metabolism

Iron metabolism is a fine balance between meeting the body's requirements for iron, while minimising the risk of iron toxicity. Iron absorption occurs in the small intestine, mainly in the duodenum (Gitlin and Cruchaud 1962, Muir and Hopfer 1985). Figure 2.1 illustrates iron absorption in an individual enterocyte for a healthy individual.

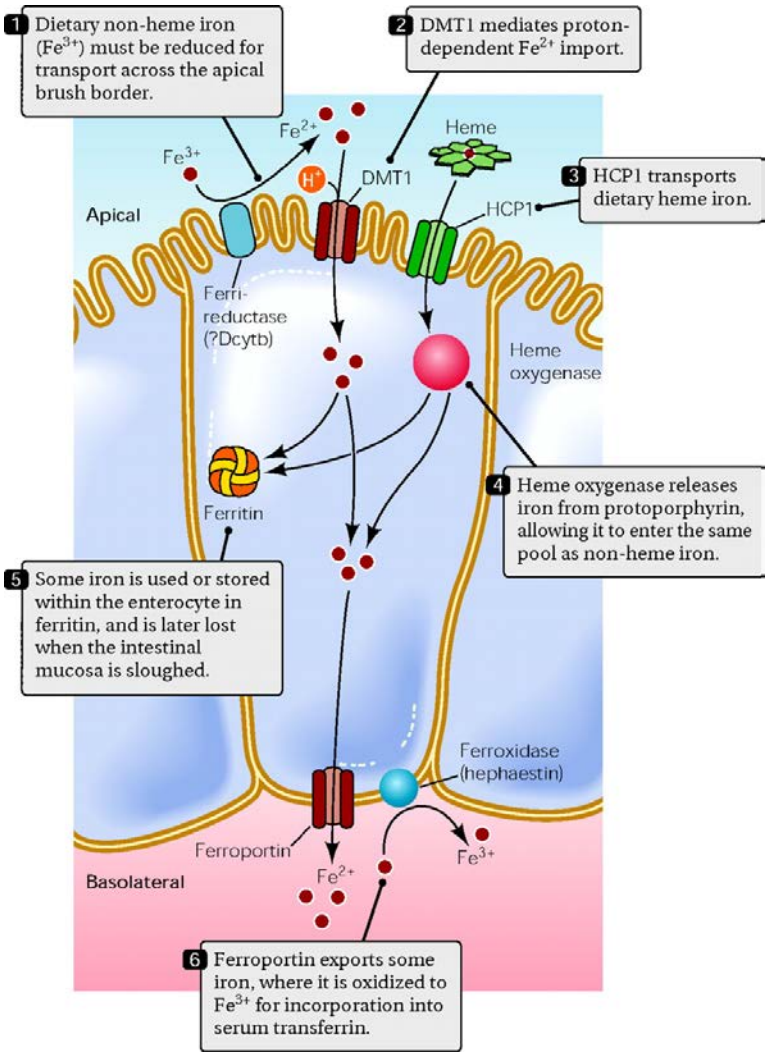

Figure 2.1. Iron absorption in an individual enterocyte (Donovan et al 2005)

Microvilli on the intestinal mucosal cell (enterocyte) form a brush border increasing the surface area available for the absorption of nutrients (Donovan et al 2005). Non-haem and haem iron enter the enterocyte by two separate pathways. It is thought that the haem carrier protein 1 (HCP1) transports haem iron into the enterocyte (Shayeghi et al 2005). Inside the enterocyte, haem oxygenase degrades haem iron to release ferrous iron.

Non-haem iron is transported into the enterocyte by Divalent Metal Transporter 1 (DMT1). Most dietary iron enters the duodenum as insoluble ferric iron (Fe^{3+}) and is reduced to the more soluble ferrous iron (Fe^{2+}) by the brush border ferric reductase, duodenal cytochrome B reductase (DcytB) (McKie et al 2001), or other reducing agents (e.g. ascorbic acid) prior to being transported into the enterocyte (Zimmermann and Hurrell 2007).

Once inside the enterocyte, the ferrous (Fe^{2+}) iron from all dietary sources enters a common labile iron pool (Scientific Advisory Committee on Nutrition 2010). Iron is either stored as ferritin, and lost from the body when the enterocyte is sloughed off into the gut lumen or transported across the basolateral membrane into the circulation by ferroportin (Wang and Pantopoulos 2011). The ferrous iron (Fe^{2+}) is oxidised to ferric iron (Fe^{3+}) by hephaestin, a membrane bound protein (Frazer et al 2001). Once released from the enterocyte, the iron binds with the iron transport protein, transferrin (Wang and Pantopoulos 2011). Transferrin transports iron to the liver for storage or to the bone marrow for the production of haemoglobin in red blood cells (MacPhail 2007). Transferrin binds to specific transferrin receptors (TfR1), enabling cellular uptake by endocytosis (Donovan et al 2005). Acidification of the endocytic vesicle via a proton pump causes iron to be released from the transferrin (Wang and Pantopoulos 2011). The transferrin receptor complex is cycled back to the cell surface where the apo-transferrin is released back into the circulation (MacPhail 2007).

At the end of their lifespan, red blood cells are cleared by macrophages of the reticulo-endothelial system (Wang and Pantopoulos 2011). The iron is either stored in ferritin or as haemosiderin or binds with transferrin and is returned to the circulation (MacPhail 2007) Most (90%) of iron entering the circulation comes from recycled red blood cells, rather than dietary iron (Coad and Conlon 2011).

Only a small amount of iron is lost from the body, which must be replaced by the absorption of dietary iron. This blood loss occurs through the surface cells of the skin, gastrointestinal and urinary tract (approximately 1mg/day), and through blood loss including menstruation (approximately 0.5mg/day over a 28 day cycle) (Food and Agricultural Organization of the United Nations/World Health Organization 2004).

2.4 Regulation of iron absorption

Approximately half the variance in iron absorption is affected by an individual's iron status and dietary factors (Reddy et al 2000), with hepcidin and other genetic or physiologic factors accounting for the remaining variation (Zimmermann et al 2010). Iron deficiency stimulates duodenal expression of DMT-1, Dcytb and ferroportin, and increases iron absorption (Collins et al 2005, Hallberg et al 1997, McKie et al 2001, Zimmermann and Hurrell 2007). Transferrin receptors on cell surfaces in the body that require iron increase during iron deficiency (Hulthen et al 1995), particularly on rapidly proliferating cells such as red blood cell precursors and the placenta (Aisen 2004).

Hepcidin, an antimicrobial peptide produced by the liver inhibits both the absorption of iron through the small intestine (Young et al 2009), and release of iron from macrophages and other cell types (Ganz 2005). Hepcidin binds to ferroportin resulting in its internalisation and subsequent degradation (Nemeth et al 2004). The production of hepcidin increases when iron stores are adequate and decreases when iron stores are low (Donovan et al 2005). Hepcidin concentrations also increase during infection and inflammation, resulting in sequestration of iron which deprives pathogens of iron and inhibits their proliferation (Nairz et al 2010). In HFE-associated haemochromatosis, hepcidin is lacking or unavailable (Bridle et al 2003), resulting in increased ferroportin and increased transport of iron out of the enterocyte (Donovan et al 2005). The physiological, genetic and dietary factors affecting iron absorption will be discussed further in section five.

3 Stages and prevalence of iron deficiency

3.1 Stages of iron deficiency

Iron deficiency follows a continuum ranging from low iron stores to iron deficiency anaemia. Three stages are commonly referred to – iron depletion, iron deficient erythropoiesis (non-anaemic iron deficiency) and iron deficiency anaemia (Gibson 2005b) (see Table 2.1). However, the terminology and cut-off values for the biochemical measures of each of these stages often vary.

Table 2.1. Stages in the development of iron deficiency and overload (Coad and Conlon 2011, Gibson 2005b, Herbert 1987, Lee and Nieman 2010a)

	Iron overload	**Normal**	**Iron depletion**	**Non-anaemic iron deficiency**	**Iron deficiency anaemia**
Total iron binding capacity (µg/dL)	<300	330±30	360	390	410
Serum ferritin (µg/L)	>300	100±60	20	10	<10
Iron absorption (%)	>15	5-10	10-15	10-20	10-20
Serum iron (µg/dL)	>175	115±50	115	<60	<40
Transferrin saturation (%)	>60	35±15	30	<15	<15
Soluble transferrin receptors	Low	Normal	Normal	High	High
Zinc/erythrocyte protoporphyrin (µg/dL)	30	30	30	100	200
Haemoglobin (g/L)	120-160	120-160	120-160	120-160	<120
Erythrocytes	Normal	Normal	Normal	Normal	Microcytic Hypochromic

Iron depletion is characterised by a progressive reduction of storage iron in the liver, reflected by a fall in serum ferritin (SF) concentrations (<20µg/L) (Gibson 2005b). The second stage **(non-anaemic iron deficiency)** is characterised by an exhaustion of iron stores, further decreases in serum ferritin concentration, a decrease in serum iron and an elevation in total iron-binding capacity, resulting in a fall in transferrin saturation (TS) (<15%). At the same time, zinc protoporphyrin concentrations increase because the supply of iron is no longer adequate for haem synthesis (Gibson 2005b). An increase in soluble transferrin receptor (STfR) concentration occurs, reflecting a reduction in tissue iron stores, where the supply of iron is unable to meet the demands required. In **iron deficiency anaemia**, iron dependent functions, such as erythropoiesis become impaired, leading to a decrease in haemoglobin (Hb) (<120g/L for females) (Gibson 2005b). In established iron deficiency anaemia, the red cells become small (microcytic) and pale (hypochromic) and oxygen transport to the cells is compromised (MacPhail 2007).

3.2 Measuring iron status

It is recommended that a combination of biochemical measures be used to measure an individual's iron status, with two or more abnormal values indicating impaired iron status (Gibson 2005b). Haemoglobin is a marker of iron deficiency anaemia when used with other measurements of iron status (World Health Organization/Centres for Disease Prevention and Control 2007). As red blood cell synthesis is protected at the cost of other iron functions, haemoglobin falls only in the later stages of iron deficiency.

Serum ferritin concentration is the only measure of iron status which assesses a continuum of iron status, including low, normal and excess iron levels. However, both serum ferritin and hepcidin are acute phase reactants and so increase during infection and inflammation (Hulthen et al 1998). The increase in hepcidin inhibits the release of iron from macrophages and results in sequestration of iron, possibly leading to iron deficiency erythropoiesis, and if inflammation is chronic, to anaemia of chronic disease (Zimmermann and Hurrell 2007). Distinguishing between anaemia of chronic disease and iron deficiency anaemia can be difficult, as the increased serum ferritin concentration observed during inflammation can occur in the presence of depleted iron stores and iron deficiency anaemia (World Health Organization/Centres for Disease Prevention and Control 2007). C-reactive protein (CRP) can be used to identify possible inflammation, however it is not known to what extent an increase in CRP invalidates the ability of serum ferritin to correctly identify iron deficiency (Zimmermann and Hurrell 2007). It has been suggested that other markers such as α_1-acid glycoprotein (AGP) should be used in combination with CRP to detect inflammation as AGP increases later in infection and remains elevated for longer (Thurnham et al 2010, Wieringa et al 2002).

The concentrations of soluble transferrin receptors, the proteolytic product of cellular transferrin receptors, are a marker of iron deficient erythropoiesis (Baynes 1996). As iron supplied to the body's tissues is reduced, the concentration of soluble transferrin receptor increases independently of the presence of adequate iron stores. During the treatment of iron deficiency, soluble transferrin receptor concentrations decrease before changes in haemoglobin occur (World Health Organization/Centres for Disease Prevention and Control 2007). The ratio of soluble transferrin saturation to serum ferritin falls on a continuum ranging from normal to tissue iron deficiency and iron deficiency anaemia

(Lynch 2011a). In countries where chronic inflammation is not endemic, a reduction in this ratio provides a more specific and sensitive indicator of worsening iron status than either measure alone (Baynes 1996, Lynch 2011b). Recent studies have shown the soluble transferrin receptor to serum ferritin ratio to be a reliable indicator of iron status in females of child-bearing age (Cogswell et al 2009, Lin et al 2008). Because soluble transferrin receptor expression is not affected by infection, soluble transferrin receptor may be useful in identifying individuals with iron deficiency anaemia versus anaemia due to chronic disease (Gibson 2005b). However, the expression of soluble transferrin receptor also increases when requirements for iron are high (e.g. growth, erythropoiesis, some malignancies). Therefore in some situations, soluble transferrin receptor may not be suitable for the detection of anaemia of chronic disease (Scientific Advisory Committee on Nutrition 2010, World Health Organization/Centres for Disease Prevention and Control 2007). The use of soluble transferrin receptor concentration is also limited by its cost (Zimmermann and Hurrell 2007) and a lack of a standard assay and reference method (World Health Organization/Centres for Disease Prevention and Control 2007).

3.3 Prevalence of iron deficiency

Anaemia is a global health concern affecting 24.8% of the population, with iron deficiency being the primary cause (McLean et al 2008, World Health Organization and Centers for Disease Control and Prevention Atlanta 2008). Iron deficiency and anaemia caused by iron deficiency are problematic in both developing and developed countries. The prevalence of anaemia worldwide is highest in preschool children (47.4%), pregnant women (41.8%), and non-pregnant women (30.2%) (McLean et al 2008). Data from the United States National Health and Nutrition Examination Survey (NHANES) 2003-2004 and 2005-2006) found 11.0% of women aged 20 to 49 years were iron deficient (≥2 of 3 abnormal concentrations of SF (<16.5µg/L), TS (<15%) or erythrocyte protoporphyrin (>1.24nmol/L red blood cells (RBCs)) and 4.7% had iron deficiency anaemia (Hb<120g/L in addition to iron deficiency criteria) (Cogswell et al 2009). In the United Kingdom, 8-16% of women aged 19 to 49 years had a serum ferritin concentration of <15µg/L, and 18-30% had a serum ferritin concentration of <20µg/L (Ruston et al 2004).

3.3.1 Iron deficiency in young women living in New Zealand

Studies undertaken in New Zealand investigating the iron status of young women are summarised in Table 2.2. These studies are difficult to compare due to the different cut-offs and terminology used to define iron depletion, non-anaemic iron deficiency and iron deficiency anaemia.

The 2008/2009 New Zealand National Adult Nutrition Survey found females aged 31 to 50 years were most at risk - 12.1% had iron deficiency (SF<12µg/L, zinc protoporphyrin>60µmol/mol, Hb≥120g/L) and 6.0% had iron deficiency anaemia (SF<12µg/L, zinc protoporphyrin>60µmol/mol, Hb<120g/L) (University of Otago and Ministry of Health 2011). For women aged 19 to 30 years, 5.2% had iron deficiency and 1.2% had iron deficiency anaemia. The New Zealand National Adult Nutrition Surveys found that in women aged 18 to 50 years, the prevalence of iron deficiency increased from 2-3% in 1997 (Russell et al 1999) to 5.2-12.1% in 2008/2009 (University of Otago and Ministry of Health 2011). In other New Zealand studies, iron depletion (SF<20µg/L) was found in 23% of women aged 18 to 44 years (Heath et al 2001b) and 15.6% of women aged 21 years (Fawcett et al 1998).

Table 2.2. Studies investigating the iron status of New Zealand females

Author	Participants	Iron status of participants
University of Otago and Ministry of Health (2011)	**New Zealand National Adult Nutrition Survey,** 240 females, 19-30y	5.2% - ID (SF<12µg/L, zinc protoporphyrin >60µmol/mol) 1.2% - IDA (SF<12µg/L, zinc protoporphyrin>60µmol/mol, Hb<120g/L)
University of Otago and Ministry of Health (2011)	**New Zealand National Adult Nutrition Survey,** 508 females, 31-50y	12.1% - ID (SF<12µg/L, zinc protoporphyrin >60µmol/mol) 6.3% - IDA (SF<12µg/L, zinc protoporphyrin >60µmol/mol, Hb<120g/L)
Heath et al (2001b)	335 females, 18-40y, not pregnant or breastfeeding, consuming Western type diet, Dunedin	23% - Mild ID (SF<20µg/L, Hb≥120g/L) (4% with iron deficient erythropoiesis (SF<12µg/L)) 2% - IDA (SF<12µg/L, Hb<120g/L) 2% - anaemia without ID (Hb<120g/L)
Schaaf et al (2000)	896 females, 14-21y, high school students, Auckland	9.6% - ID only (2 or more abnormal indicators of iron status- SF<12µg/L, TS<14%, or red cell distribution width >14.5%) 8.7% - IDA 2.8% - anaemia only (Hb<120g/L)
Fawcett et al (1998)	357 females, 21y, Dunedin	15.6% - low iron stores (SF<20µg/L) 6.7% - ID (SF<12µg/L, Hb>120g/L) 2.2% - IDA (SF<12µg/L, Hb<120g/L) 5.8% - anaemia (Hb<120g/L)
Russell et al (1999)	**New Zealand National Adult Nutrition Survey,** 146 females, 19-24y	4% - low iron stores (SF<12µg/L) 2% - ID (SF<12µg/L, zinc protoporphyrin>60µmol/mol) 1% - IDA (SF<12µg/L, zinc protoporphyrin >60µmol/mol, Hb<120g/L)
Russell et al (1999)	**New Zealand National Adult Nutrition Survey,** 867 females, 25-44y	7% - low iron stores (SF<12µg/L) 3% - ID (SF<12µg/L, zinc protoporphyrin >60µmol/mol) 2% - IDA (SF<12µg/L, zinc protoporphyrin >60µmol/mol, Hb<120g/L)

Hb – Haemoglobin; ID – Iron deficiency; IDA - Iron deficiency anaemia; SF – Serum ferritin; TS – Transferrin saturation

3.3.2 Iron deficiency in female students

University female students may also be at risk of iron deficiency due to the consumption of low energy diets (Hendricks et al 2004), limited time and financial resources, and making the transition from home to independent living. However, no one study has compared the iron status of student versus non-student populations. A study in New Zealand found 10

percent of female university students had serum ferritin concentrations <12µg/L (Horwath 1991). Haemoglobin concentrations were not investigated. Studies in Australia, the United States and France investigating the iron status of female students have found the prevalence of iron deficiency anaemia ranged from 4.5 to 6% (SF<12µg/L, Hb<120g/L) (Houston et al 1997, Rangan et al 1997), iron deficiency (SF<12µg/L) from 12.5 to 16% (Galan et al 1985, Horwath 1991, Rangan et al 1997) and iron depletion (SF<20µg/L) from 19.8 to 30.7% (Grondin et al 2008, Rangan et al 1997) (see Table 2.3).

Table 2.3. Studies investigating the iron status of female students

Author	Participants	Iron status of participants
Grondin et al (2008)	543 female students at university or secondary school, 17-38y, France	11.4% - iron depletion borderline (SF 15-20µg/L) 19.3% - ID (SF<15µg/L)
Houston et al (1997)	80 female university students, 19-26y, null para, null gravid, US	34% - iron depletion (SF<15µg/L) 24% – impaired iron status (2 abnormal indicators of iron status – not defined) 6% - IDA (SF<12µg/L, Hb<120g/L) 9% – anaemia (Hb<120g/L)
Rangan et al (1997)	265 female university or secondary school students, 15-30y, not pregnant, post partum, breastfeeding or resided in Australia <1 year, Australia	19.8% - low iron stores (SF<20µg/L) 12.5% – ID (SF≤12µg/L) 4.5% – IDA (SF≤12µg/L, TS<16%, Hb<120g/L) 10.2% – anaemia (Hb<120g/L)
Horwath (1991)	84 first year female university students studying nutrition, Dunedin, NZ	10% - SF<12µg/L
Galan et al (1985)	476 female students, 17-42y, not pregnant or breastfeeding, non-blood donors, no medication influencing iron status in past 6 months, France	16% - SF≤12µg/L 10.1% – transferrin IBC <90µmol/L 1.3% – anaemic (Hb<120g/L)

Hb – Haemoglobin; ID – Iron deficiency; IDA - Iron deficiency anaemia; IBC – Iron binding capacity; NZ – New Zealand; SF – Serum ferritin; TS – Transferrin saturation; US – United States

3.4 Recommended dietary iron intakes for adult females

It is difficult to determine recommended dietary intakes for iron. This is due to difficulties in measuring menstrual blood loss, inaccuracies in assessing iron intake, and the wide range of iron bioavailability of various diets. Individuals in most developed countries, including New Zealand, consume diets of high iron bioavailability (>2.1mg or >15% of iron absorbed daily) (Gibson 2005e). Diets of high iron bioavailability contain generous amounts of meat and foods that enhance iron absorption (e.g. ascorbic acid) and low levels of foods that inhibit iron absorption (e.g. phytic acid). The RDI for iron in women aged 19 to 50 years in New Zealand and Australia is 18mg per day (Commonwealth Department of Health and Ageing Australia et al 2006) (Table 2.4). Vegetarians may require more iron due to the lack of meat and therefore decreased iron bioavailability in their diets (Commonwealth Department of Health and Ageing Australia et al 2006). However, many women in New Zealand do not meet the RDI, with a median iron intake of 10.2mg per day for females aged 19 to 50 years (University of Otago and Ministry of Health 2011). This was slightly above the estimated average requirement of 8mg per day. The estimated prevalence of inadequate intake (calculated using probability analysis) was 6.0% for females aged 19 to 30 years and 15.4% for females aged 31 to 50 years (University of Otago and Ministry of Health 2011).

Table 2.4. Recommendations for iron intake for women aged 19 to 50 years (Commonwealth Department of Health and Ageing Australia et al 2006, Department of Health 1991, Food and Nutrition Board: Institute of Medicine 2001)

	NZ and Australia – RDI (2006)	**UK – RNI (1991)**	**USA & Canada - RDA (2001)**
19-30 years	18mg	14.8mg	18mg
31-50 years	18mg	14.8mg	18mg

RDA - Recommended Dietary Allowance; RDI - Recommended Dietary Intake;
RNI - Reference Nutrient Intake

4 Consequences of iron deficiency

4.1 Introduction

Iron deficiency in women of reproductive age has been linked to impaired physical work capacity, deficits in mood and cognitive function, and poor pregnancy outcome (Food and Nutrition Board: Institute of Medicine 2001). These symptoms or iron deficiency itself may impact on quality of life, including effects on self-perceived health and well-being, and fatigue. The importance of a woman's perception of her own health and well-being is increasingly being recognised alongside more traditional measures of health such as disease prevalence (Patterson et al 2000). However, the relationship between non-anaemic iron deficiency and self-perceived health and well-being remains unclear (Grondin et al 2008, Patterson et al 2000, Patterson et al 2001a).

Fatigue is a subjective experience, and can be defined as extreme and persistent tiredness, weakness or exhaustion (physical, mental or both) (Dittner et al 2004). Fatigue is a common complaint of young women (Ridsdale et al 1993) and female students (Rangan et al 1998), and is often attributed to iron deficiency by the general population and the medical profession (Patterson et al 2000). Some studies have found a relationship between iron deficiency and fatigue (Krayenbuehl et al 2011, Patterson et al 2000, Patterson et al 2001a, Verdon et al 2003), while others have found no association (Grondin et al 2008, Mansson et al 2005, Rangan et al 1998, Waldvogel et al 2012). It is not clear whether fatigue is associated with iron deficiency in the absence of anaemia (Verdon et al 2003).

4.2 Assessment of self-perceived health, well-being and fatigue

Previous studies have used a range of tools to investigate the link between iron status and self-perceived health and well-being. Some studies rely on self-reporting, while others have used validated scales such as the Duke Health Profile (Duport et al 2003), the General Health Questionnaire (GHQ) (Fordy and Benton 1994, Rangan et al 1998), the Profile of Mood States (POMS) questionnaire (McClung et al 2009) and the SF-36v2 Health Survey (SF-36 questionnaire) (Grondin et al 2008, Patterson et al 2000, Patterson

et al 2001a). Associations between iron deficiency and fatigue have been measured using the vitality (VT) scale of the SF-36 questionnaire, the fatigue scale of the POMS questionnaire (McClung et al 2009), the Piper Fatigue Scale (PFS) (Patterson et al 2001a), visual analogue scales (VAS) (Verdon et al 2003, Waldvogel et al 2012), the Brief Fatigue Inventory (BFI) Questionnaire (Krayenbuehl et al 2011), the Fatigue Severity Scale (FSS) (Waldvogel et al 2012) and self-reported fatigue (Mansson et al 2005, Rangan et al 1998).

The SF-36 questionnaire is one of the most extensively validated and used generic instruments for measuring quality of life (Contopoulos-Ioannidis et al 2009). It measures an individual's perception of his or her health status and functioning (Patterson et al 2000) and is often used to provide normative data in large population surveys, including the New Zealand Health Survey (Ministry of Health 2008). It contains 36 items scored as eight multi-item scales (physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health) scored from zero to 100. These scores are combined to produce a physical component summary score (PCS) and a mental component summary score (MCS) (Ware et al 2007). The VT subscale is recognised as an established measure of fatigue, and includes four items asking participants to indicate the extent to which they have felt "tired and worn out" versus "energetic". The SF-36 questionnaire has been used previously to investigate the relationship between iron status and quality of life in young women (Grondin et al 2008, Patterson et al 2000, Patterson et al 2001a).

A systematic review found no one scale met all of the criteria as an ideal tool to measure fatigue (Whitehead 2009). Fatigue scales should be usable (minimal participant burden and easy to understand and complete), able to discriminate cases from non-cases with acceptable sensitivity and specificity, and provide a full description of fatigue severity and impact (Whitehead 2009). As fatigue presents in a variety of ways, it is vital to recognise and assess its multidimensional aspects (Lim et al 2005). Tools which treat the symptoms of fatigue as a one dimensional concept provide limited information about the participant's experience and tend to lack reliability (Stein et al 1998). Scales should also demonstrate robust psychometric properties, including reproducibility and validity (Whitehead 2009).

The Multidimensional Fatigue Symptom Inventory – Short Form (MFSI-SF) demonstrates good psychometric properties (Stein et al 2004, Whitehead 2009) and is increasingly being used as a measure of fatigue. The MFSI-SF measures multiple characteristics and manifestations of fatigue (Lim et al 2005). While the MFSI-SF has mainly been used in cancer patients, it may also be useful in healthy populations, as it contains no reference to any medical diagnosis or disease (Dittner et al 2004), nor does it assume the presence of fatigue (Stein et al 2004). The MFSI-SF contains 30 items which produce five subscales (general, physical, emotional and mental fatigue; and vigour). Higher scores on the MFSI-SF subscales, other than vigour, are indicative of more fatigue. The MFSI-SF has not previously been used to investigate the relationship between iron status and fatigue.

4.3 Iron status and health, well-being and fatigue

In Tables 2.5a and 2.5b studies that have been undertaken to investigate the relationship between iron status and health, well-being and fatigue are displayed. Early studies investigating the effect of iron supplementation on health, well-being and fatigue were limited by the sole measurement of haemoglobin as a measure of improved iron status (Beutler et al 1959, Elwood and Hughes 1970) and self-reporting of fatigue (Ballin et al 1992, Beutler et al 1959, Elwood and Hughes 1970, Morrow et al 1968). Beutler et al (1959) reported improvements in symptoms, including tiredness, after iron supplementation in women with chronic fatigue. In other studies, despite haemoglobin concentrations increasing with iron supplementation, symptoms including fatigue did not change (Elwood and Hughes 1970) or no differences were found in symptoms between iron and placebo treatments (Morrow et al 1968).

Most cross-sectional studies using validated questionnaires, in which participants were unaware of their iron status and where the majority of participants did not have iron deficiency anaemia, have not found a relationship between iron status and health and well-being, or fatigue (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Mansson et al 2005, Rangan et al 1998). Rangan et al (1998) found no association in female students between iron status and the frequency of non-specific symptoms including fatigue. Using the GHQ (a 12-item validated questionnaire and mental health assessment tool), no relationship was found in two studies investigating the relationship between iron status and psychological distress in female students (Fordy and Benton

1994, Rangan et al 1998). However, students with anaemia (Hb<120g/L) did report increased psychological stress (Rangan et al 1998). No relationship was observed between iron status and quality of life in menstruating women as determined using the Duke Health Profile (Duport et al 2003); and only vertigo/dizziness was associated with iron deficiency in students completing a standardised 30-item quality of life questionnaire (Mansson et al 2005). Using the SF-36 questionnaire, Grondin et al (2008) found no significant differences between the PCS, MCS and VT scores from the SF-36 questionnaire in iron replete and deficient French female students. The general health scale was however significantly lower in iron deficient students. Haemoglobin concentrations were investigated in a small proportion of participants only, some of whom had iron deficiency anaemia (Grondin et al 2008).

In a randomised placebo controlled double blind (RPCDB) trial in female soldiers (including those with iron deficiency anaemia), supplementation with iron significantly reduced the decrement in iron status (serum ferritin and soluble transferrin receptor) observed with eight weeks of basic combat training (McClung et al 2009). There were no significant differences between iron or placebo groups regarding changes in total or fatigue scores as measured by the POMS questionnaire (McClung et al 2009). In another study, Waldvogel et al (2012) observed no improvement in fatigue or vitality scores in non-anaemic iron deficient female blood donors who improved their iron status after four weeks of iron supplementation.

In contrast, Patterson et al (2001a) found MCS and VT scores measured using the SF-36 questionnaire were lower in iron deficient women compared with iron replete women. Iron deficient women reported more fatigue using the Piper Fatigue Scale (PFS), a multidimensional measure of fatigue (Piper et al 1989). However, the PFS assumes the presence of fatigue, and women were aware of their iron status prior to completing the PFS, which may have influenced their response (Patterson et al 2001a). In addition, the iron deficient group included women with iron deficiency anaemia (Hb 90-120g/L). The SF-36 MCS and VT scores, and fatigue (measured using the PFS) improved following treatment with either diet or iron supplements (Patterson et al 2001a). However, no blinding was used nor was a placebo group included and similar improvements were seen in all scores, despite serum ferritin increasing more in the supplemented group. Participants may have assumed their iron status improved due to the intervention, and

changes in the diet group's intake may have had other benefits in relation to general health and well-being, which were not solely linked to iron status (Patterson et al 2001a). Furthermore, the improvements in VT in the supplement group and PFS scores in the diet and supplement group were not significant when women with anaemia were excluded (Patterson et al 2001a).

In studies where a relationship was observed between iron status and quality of life, the inclusion of anaemic participants may have influenced the results (Ando et al 2006, Ballin et al 1992, Verdon et al 2003). Iron supplementation improved haemoglobin concentrations and all scales of the SF-36 questionnaire (except for the role emotional scale) in women who had iron deficiency anaemia (Ando et al 2006). However, no control group was included. In a randomised controlled trial, Verdon et al (2003) found fatigue in non-anaemic women improved with four weeks of iron supplementation as measured on a unidimensional VAS. However, four weeks is a short time in which to see an improvement in iron status and haemoglobin concentrations were not reported (that is, some participants in the placebo group could potentially have had anaemia at the end of the study). Ballin et al (1992) found significant improvements in self-reported lassitude and mood (but not fatigue) in female students with iron deficiency and iron deficiency anaemia who took iron supplements over a two month period. Participants in these studies may have known which group they were in due to possible side-effects of iron supplementation. In an uncontrolled trial, Mansson et al (2005) observed reduced symptoms of vertigo/dizziness, irritability, indisposition and depression (but not fatigue) in women with iron deficiency and iron deficiency anaemia following three months of iron supplementation.

In a large cross-sectional study, Patterson et al (2000) found women who reported (ever) having had low iron levels diagnosed by a doctor had a greater prevalence of constant tiredness and significantly lower PCS, MCS and VT scores on the SF-36 questionnaire than women without a history of iron deficiency. The PCS, MCS and VT scores were significantly lower among women without iron deficiency, but who reported iron deficiency two years later. A major limitation of this study was that all data regarding iron deficiency were self-reported with no actual measures of iron status being made. Kallich et al (2006) found participant knowledge of haemoglobin concentrations had a modest association

with aspects of health related quality of life. This may also be true for a woman's knowledge of whether she has had previous iron deficiency.

More recently, in a RPCDB trial, a total of 800mg intra-venous iron provided over two weeks did not improve fatigue (as measured by the BFI Questionnaire) in women after six and 12 weeks compared with a placebo group (Krayenbuehl et al 2011). However, fatigue did improve in those women being treated with iron who had a serum ferritin concentration of <15µg/L and normal haemoglobin concentrations.

4.4 Conclusion

It remains unclear whether self-perceived health, well-being and fatigue is associated with iron deficiency in the absence of anaemia, although recent research suggests a likely relationship for women who have very low serum ferritin concentrations (Krayenbuehl et al 2011). In previous studies, iron status has been determined using a range of biochemical indicators and cut-off points (Ando et al 2006, Ballin et al 1992, Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Krayenbuehl et al 2011, Mansson et al 2005, McClung et al 2009, Patterson et al 2001a, Rangan et al 1998, Verdon et al 2003, Waldvogel et al 2012) or through self-reporting of previous iron deficiency (Patterson et al 2000). In some studies, non-validated questionnaires have been used (Ballin et al 1992, Beutler et al 1959, Elwood and Hughes 1970, Morrow et al 1968) and participants have known their iron status prior to completing questionnaires (Patterson et al 2001a), which may have influenced their response. The use of varying methodologies and lack of clear consensus regarding the impact of iron deficiency on health, well-being and fatigue suggest further investigation is required. This research should be undertaken in non-anaemic women using validated questionnaires and women should remain unaware of their iron status prior to testing.

Table 2.5a. Observational studies investigating the relationship between iron status and health, well-being and fatigue

Author, year, country	Population group	Study design	Questionnaire(s) used	Definition/groupings for ID	Conclusions
Ballin et al (1992) Israel	222 female high school students, 16-17y	Cross-sectional	Questions about symptoms including fatigue	ID - SF<10µg/L, TS<16%, or serum iron<12µmol/L IDA – as above & Hb<120g/L	Significant correlation between Hb & ability to concentrate
Fordy & Benton (1994) UK	297 male & female students, 17-27y	Cross-sectional	GHQ (psychological stress)	SF≤5µg/L; 5.1-20µg/L; >20µg/L Hb<120g/L (females); <130g/L (males)	No association between SF & mood for males & females not taking OCP; in those taking OCP, SF<5µg/L associated with poorer mental health
Rangan et al (1998) Australia	255 female school or university students, 15-30y	Cross-sectional	GHQ & frequency of non-specific symptoms including chronic fatigue	ID - SF≤12µg/L, Hb ≥120g/L IDA - SF≤12µg/L, Hb<120g/L	No association between ID & psychological stress or non-specific symptoms; participants with IDA had increased psychological stress

Author, year, country	Population group	Study design	Questionnaire(s) used	Definition/groupings for ID	Conclusions
Patterson et al (2000) Australia	14,762 females, 18-23y; 14,072 females, 45-50y 12,328 of females 45-50y 2 years later	Cross-sectional & prospective	SF-36 questionnaire Questions included about constant or severe tiredness	Self-reporting - "Have you ever been told by your doctor that you have low iron?"	Participants with low iron previously had a greater prevalence of constant tiredness & significantly lower PCS, MCS & VT scores than participants with no history of ID; PCS, MCS and VT scores were significantly lower in participants who reported ID in the past 2y than for participants who reported past ID or no history of ID
Patterson et al (2001a) Australia	66 females 18-50y	Cross-sectional	SF-36 questionnaire PFS	ID - SF<15µg/L or SF 15-20µg/L with 2 other values indicative of ID (TS<15%, serum iron <10µmol/L, TIBC>68µmol/L, Hb>90g/L) Iron replete - SF≥20µg/L, Hb≥120g/L	MCS & VT scores lower, PFS higher (greater fatigue) in ID women; PCS not related to iron status
Duport et al (2003) France	865 females, 35-51y	Cross-sectional	Duke profile - quality of life (physical, mental, social, general, perceived, self-esteem, anxiety, depression, pain, disability)	Iron deplete - SF<15µg/L, Hb < or ≥120g/L Iron sufficient - SF 30-80µg/L	No significant association between iron status & quality of life

Author, year, country	Population group	Study design	Questionnaire(s) used	Definition/groupings for ID	Conclusions
Mansson et al (2005) Sweden	339 male & female secondary school students, 16-19y	Cross-sectional	Standardised questionnaire of 30 questions related to quality of life (including general fatigue) for symptoms experienced in past 3 months	ID – 1 or more abnormal values - Hb<115g/L, iron saturation index <17%, SF <20µg/L, serum transferrin >3.30g/L	Vertigo/dizziness significantly higher in those with ID
Grondin et al (2008) France	543 female school or university students, 17-38y	Cross-sectional	SF-36 questionnaire	ID - SF<15µg/L Iron depletion - SF 15-20µg/L Iron replete - SF>20µg/L	General health significantly lower but no differences in PCS, MCS or VT in ID versus iron replete

GHQ – General health questionnaire; Hb – Haemoglobin; ID – Iron deficiency; IDA - Iron deficiency anaemia; MCS - Mental component summary score; OCP – Oral contraceptive pill; PCS - Physical component summary score; PFS – Piper fatigue scale; SF – Serum ferritin; SF-36 - SF-36v2 Health Survey; TIBC – Total iron binding capacity; TS – Transferrin saturation; VT - Vitality

Table 2.5b. Intervention studies investigating the relationship between iron status and health, well-being and fatigue

Author, year, country	Population group	Study design	Questionnaire(s) used	Effects of intervention on iron status[a]	Effects of intervention on health, well-being and fatigue
Beutler et al (1959) USA	30 healthy females with chronic fatigue, Hb>120g/L	Cross-over – RPCDB - 300mg ferrous sulphate/d for 3mths	Questions about symptoms including tiredness/fatigue	In more iron depleted participants (bone marrow analysis), Hb increased significantly with iron but not placebo In less iron depleted participants, neither iron or placebo increased Hb	Symptoms improved in all but two participants receiving iron & placebo In the more iron depleted participants, the improvement in symptoms was greater for iron than for placebo
Morrow et al (1968) Scotland	20 females with sideropenia & symptoms, 15-63y, Saturation plasma IBC <16%, Hb 117-131g/L	Cross-over – RPCDB - 180mg ferrous sulphate/d for 3mths	Questions about symptoms including excessive tiredness	Hb increased significantly with iron but not placebo	Decreased incidence of excessive tiredness in iron & placebo; no significant difference between iron & placebo on excessive tiredness
Elwood & Hughes (1970) Wales	47 females, >20y, Hb<105g/L	RPC - 150mg/d iron as ferrous carbonate for 8wks	Questions about symptoms including fatigue on visual scale	Hb increased significantly with iron	No beneficial effects of increased Hb on fatigue; no significant difference between iron & placebo on fatigue

Author, year, country	Population group	Study design	Questionnaire(s) used	Effects of intervention on iron status[a]	Effects of intervention on health, well-being and fatigue
Ballin et al (1992) Israel	59 female high school students, 16-17y, all iron status	RPCDB - 105mg elementary ferrous iron/d for 2mths	Questions about symptoms including fatigue	Iron – 27.6% had SF<10 (B), 6.9% (E) Placebo – 23.3% had SF<10 (B), 16.7% (E)	Significant improvement in lassitude, ability to concentrate in school & mood with iron compared with placebo Majority of participants with improvement had hypoferremia before & normoferremia after study
Patterson et al (2001a) Australia	66 females 18-50y, ID - SF<15µg/L or 15-20µg/L with 2 other indicators of ID (TS<15%, serum iron <10µmol/L, TIBC>68µmol/L), Hb>90g/L Control - SF≥20µg/L, Hb≥120g/L	Randomised intervention – ID - 350mg ferrous sulphate/d or high iron diet for 12wks Control - no intervention	SF-36 questionnaire PFS	Iron status improved in diet & supplement groups, but not in control group <u>SF</u> Control - 49.4 (B), 44.5 (E) Diet - 8.9 (B), 11.0 (E)* Supplement - 9.0 (B), 24.8 (E)* <u>Hb</u> Control - 135.9 (B), 134.0 (E) Diet - 127.6 (B), 130.6 (E) Supplement - 125.2 (B), 130.4 (E)*	Similar improvements in MCS, VT & PFS for diet & supplement groups PCS did not change significantly in any group

Author, year, country	Population group	Study design	Questionnaire(s) used	Effects of intervention on iron status[a]	Effects of intervention on health, well-being and fatigue
Verdon et al (2003) Switzerland	136 females, 18-55y, fatigued, Hb>117g/L, 51% had SF<20µg/L	RPCDB - 80mg ferrous sulphate/d for 4wks	10 point VAS - fatigue	Change in SF from B to E not clear, Hb at E not reported	Fatigue improved more with iron supplementation compared with placebo
Mansson et al (2005) Sweden	127 male & female secondary school students, 16-19y ID – 1 or more - Hb<115g/L, TS<17%, SF<20µg/L, sTfR >3.30g/L	100mg iron/d as ferrous sulphate for 3mths	Standardised questionnaire of 30 questions related to quality of life (including general fatigue) for symptoms in past 3mths	Iron supplementation improved SF (11 (B), 22 (E))*	Iron supplementation reduced fatigue, vertigo/dizziness, irritability, over-exertion, depressive symptoms, indisposition, sensitiveness to cold (participants with ≥1 abnormal values) & vertigo/dizziness, irritability, depressive symptoms, indisposition (participants with ≥2 abnormal values)
Ando et al (2006) Japan	84 females, 19-54y, IDA – SF <20µg/L, Hb 60-110g/L	100mg iron/d as sodium ferrous citrate for 3mths	SF-36 questionnaire	Iron supplementation improved Hb (92 (B), 130 (E))	Iron supplementation improved all sub scales of SF-36, except role emotional

Author, year, country	Population group	Study design	Questionnaire(s) used	Effects of intervention on iron status[a]	Effects of intervention on health, well-being and fatigue
Krayenbuehl et al (2011) Switzerland	90 females (premenopausal) with fatigue, ≥18y, non-anaemic ID - SF≤50µg/L, Hb≥120g/L	RPCDB - 800mg intra-venous iron as iron (III)-hydroxide sucrose for 2wks	BFI SPI	Change in SF from B to 6/12wks Iron – 98*/81* Placebo – 1/-1 Change in Hb from B to 6wks Iron – 1 Placebo - 0	BFI – At 6 & 12wks NS difference in fatigue between iron & placebo; fatigue improved in participants with SF<15µg/L in iron versus placebo SPI – Iron group reported greater improvement in fatigue at 6 & 12wks compared with placebo
Waldvogel et al (2012) Switzerland	154 females (donated blood 1wk prior), 18-50y, non-anaemic ID – SF<30µg/L, Hb≥120g/L	RPCDB – 80mg ferrous sulphate /d for 4wks	10 point VAS – fatigue FSS SF-12 questionnaire	Iron status improved with iron, but not placebo * <u>SF</u> Iron – 15.3 (B), 28.0 (E) Placebo – 14.8 (B), 12.9 (E) <u>Hb</u> Iron – 126 (B), 135 (E) Placebo – 126 (B), 130 (E)	No significant improvement in fatigue or vitality in iron versus placebo Physical but not mental health improved in iron compared with placebo – main change due to less pain

[a]Serum ferritin concentrations reported in µg/L; Haemoglobin concentrations in g/L; [*]Indicates a significant change ; B – Baseline; BFI - Brief fatigue inventory; E – End; FSS – Fatigue severity scale; Hb – Haemoglobin; IBC - Iron binding capacity; ID – Iron deficiency; IDA - Iron deficiency anaemia; MCS - Mental component summary score; NS – not significant; PCS - Physical component summary score; PFS – Piper fatigue scale; POMS – Profile of mood states; RPC - Randomised placebo controlled; RPCDB – Randomised placebo controlled double-blind; SF – Serum ferritin; SF-36 - SF-36v2 Health Survey; SfTR – Soluble transferrin receptor; SPI – Short performance inventory; TS – Transferrin saturation; VAS - visual analogue scale; VT - Vitality

5 Determinants of iron status in young women

5.1 Introduction

A number of factors contribute to the iron status of young healthy women living in developed countries, including dietary and non-dietary factors. It is important to identify these factors, so they can be targeted in the prevention and treatment of iron deficiency.

5.2 Dietary factors affecting iron status

5.2.1 Dietary factors and iron absorption

Dietary factors have a major influence on iron absorption. These include the amount and type of iron (haem versus non-haem iron) in the diet, and the effect of dietary enhancers and inhibitors on iron absorption.

Haem iron is derived from haemoglobin and myoglobin in meat, poultry and fish. It has been suggested that haem iron contributes up to 15% of total iron intake (Hallberg 2002). In the New Zealand Adult National Nutrition Survey, 19.5% of dietary iron consumed by females was provided from meat sources: beef and veal (6.3%), poultry (3.8%), fish and seafood (3.3%), pork (2.1%), sausages and processed meats (2.1%), lamb and mutton (1.4%) and other meats (0.5%) (University of Otago and Ministry of Health 2011). The average absorption of haem iron from meals containing meat is approximately 25% (Hallberg et al 1979), but may vary from 10% during iron repletion to approximately 40% during iron deficiency (Hercberg et al 2001). Only a few dietary factors impact on haem iron absorption (see Table 2.6). Meat and soy protein may enhance (Hallberg et al 1979, Lynch et al 1985), while calcium inhibits haem iron absorption (Hallberg et al 1991, Hallberg 1998). Haem iron is converted to non-haem iron if meat is cooked at too high a temperature for too long (Baech et al 2003, Food and Agricultural Organization of the United Nations/World Health Organization 2004).

Non-haem iron is the major contributor of dietary iron and is found in both animal and plant foods including meat, eggs, cereals, pulses, legumes, and fruit and vegetables.

Approximately two to 20% of non-haem iron is absorbed (Gibson et al 1997), depending on a number of factors (see Table 2.6). Non-haem iron absorption is affected by an individual's iron status (increased absorption with iron deficiency) and by dietary factors, with bioavailability varying greatly across meals which contain the same amount of iron (Hallberg 1981). Dietary factors enhance non-haem iron absorption by converting ferric (Fe^{3+}) iron to the more soluble ferrous (Fe^{2+}) form (Fairweather-Tait 1995), or by maintaining the iron released from food during digestion in a soluble form. Ascorbic acid (Cook and Monsen 1977) and an unidentified factor in meat, fish and poultry (Cook and Monsen 1975) are strong enhancers of non-haem iron absorption. Other enhancers of non-haem iron absorption include organic acids (Gillooly et al 1983), alcohol (Cook et al 1995) and fermented foods (Baynes et al 1990). Vitamin A and carotenoids may enhance non-haem iron absorption but evidence is conflicting (Garcia-Casal et al 1998, Layrisse et al 1997, Layrisse et al 1998, Walczyk et al 2003).

Inhibitors of non-haem iron absorption include phytic acid (Hallberg et al 1989), polyphenols (Hurrell et al 1999), calcium (Hallberg et al 1991) and proteins (Cook et al 1981). These bind non-haem iron, forming insoluble complexes which are poorly absorbed and unable to be taken up into the enterocyte (Disler et al 1975, Oke 1969). It is thought that calcium's inhibition occurs within the enterocyte as calcium inhibits both haem and non-haem iron absorption (Hallberg et al 1991, Hallberg 1998). High doses of organic elements (zinc, cobalt, cadmium, manganese and copper) interfere with non-haem iron absorption (Monsen 1988, Rossander-Hulten et al 1991, Solomons 1986) by competing for transport by DMT1. However the levels of these elements found in foods are unlikely to inhibit iron absorption. Factors affecting haem and non-haem iron absorption are summarised in Table 2.6.

Table 2.6. Factors influencing dietary iron absorption (adapted from Hallberg (2002))

	Factors influencing iron absorption	Dietary enhancers of iron absorption	Dietary inhibitors of iron absorption
Non-haem iron	Iron status of participant Gastric acid production Amount of non-haem iron Balance between dietary enhancers and inhibitors of iron absorption	MFP factor Ascorbic acid Organic acids Alcohol Vitamin A and β-Carotene Some fermented products and soy sauces	Polyphenols Oxalate Calcium Proteins Phytates and other inositol phosphates
Haem iron	Iron status of participant Amount of dietary haem iron Food preparation (time, temperature)	Meat Soy protein	Calcium

MFP=Meat/fish/poultry

Due to the number of factors influencing iron bioavailability (haem versus non-haem iron; food preparation methods; enhancers and inhibitors of iron absorption), it is important to consider the whole diet when investigating dietary factors associated with iron status.

5.2.2 Dietary factors and iron status - cross-sectional studies

Dietary factors affecting iron absorption (as described above) do not always translate into having an effect on iron status. Studies investigating the effect of dietary factors on iron status in healthy young premenopausal women living in developed countries have been cross-sectional rather than prospective studies. Most of these studies have not found an association between total iron intake and iron status (Asakura et al 2009, Blanco-Rojo et al 2011a, Brussard et al 1997, Cade et al 2005, Frith-Terhune et al 2000, Galan et al 1985, Harvey et al 2005, Heath et al 2001b, Pynaert et al 2009, Rangan et al 1997, Razagui et al 1991, Ruston et al 2004, Takkunen and Seppanen 1975). Those studies showing a positive relationship have included men and women across a wide range of ages (Preziosi et al 1994), adolescents (Bairati et al 1989, Deegan et al 2005), women aged 35 to 60 years (Galan et al 1998), and one study showed a positive relationship in smokers only (Houston et al 1997). The type of iron (haem versus non-haem) rather than the amount of iron consumed appears to be more important in determining iron status.

Most studies in young women have observed a positive association between iron status and meat (Brussard et al 1997, Cade et al 2005, Galan et al 1998, Heath et al 2001b, Leggett et al 1990, Takkunen and Seppanen 1975, Worthington-Roberts et al 1988) or haem iron intake (Brussard et al 1997, Cade et al 2005, Galan et al 1998, Preziosi et al 1994, Ramakrishnan et al 2002, Rangan et al 1997). In New Zealand, Heath et al (2001b) found premenopausal women with mild iron deficiency (SF<20µg/L, Hb≥120g/L) had a significantly lower daily intake of meat, fish and poultry compared with women who had normal iron stores (86g versus 111g). This was the only dietary factor associated with mild iron deficiency (Heath et al 2001b). Only a few studies have found no association between meat or haem iron intake and iron status (Asakura et al 2009, Houston et al 1997, Pynaert et al 2009, Ruston et al 2004).

Female vegetarians tend to have lower serum ferritin concentrations than non-vegetarians (Alexander et al 1994, Ball and Bartlett 1999, Helman and Darnton-Hall 1987, Hua et al 2001, Reddy and Sanders 1990). In New Zealand Seventh Day Adventists, similar serum ferritin and haemoglobin concentrations were observed in vegetarians and non-vegetarians, although more vegetarians had serum ferritin concentrations at the lower end of the reference range (Harman and Parnell 1998). In the United States, premenopausal women who consumed red meat had a higher iron status (determined using serum ferritin, haemoglobin and total iron binding capacity) than lacto-ovo vegetarians or those consuming fish or poultry as their main protein source (Worthington-Roberts et al 1988). However, in the United Kingdom, consumption of a lacto-ovo vegetarian diet was not associated with a lower iron status (Harvey et al 2005) and serum ferritin concentrations were lower in women who ate red meat compared with those who ate fish and poultry (Harvey et al 2005). Several studies have found a similar incidence of iron deficiency anaemia in vegetarians and vegans compared with non-vegetarians (Ball and Bartlett 1999, Haddad et al 1999, Hunt 2002, Reddy and Sanders 1990). However, a report by the Meat and Livestock Commission in Britain found iron deficiency anaemia in women was more common in non-meat eaters (38%) compared with those who consumed average (90g meat/day) (14%) or high amounts of meat (>140g meat/day) (6%) (Gibson and Ashwell 2003).

The majority of studies in young women have not found any association between total daily ascorbic acid intake and iron status (Asakura et al 2009, Bairati et al 1989, Blanco-

Rojo et al 2011a, Brussard et al 1997, Galan et al 1985, Galan et al 1998, Heath et al 2001b, Houston et al 1997, Preziosi et al 1994, Pynaert et al 2009, Ramakrishnan et al 2002, Rangan et al 1997, Razagui et al 1991). Supporting the finding that ascorbic acid is effective in enhancing iron absorption only when taken with meals (Cook and Monsen 1977), Razagui et al (1991) found the amount of ascorbic acid consumed at meal times was more important than total daily ascorbic acid intake in determining the iron status of women living in an institutionalised setting.

Most studies have found no association between iron status and fruit or vegetable intake (Asakura et al 2009, Cade et al 2005, Galan et al 1998). A study in females aged 35 to 69 years found a negative association between intake of fruit juice and iron status, but a positive association between ascorbic acid intake and iron status (Cade et al 2005). Peneau et al (2008) found no association between serum ferritin and consumption of ascorbic acid-rich fruit, vegetables and juices. However, a higher consumption of fibre-poor fruit, vegetables and juices was associated with higher serum ferritin concentrations and a lower risk of iron deficiency. It was suggested that in diets containing varied fruits and vegetables, the effects of varying levels of ascorbic acid and fibre counteract each other, leading to an unchanged serum ferritin concentration (Péneau et al 2008). The timing of intake (i.e. whether fruit, vegetables and juices were consumed at or in between meal times) was not investigated.

While serum ferritin was negatively associated with fibre intake in one study (Galan et al 1998), most studies have not reported any association between phytate (Asakura et al 2009, Heath et al 2001b, Ramakrishnan et al 2002) or fibre (Asakura et al 2009, Blanco-Rojo et al 2011a, Brussard et al 1997, Cade et al 2005, Houston et al 1997) intake and iron status in young women.

Several studies in young women have found a negative association between iron status and calcium (Galan et al 1998, Rangan et al 1997) or dairy product (Galan et al 1985, Galan et al 1998, Takkunen and Seppanen 1975) intake. Across six European countries, van de Vijver et al (1999) found dietary calcium intake was inversely associated with iron status, irrespective of whether calcium was taken simultaneously with iron. Other studies however, have found no association between calcium (Asakura et al 2009, Blanco-Rojo et al 2011a, Cade et al 2005, Galan et al 1985, Heath et al 2001b, Pynaert et al 2009) or

dairy product intake (Asakura et al 2009, Brussard et al 1997, Pynaert et al 2009) and iron status. In studies where dairy products or calcium intake impact negatively on iron status, it is possible that this may be due to these foods displacing foods high in bioavailable iron (meat) rather than the inhibitory effect of calcium itself (Heath et al 2001b), although this has not been confirmed.

Most studies have not found an association between tea and coffee intake (Brussard et al 1997, Galan et al 1998, Heath et al 2001b, Mennen et al 2007, Pynaert et al 2009) or polyphenol intake and iron status (Cade et al 2005, Ramakrishnan et al 2002). However, three studies found a negative association between serum ferritin concentration and tea intake (Galan et al 1985, Pynaert et al 2009, Razagui et al 1991). A meta-analysis found tea consumption does not appear to affect iron status in populations who mostly have adequate iron status (Temme and Van Hoydonck 2002). However, in populations with marginal iron status, there appears to be a negative association between tea consumption and iron status.

Studies that have investigated alcohol intake have found either a positive association (Broderstad et al 2007, Brussard et al 1997, Cade et al 2005, Leggett et al 1990, Pynaert et al 2009, Whitfield et al 2001) or no association (Asakura et al 2009, Heath et al 2001b, Rangan et al 1997) between alcohol intake and iron status. The type of alcohol may be important, with beer rather than wine or spirits being associated with a higher serum ferritin concentration in two studies (Leggett et al 1990, Whitfield et al 2001). Using data from the third National Health and Examination Survey in the United States, Ioannou et al (2004) concluded that consumption of up to two alcoholic drinks per day is associated with reduced risk of iron deficiency and iron deficiency anaemia. Alcohol may increase non-haem iron absorption by stimulating the secretion of gastric hydrochloric acid (Hallberg and Rossander 1982).

Two studies found a negative association between serum ferritin concentrations and energy intake (Brussard et al 1997, Cade et al 2005), however the majority of studies have found no association (Blanco-Rojo et al 2011a, Heath et al 2001b, Houston et al 1997, Pynaert et al 2009, Rangan et al 1997, Takkunen and Seppanen 1975). One study observed a negative association between vegetable protein intakes and iron status (Brussard et al 1997). Only one cross-sectional study has considered vitamin A intake

and found no association with iron status (Asakura et al 2009). No cross-sectional studies in young women have investigated associations between carotenoid intake and iron status. The effect of vitamin A and carotenoids on iron absorption is further discussed in section 6.2.4.

In females aged 35 to 69 years, Cade et al (2005) found the consumption of white and wholemeal bread, and nuts and seeds were negatively associated with serum ferritin concentrations. No relationship was found between iron status and cereal and pulse intake (Asakura et al 2009), or between consumption of beans and pulses, iron-rich food or fortified breakfast cereals and iron status (Cade et al 2005). No association has been found between iron status and egg intake (Asakura et al 2009, Brussard et al 1997).

In summary, most cross-sectional studies show meat consumption to be associated with an increased iron status. Some, but not all, studies have observed a negative association between calcium/dairy product intake and iron status. Studies have found either a positive association or no association between alcohol intake and iron status. Tea intake appears to only affect iron status in women with marginal iron status. The majority of cross-sectional studies in young women have observed no relationship between iron status and intakes of ascorbic acid, fruit and vegetables, fibre and phytate. Few studies have considered how and when these foods/nutrients were consumed (e.g. whether they were consumed with iron containing meals).

5.2.2.1 Dietary patterns, practices and iron status

The focus on individual nutrients and food in cross-sectional studies has several limitations (section 5.2.2.2 below). Dietary pattern analysis considers the whole diet and is an alternative and complementary approach to studying individual foods and nutrients and their association with iron status (Hu 2002, Newby and Tucker 2004). Only two studies have explored the association between dietary patterns and iron status (Broderstad et al 2011, Shi et al 2006). A study in China, found 'traditional' (rice, vegetable, wheat flour) and 'sweet tooth' (drinks, cakes) patterns were positively associated with anaemia, while a 'healthy' dietary pattern (whole grains, fruits, vegetables) was negatively associated with anaemia (Shi et al 2006). No association was found between a 'macho' (meat and alcohol) dietary pattern and iron status, however the

average daily meat consumption was low (Shi et al 2006). In Norway, high serum ferritin concentrations were associated with a 'reindeer meat' dietary pattern. The reindeer meat pattern consisted of reindeer meat and other reindeer products, moose meat, cured/salted fish and boiled coffee (Broderstad et al 2011). None of the other dietary patterns including 'fish' (all marine foods), 'average' (average intakes of most food items, except for a high intake of whole milk, processed fish, coffee, sausages, pork and mutton), 'fruit and vegetables' (fruit, vegetables, water, tea, pasta, chicken), and 'Westernised/traditional marine' (hamburgers, pizza, sausages, casseroles, pork, beef, fish liver, hard roe, whale meat, filtered coffee) were associated with iron status (Broderstad et al 2011).

In addition, the various combinations of foods and beverages eaten at meal times may be an important determinant of iron status, as foods are most likely to impact on non-haem iron absorption when consumed simultaneously (Cook and Monsen 1977, Gleerup et al 1993, Gleerup et al 1995). In a small study (n=15), mentally handicapped women living in an institutionalised setting who had depleted iron stores (SF<12µg/L) had a significantly higher tea and lower ascorbic acid intake at meal times (Razagui et al 1991) compared to women who had higher iron stores (SF>12µg/L). Mennen et al (2007) found tea drinking with or between meals was not associated with serum ferritin concentrations or iron depletion in French adults. In another study, the timing of calcium intake (i.e. consumed with iron or not) had no effect on iron status (van de Vijver et al 1999).

5.2.2.2 Limitations of cross-sectional studies

There are difficulties in comparing cross-sectional studies due to the different populations studied (age, sex, country, ethnicity), different biochemical indices and cut-offs used to measure and define iron deficiency, varying (and sometimes inadequate) methods of dietary assessment, and analysis of results which does not take into account potential confounders of iron status (such as blood loss) (Scientific Advisory Committee on Nutrition 2010). Other limitations of cross-sectional studies may include small sample sizes and the difficulty in identifying a causal relationship, as exposure (e.g. dietary intake) and outcome (iron status) are measured at the same time (Pynaert 2007). A cohort study where participants are followed up over time would provide a better insight into causal relationships. However these studies are more expensive due to increased time required from both participants and the researcher (Pynaert 2007).

5.2.3 Dietary factors and iron status – intervention studies

A better indicator of the causal effects of various dietary factors on iron status may be provided by intervention studies. These include general dietary interventions (Heath et al 2001a, Patterson et al 2001b) and interventions which have added specific nutrients or foods to participants' diets.

The addition of a daily iron supplement (amino acid chelate providing 50mg elemental iron) or a dietary intervention improved serum ferritin concentrations by 59% (*P*=0.001) and 26% (*P*=0.068) respectively compared with a placebo in women with mild iron deficiency (SF<20µg/L, Hb≥120g/L) following a 16-week intervention (Heath et al 2001a). The dietary intervention included advice to: increase the intake of iron-containing foods and foods known to enhance non-haem iron absorption, decrease the intake of foods that inhibit iron absorption, and modify eating patterns so enhancers of non-haem iron absorption were eaten with meals, and inhibitors between meals. Women in the dietary intervention group significantly increased their intake of meat, fish and poultry (31g/day compared with the placebo group), haem iron (0.36mg/day), ascorbic acid (136mg/day) and foods cooked using cast iron cook ware; and significantly decreased their intake of calcium (169mg/day) and their phytate:iron molar ratio (5.6 compared with 8.5 in the placebo group), but there was no significant change in total iron intake (range 11.0-12.4mg/day across groups during the intervention) (Heath et al 2001a). In another study, Patterson et al (2001b) found a highly iron-bioavailable diet (aimed at providing 2.25mg absorbable iron/day) produced smaller increases in serum ferritin than daily iron supplementation (105mg iron as ferrous sulphate) in a 12-week intervention in women with a SF<20µg/L and Hb>90g/L. Participants in the diet group were given advice on combinations of high, medium and low iron foods to consume each day, were encouraged to consume enhancers of iron absorption at each meal, and to avoid tea, coffee or milk at lunch and dinner time. However, no placebo group was used, and women in the diet group did not significantly change their intake of haem iron, non-haem iron, ascorbic acid or phytate. Total iron intake at the end of the intervention ranged from 10.7-12.8mg iron/day across each group.

Intervention studies have shown varied results with regard to the effect of meat on iron status. Women with low iron stores (SF≤30µg/L, Hb>120g/L) who consumed 150g meat

per day (approximately 2.4mg iron) in addition to their usual diet for 20 weeks had no significant changes in serum ferritin concentrations (Tetens et al 2007). However, women who consumed vegetable products of similar energy and iron content (to the meat products) and consumed less than 250g meat per week had a significant decrease in serum ferritin concentrations (Tetens et al 2007). Lyle et al (1992) found an increased intake of meat (11.8mg total; 1.8mg haem iron/day) or a 50mg iron supplement/day (57.8mg total; 0.6mg haem iron) better compensated for initial decreases in iron status in women who exercised over a 12-week period when compared to women who ingested a 10mg iron supplement (17.5mg total; 0.4mg haem iron/day), a placebo (8.8mg total; 1.0mg haem iron/day) or a diet of free choice (8.0mg total; 0.8mg haem iron/day) (Lyle et al 1992). Snetselaar et al (2004) found serum ferritin concentrations remained unchanged in adolescents who consumed beef five times per week and poultry/fish ≤ two times per week (increased beef intake by 26g/day; decreased poultry and fish intake by 16g/day between baseline and end), but decreased in adolescents who consumed poultry/fish five times per week and beef ≤ two times per week (increased poultry and fish intake by 7g/day; decreased beef intake by 8g/day) over a three month period. Iron intake in both groups was similar, meeting the dietary reference intake (DRI) for iron. The reduction in serum ferritin concentrations in the predominantly poultry/fish group was attributed to a lower intake of haem iron. In a cross-over study, no significant differences in iron status were observed in iron deficient women who consumed five of eight portions of meat as oily fish (11.5mg iron/day) versus red meat (13.9mg iron/day) for eight weeks (Navas-Carretero et al 2009). Two cross-over studies of short duration where participants with a range of serum ferritin concentrations acted as their own control, found increasing meat intake did not improve serum ferritin concentrations (Hunt et al 1995, Hunt and Roughead 1999). In one study, women consumed either a lacto-ovo vegetarian diet (0g meat; 17.8mg total iron/day) or a non-vegetarian diet (184g meat; 17.5mg total; 1.5mg haem iron/day) for eight weeks (Hunt and Roughead 1999). In the other study, a high meat (289g meat; 12.1mg total iron/day) diet was found to be unexpectedly associated with a lower iron status than a low meat (38.5g meat; 8.8mg total iron/day) and a low meat diet with supplement (38.5g meat; 12.3mg total iron/day) in women who consumed each diet for seven weeks in random order (Hunt et al 1995).

Most studies have found the addition of ascorbic acid to the diet does not impact on iron status (Cook et al 1984, Cook and Reddy 2001, Hunt et al 1990, Hunt et al 1994, Kandiah

2002, Malone et al 1986). The effect of adding ascorbic acid to the diet will be discussed in section 6.2.3.

Calcium supplementation in addition to following a normal diet does not appear to affect iron status. In all the studies described, calcium was provided in the form of calcium carbonate (Kalkwarf and Harrast 1998, Minehane and Fairweather-Tait 1998, Molgaard et al 2005, Sokoll and Dawson-Hughes 1992), with the exception of Ilich-Ernst et al (1998) who provided calcium as calcium citrate malate. No changes in iron stores were observed in premenopausal women when 500mg calcium was given with two meals per day for 12 weeks (Sokoll and Dawson-Hughes 1992) or when 400mg calcium was given three times per day to participants over a six month period (Minehane and Fairweather-Tait 1998). Supplementation with 500mg of calcium twice a day with meals for six months had no effect on serum ferritin concentrations in both lactating and non-lactating women (Kalkwarf and Harrast 1998). No significant difference was seen in serum ferritin concentrations in young girls who received 1000mg calcium per day for four years compared with girls who received a placebo (Ilich-Ernst et al 1998) or in young girls who received 500mg calcium per day for one year in addition to a relatively high habitual intake of calcium (Molgaard et al 2005). A review by Bendich (2001) concluded that calcium supplementation as high as 1200mg per day does not affect iron status in healthy premenopausal women. Further studies are needed to determine whether calcium intake affects iron status in women who are anaemic or have low iron stores (Bendich 2001).

Intervention studies investigating the effect of adding protein to the diet have shown mixed results (Bodwell et al 1987, Hanson et al 2006, Morris et al 1987, Swain et al 2002, Zhou et al 2011). The addition of 40g soy protein isolate containing native phytate to the diet significantly reduced iron status, whereas soy protein with native isoflavones had no effect on iron status in postmenopausal women over six weeks (Hanson et al 2006). In contrast, Swain et al (2002) found iron status increased when women consumed either 40g of soy protein isolate or whey protein per day over a 24-week period. No difference in iron status was observed between groups of participants who consumed one of seven beef products extended with varying amounts and types of soy protein (Bodwell et al 1987) or in participants who consumed all beef or beef patties extended with soy isolate concentrate or flour in one or two meals per day as the main protein source over a six month period (Morris et al 1987). A recent study found adding soy-based foods (19g soy protein/day) to

the diet had no effect on iron status in premenopausal women compared with the addition of animal-based foods (e.g. meat) (Zhou et al 2011).

Only one intervention study had investigated the effect of fibre on iron status. Bach-Kristensen et al (2005) found consumption of a high fibre bread (to recommended dietary intake levels) resulted in a decreased iron status in women with replete iron stores. Reducing the bread's phytic acid content (from a phytic acid: iron molar ratio of 8.5:1 to 6.7:1) was not adequate to maintain iron status.

In summary, intervention studies investigating the impact of diet on iron status have showed mixed results. Limitations of these studies include small sample sizes and therefore possibly insufficient power to show any effect, studies of short duration and in some cases, the use of iron replete women who are less likely to absorb dietary iron.

5.3 Non-dietary factors affecting iron status

In developing countries, increased blood loss from gastrointestinal parasites (e.g. hookworm) and limited dietary diversity (low iron bioavailability due to plant-based diets) are major causes of iron deficiency (Zimmermann et al 2005, Zimmermann and Hurrell 2007). In developed countries, both dietary factors and non-dietary factors including genetics, ethnicity and blood loss are associated with iron status. Any associations between iron status and contraceptive use, body composition, parity, age, socioeconomic status, smoking, physical activity and supplement use are less clear.

5.3.1 Genetics and ethnicity

Genetic factors may explain the variation observed in iron absorption and status among young women. A positive association between body iron in mothers and young children has been reported (Cook et al 2005), and genetic factors accounted for a high percentage of non-haem iron absorption in mother-child pairs (Zimmermann et al 2010). While the specific genes affecting iron status in the general population are not known, single nucleotide polymorphisms (SNPs) affecting iron absorption are common (Whitfield et al 2003). A common polymorphism in the transferrin protein (G277S) has been associated with iron deficiency in women (Lee et al 2001). However, in a stable isotope study no

significant differences in iron absorption were seen in non-anaemic women who were either heterozygous G277S/G277G or wild type G277G/G277G genotype (Sarria et al 2007). Most people with haemochromatosis are homozygous for the C282Y mutation of the HFE gene, although other mutations (e.g. H63D) have been identified. (Roe et al 2005). Other mutations, including the gene for the DMT-1 and ferroportin may also affect iron absorption (Zimmermann et al 2010).

Two cross-sectional studies have investigated associations between iron status and genetic factors in young women. Whitfield et al (2003) found genetic differences in female twins had more influence on iron stores than self-reported magnitude of menstrual blood loss, duration of menstrual periods and parity. However, the variance in the HFE genotype accounted for less than two percent of variance in serum ferritin concentrations (Whitfield et al 2003). More recently, a study in premenopausal Caucasian women (Blanco-Rojo et al 2011a) found four SNPs to be associated with soluble transferrin receptor concentrations (explaining 35% of the genetic variation). Women carrying the transferrin gene rs3811647 had a reduced supply of iron to the tissue, while the presence of the transferrin gene rs1799852 and HFE genes (C282Y and H63) were associated with a lower soluble transferrin receptor concentration (indicating less risk of iron deficiency). Nutrient intake had no effect on any of the measures of iron status (Blanco-Rojo et al 2011a). In other studies, postmenopausal (but not premenopausal) C282Y homozygote women had higher serum ferritin concentrations than women carrying the wild-type gene (Cade et al 2005), while in men, C282Y heterozygosity was not associated with serum ferritin or soluble transferrin receptor concentrations, but was associated with slightly higher transferrin saturation (Heath et al 2008). Further research is needed to identify the nature and location of the genes affecting iron status, and the extent to which they influence iron status.

Research on the effect of ethnicity supports the influence of genetics on iron status. In the United States, iron deficiency was two to three times higher in Mexican American women compared with non-Hispanic white females of reproductive age (Frith-Terhune et al 2000, Looker et al 1997). Mexican American women remained at increased risk of low iron status even after adjustment for dietary and socio-demographic factors including income, education, body mass index (BMI), parity, oral contraceptive use and supplement use (Ramakrishnan et al 2002). A study in New Zealand found iron deficiency was two to

three times, and anaemia three to four times, more common in Māori, Pacific Island and Asian high school students compared with Europeans (Schaaf et al 2000). However, it is not known whether this was due to genetic or lifestyle factors, as dietary intake was not taken into account. In the 2008/2009 New Zealand National Nutrition Survey (University of Otago and Ministry of Health 2011) a higher prevalence of iron deficiency (with and without anaemia) was observed in Māori (10.8%) and Pacific Island (8.9%) women compared with European (3.8%) women aged 19 to 30 years. Similar levels of iron deficiency (with and without anaemia) were observed between ethnicities in women aged 31 to 50 years (range 11.1-12.6%) (University of Otago and Ministry of Health 2011). No studies have investigated the effect of dietary acculturation on iron status.

5.3.2 Blood loss (including contraceptive use)

Only one study has investigated the effect of nose bleeds on iron status and found them to be risk factor for mild iron deficiency (Heath et al 2001b). Several studies have found blood donors to have lower serum ferritin concentrations than non-blood donors (Brussard et al 1997, Cade et al 2005, Heath et al 2001b, Leggett et al 1990, Pynaert et al 2009, Rangan et al 1997). Time since the previous blood donation appears to play an important role, with blood donation in the previous six months (Brussard et al 1997, Rangan et al 1997) or year (Pynaert et al 2009) associated with decreased serum ferritin concentrations. The usual amount of blood donated (450ml) equates to an iron loss of 1-1.35mg per day assuming six months between each blood donation (Coad and Conlon 2011). In New Zealand, women are able to donate blood every four months (New Zealand Blood Service 2010), potentially making it extremely difficult to recoup iron lost if blood donation is that frequent. Heath et al (2001b) found women who donated blood in the past 4 months had a significantly higher risk of mild iron deficiency compared with women who did not donate blood. However, this difference was not significant for women who donated blood in the previous four to twelve or >12 months. A higher frequency of blood donation is also associated with depleted iron stores (Badami and Taylor 2008, Cable et al 2011, Cable et al 2012, Leggett et al 1990).

Menstrual blood loss (MBL) also appears to impact negatively on iron status. An increased duration and extent of menstrual bleeding, assessed using a validated menstrual recall, increased the risk of mild iron deficiency (Heath et al 2001b), while MBL

determined by direct measurement was negatively correlated with serum ferritin concentrations (Harvey et al 2005). Most studies have found duration of menstrual period to be inversely related to serum ferritin concentrations (Galan et al 1985, Rangan et al 1997, Razagui et al 1991). While Whitfield et al (2003) did not find this association, a higher magnitude of menstrual bleeding was associated with reduced serum ferritin concentrations. Asakura et al (2009) found an increased risk of iron deficiency in women with heavy compared with average self-reported menstrual flow, and in women with infrequent or no menstrual cycles compared with women who reported regular menstrual cycles.

The choice of contraceptive method may affect iron status indirectly by impacting on MBL. Oral contraceptive agents decrease MBL and their use has been shown to be positively associated with serum ferritin concentration (Galan et al 1998). Non-hormonal intra-uterine devices (IUDs) increase blood loss and are associated with lower serum ferritin concentrations (Galan et al 1998, Pynaert et al 2009), while hormonal IUDs which reduce menstrual bleeding may have beneficial effects on iron status (Fraser 2010). Heath et al (2001b) found once the extent and duration of menstrual bleeding were controlled for, there was no effect of oral contraceptive use on risk of mild iron deficiency. Other studies have found no relationship between oral contraceptive use and iron status (Brussard et al 1997, Casabellata et al 2007, Galan et al 1985, Rangan et al 1997).

5.3.3 Body weight and composition

Hypoferremia (low serum iron) appears to be more common in obese adults (Menzie et al 2008, Yanoff et al 2007). Mechanisms are not clear, but may include a poor dietary intake of iron (Seltzer and Mayer 1963), or a larger blood volume in the obese (Pinhas-Hamiel et al 2003). Another explanation is the inflammation associated with obesity (Greenberg and Obin 2006) results in expression of pro-inflammatory cytokines, which increase the expression and release of hepcidin. Hepcidin inhibits iron absorption and results in the sequestration of iron (Cepeda-Lopez et al 2011, McClung and Karl 2009, Yanoff et al 2007), leading to decreased iron stores and hypoferremia (McClung and Karl 2009). Tussing-Humphreys et al (2010) found increased serum hepcidin and higher rates of iron depletion in obese versus non-obese premenopausal women, while Zimmermann et al (2008) found obesity to be a predictor of lower iron absorption in young women.

A recent study found iron status (determined using soluble transferrin receptor) did not differ between healthy overweight (BMI 25-30 kg/m^2) or over-fat (percentage body fat ≥ 30.0%) premenopausal women compared to women of normal weight (Karl et al 2009). It was suggested that a certain level of fat mass may be needed to support an association between body composition and low iron status (Karl et al 2009). Other cross-sectional studies in premenopausal women have found serum ferritin to be positively associated with BMI (Broderstad et al 2011, Cade et al 2005, Pynaert et al 2009, Whitfield et al 2003), and Heath et al (2001b) found women with mild iron deficiency (SF<20µg/L) had a slightly but significantly lower BMI than participants without mild iron deficiency. Other studies have found no association between BMI and iron status as determined using serum ferritin (Asakura et al 2009, Brussard et al 1997, Houston et al 1997). However, because obesity is a chronic state of inflammation, serum ferritin (an acute phase protein) tends to be high in people with obesity (Lecube et al 2008), and must be used with caution when investigating associations between iron status and BMI. A systematic review by Cheng et al (2012) found higher concentrations of serum ferritin and haemoglobin concentrations and lower transferrin saturation concentrations in obese compared with non-obese groups. Data were insufficient to make conclusions regarding soluble transferrin receptor, hepcidin or CRP (Cheng et al 2012). Further research is needed to investigate the complex relationship between body weight/composition and iron status. Ideally, iron status should be determined using soluble transferrin receptor rather than serum ferritin, as soluble transferrin receptor concentration is not affected by inflammation or infection.

5.3.4 Other non-dietary factors

It is not clear to what extent recent pregnancy or parity determines iron status. The risk of iron deficiency is thought to reduce post delivery, however post partum iron deficiency often remains a concern (Bodnar et al 2005). In Belgium, women who had been pregnant in the past year had lower serum ferritin and higher soluble transferrin receptor concentrations (Pynaert et al 2009), while in the United States iron deficiency and iron deficiency anaemia was more common in women with higher parity (Frith-Terhune et al 2000, Looker et al 1997). Other studies have found no association between having children (Heath et al 2001b) or number of children (Whitfield et al 2003) and iron status.

Excluding menstrual blood loss and parity, there appears to be no reason for a positive relationship between age and iron status (Heath et al 2001b). Studies in premenopausal women have not found age to be associated with iron deficiency (Broderstad et al 2011, Pynaert et al 2009, Rangan et al 1997). In studies where age was associated with an increased iron status (Brussard et al 1997, Cade et al 2005, Leggett et al 1990, Whitfield et al 2003), postmenopausal women were included, which is likely to explain the association found.

In the 1997 New Zealand National Nutrition Survey (Russell et al 1999), women of lower socio-economic status had a slightly higher prevalence of iron deficiency. The relationship between iron status and socio-economic status was not reported in the 2008/2009 New Zealand Adult Nutrition Survey (University of Otago and Ministry of Health 2011). Iron deficiency was found to be more common among the poor and less educated in the United States (Looker et al 1997). Possible explanations could include a lack of food security (including less money to spend on meat), limited dietary diversity (Lynch 2011b) and inadequate or no access to health care (Looker et al 1997, United Nations Children's Fund/World Health Organization 1999). However, in one Australian study a higher social status was associated with a reduced iron status (Rangan et al 1997). Other cross-sectional studies have found no association between education (Cade et al 2005, Pynaert et al 2009) or socio-demographic status (Heath et al 2001b) and iron deficiency.

The association between dietary supplements and iron status is not clear from cross-sectional studies. Blanck et al (2005) found no association between supplemental iron and serum ferritin concentrations in young women. However, another study found females taking iron supplements which provided 15- 30mg iron per day were less likely to have iron deficiency than females who did not take supplements (Frith-Terhune et al 2000). The use of a multivitamin and mineral supplement in the past year reduced the risk of mild iron deficiency in premenopausal women living in New Zealand (Heath et al 2001b). Other cross-sectional studies have not found any association between supplement use and iron status (Asakura et al 2009, Brussard et al 1997, Leggett et al 1990, Rangan et al 1997). These differences in findings may be due to variations in the type and amount of iron in the supplement, other nutrients or non-nutrients contained in the supplement, or the frequency and duration of supplement consumption.

Most studies have found no association between physical activity or exercise and iron status (Asakura et al 2009, Broderstad et al 2011, Houston et al 1997, Leggett et al 1990, Pynaert et al 2009, Rangan et al 1997). Leggett et al (1990) found no relationship between frequency of active exercise and iron status, while Rangan et al (1997) found no association between iron deficiency and exercise frequency, duration or intensity. Iron status may be affected in athletes due to an increased red cell turnover, poor dietary choices and in runners, gastrointestinal blood loss and foot strike haemolysis (Beard and Tobin 2000). A review study undertaken nearly 20 years ago found the prevalence of low serum ferritin concentrations to be more common in female athletes compared with untrained female controls (Fogelholm 1995), although more recently Malczewska et al (2000) observed higher serum ferritin concentrations in female endurance athletes compared with untrained controls.

Most studies have not found smoking to be associated with iron status (Asakura et al 2009, Broderstad et al 2007, Cade et al 2005, Heath et al 2001b, Leggett et al 1990, Péneau et al 2008). However, in one small study, smoking was negatively associated with serum ferritin concentrations (Houston et al 1997). Pyneart et al (2009) found no relationship between smoking and serum ferritin concentrations, however non-smokers had higher soluble transferrin receptor concentrations (indicating increased functional or tissue iron deficiency) (Pynaert et al 2009).

5.4 Conclusion

Given that a number of dietary factors impact on iron absorption and status, it makes sense that the whole diet is considered when investigating dietary factors associated with iron status. The use of dietary patterns and practices considers the whole diet, which is particularly relevant to iron nutrition. Dietary patterns should however be considered in the context of non-dietary factors (e.g. blood loss, ethnicity), as these are also likely to impact on iron status.

6 Solutions to improve iron status

Iron deficiency can be treated through a range of measures including dietary interventions and education to increase the intake and bioavailability of iron in the diet (Food and Agricultural Organization of the United Nations/World Health Organization 2004), iron fortification of food or through iron supplementation.

6.1 Iron supplementation

Iron supplementation is necessary for the treatment of iron deficiency anaemia in order to restore haemoglobin concentrations and replenish body iron stores (Goddard et al 2011). A range of iron supplements are available with varying levels of bioavailability. The benefits are limited to the period of supplementation and for a few months afterwards in individuals with high requirements (Lynch 2011b). Iron supplementation may erode the gut, causing unpleasant side-effects (Cook and Reusser 1983) such as nausea, abdominal discomfort, diarrhoea, constipation, dizziness, headaches and fatigue (Galloway and McGuire 1994). While much has been made about these side-effects reducing patient compliance (Pena-Rosas and Viteri 2009, Seck and Jackson 2008), this is controversial (Galloway and McGuire 1994, Hyder et al 2002). In non-pregnant women, weekly supplementation has been shown to be as effective as daily supplementation and may enhance compliance (Lynch 2011b).

6.2 Dietary treatment of iron deficiency

While iron supplementation is necessary to improve iron status in iron deficiency anaemia, the best treatment for depleted iron stores or non-anaemic iron deficiency is less clear (Heath et al 2001a). Dietary intervention is often recommended as the first line of treatment for depleted iron stores (Heath et al 2001a). This may include an increased intake of iron in the diet including the use of iron-fortified foods and increasing the iron bioavailability of the diet.

6.2.1 Iron fortification

Food fortification involves the addition of one or more essential nutrients to a food, for the purpose of preventing or correcting a deficiency of one or more nutrients in the population (Food and Agriculture Organization of the United Nations 1996). Fortifying food with iron can be a cost-effective, long-term approach to combating iron deficiency in populations with a high prevalence of dietary iron deficiency (Lynch 2011b). Depending on the choice of food, mandatory fortification may enable all sectors of the population to be reached. Furthermore, success is not dependent on individual motivation and compliance (Baker and DeMaeyer 1979). However, the risks of food fortification include toxicity (Lynch 2011b) (e.g. iron toxicity in people with haemochromatosis) and a possible increased risk of cancer due to iron's role as a prooxidant (Knekt et al 1994).

Food fortification serves to meet a variety of objectives. Market-driven fortification is common in developed countries. It includes voluntary initiatives led by manufacturers to enhance sales such as adding micronutrients to breakfast cereals, usually within regulatory limits. Targeted fortification is aimed at meeting the needs of specific groups (e.g. infant weaning foods), while mass fortification is usually mandatory and involves the addition of micronutrients to foods commonly consumed by the general population (World Health Organization/Food and Agriculture Organization of the United Nations 2006). Foods that have been fortified with iron include wheat flour, cereals, milk and condiments such as sugar, salt, fish sauce, soy sauce and curry powder (Hurrell 1997).

The development of an iron-fortified food involves selecting an iron compound that is adequately absorbed but does not change the taste and appearance of the food to which it is added. The iron chosen must also be able to overcome the inhibitory effects of some food components (e.g. phytic acid) on iron absorption (Hurrell 2002). Water soluble iron compounds such as ferrous sulphate are highly bioavailable and therefore desirable to use as food fortificants. However they often lead to sensory changes (including taste and colour changes, precipitation, fat oxidation and rancidity (Hurrell 2002)), making them unsuitable for use in many foods (Hurrell et al 2004). Encapsulated ferrous sulphate has the potential to maintain iron bioavailability, while preventing unwanted sensory changes. It has been observed that the bioavailability of encapsulated ferrous sulfate is similar to ferrous sulfate in rodent studies (Hurrell et al 1989). Bioavailability is also dependent on

the coating material used and thickness of the capsule (Hurrell 2002). Encapsulated iron has been used in infant formulas and infant cereal, but few other foods (Hurrell 2002). Problems still remain with the heat instability of the capsule and the increased cost of encapsulation must be considered (Hurrell 2002).

Elemental iron powders have the lowest and most variable bioavailability (Hurrell 2002), but are commonly used to fortify food products as they are relatively inexpensive and do not cause sensory changes, increasing shelf life and consumer acceptability (Hurrell 1997). Most breakfast cereals have been fortified with elemental iron, but the effect of elemental iron powders on iron status remains controversial. Hurrell (1997) suggested that the fortification of breakfast cereal products (high phytic acid content) with reduced elemental iron may not be useful, particularly in the absence of ascorbic acid. A recent study however, found that adding 12 mg of iron per day to snack foods over 35 weeks (as either ferrous sulphate, or the elemental iron powders, electrolytic iron or hydrogen-reduced iron) increased iron status in women with low iron stores (Zimmerman et al 2005). Ferrous sulphate increased body iron stores to a greater extent than electrolytic iron and hydrogen-reduced iron (Zimmerman et al 2005). The amount of iron needed in food fortification is difficult to quantify as the amount of iron absorbed depends on several factors including the iron fortificant itself and the bioavailability of the food to which the iron is added (Hurrell et al 2004). However, it has been recommended that the amount of iron added to complementary foods for infants should be sufficient to meet their DRI (Lynch and Stoltzfus 2003). Iron status improved in a 16-week RPCDB trial in iron deficient premenopausal women when 18mg iron per day (DRI-equivalent) was added to the diet as microencapsulated iron pyrophosphate in the form of a fruit juice (Blanco-Rojo et al 2011b).

6.2.2 Improving the bioavailability of fortification iron

Strategies to counteract inhibitors of iron absorption include the addition of fortified iron in a form that is protected from combining with dietary inhibitors (e.g. NaFeEDTA or bovine haemoglobin), the degradation of phytic acid or the addition of enhancers of iron absorption (e.g. ascorbic acid) to the food product (Hurrell 1997).

6.2.2.1 Ascorbic acid

Ascorbic acid is the most widely used enhancer of fortified iron. Both synthetic and ascorbic acid in foods increase iron absorption (Cook and Monsen 1977, Diaz et al 2003, Hallberg et al 1986). Ascorbic acid acts identically on iron in food and fortification iron such as ferrous sulphate (Lynch and Stoltzfus 2003).

Ascorbic acid has been shown to increase the absorption of ferrous sulphate, ferric ammonium citrate, ferric orthophosphate, ferrous fumerate and electrolytic iron (Hurrell 2002), with most studies investigating its effect on ferrous sulphate absorption (Lynch and Stoltzfus 2003). The enhancing effect of ascorbic acid on iron absorption appears to be dose-related (Bjorn-Rasmussen 1974, Cook and Monsen 1977, Siegenberg et al 1991). Cook and Monsen (1977) observed a linear increase in iron absorption (25mg ascorbic acid added to a semi-synthetic meal containing 4.1mg iron as ferrous sulphate increased iron absorption by 65%, while 1000mg ascorbic acid increased iron absorption by 857%). Low levels of ascorbic acid (<25mg) are less likely to increase iron absorption (Bjorn-Rasmussen 1974, Fairweather-Tait et al 2000, MacPhail et al 1981), while 25 to 50mg ascorbic acid should promote a measureable effect on iron absorption when added to meals (Hallberg et al 1986, Tuntawiroon et al 1991). However, this will depend on the other components in the meal. For iron soluble compounds (such as ferrous sulphate), it has been suggested that meals containing low to medium levels of inhibitors require the addition of ascorbic acid to iron at a molar ratio of 2:1. However, a ratio of at least 4:1 is needed to increase iron absorption from foods high in phytic acids (e.g. whole grain breads and cereals) or polyphenols (e.g. tea and coffee) (Hurrell 2002).

Ascorbic acid is only effective in enhancing iron absorption when taken with meals (Cook and Monsen 1977). Cook and Monsen (1977) found ascorbic acid taken in the morning did not influence iron absorption from meals eaten four and eight hours later. The instability of ascorbic acid limits the number of foods it can be successfully added to. The addition of fruits and vegetables high in ascorbic acid to iron-fortified dried blended foods has been recommended (Teucher et al 2003). However, ascorbic acid in fruits and vegetables will degrade with exposure to air during storage, cooking and food processing (Teucher et al 2003). The bioavailability of encapsulated iron consumed with ascorbic acid requires further investigation.

The effect of ascorbic acid on iron absorption from complete diets appears to be lower than that from single meals (Cook and Reddy 2001, Hunt et al 1994). This may help to explain why prolonged supplementation with ascorbic acid does not appear to affect iron status (Cook et al 1984, Cook and Reddy 2001, Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986), as shown in Table 2.7. Cook et al (1984) found consuming 1000mg ascorbic acid with meals twice per day for 16 weeks did not improve serum ferritin concentrations in young healthy adults (n=17) who had a range of iron stores and ate self-selected diets. This was not caused by adaptation to the ascorbic acid intake, as iron absorption from single test meals was still observed after 16 weeks of supplementation. Four participants whose initial serum ferritin was <10µg/L did however, improve their iron status. However, no significant improvement was seen in the serum ferritin concentrations of five iron replete and four iron deficient participants (SF<6µg/L) who continued to receive ascorbic acid for 20 months. The authors concluded that ascorbic acid may have little effect on iron status when the diet contains substantial amounts of meat (Cook et al 1984).

In a RPCDB study no changes in serum ferritin concentrations were observed in 48 healthy young women supplemented with 100mg of ascorbic acid three times per day with meals for eight weeks (Malone et al 1986). The iron status of these women was not reported.

Hunt et al (1994) found 500mg ascorbic acid consumed with meals three times per day for five weeks did not significantly improve serum ferritin concentrations in 25 iron depleted (SF 3.5-17.7µg/L) premenopausal women consuming typical Western diets or diets of poor iron bioavailability when compared with a placebo in a double blind cross-over study.

In another study, 11 premenopausal women (SF<8.5µg/L achieved through phlebotomy and a low iron diet) consuming a diet of low iron bioavailability were provided with 500mg ascorbic acid three times per day with meals for five and a half weeks in a controlled environment. Haemoglobin concentrations improved significantly in the ascorbic acid group, but serum ferritin concentrations did not change (Hunt et al 1990), indicating that absorbed iron may have preferentially been used for haemoglobin synthesis.

No changes in serum ferritin concentrations were seen in 14 vegetarian women who consumed tofu alone or tofu and orange juice for 30 days in a cross-over study (Kandiah 2002).

In a well-designed intervention study, Garcia et al (2003) found the addition of 25mg of ascorbic acid as lime juice to two meals per day six days per week for eight months did not improve iron status (serum ferritin or haemoglobin) in 18 iron deficient (SF<12µg/L) women compared to a control group consuming a lime-flavoured beverage with no ascorbic acid (Garcia et al 2003). This was despite an earlier study showing lime juice containing 25mg ascorbic acid more than doubled iron absorption in women with serum ferritin concentrations less than 12µg/L (Diaz et al 2003). These women consumed diets high in phytate and non-haem iron, and both groups had a similar intake of iron. Although not measured, it is likely these women had high menstrual blood losses (leading to low iron stores), meaning that the improved iron absorption seen (Diaz et al 2003) was insufficient to increase iron stores (Garcia et al 2003). In addition, it was suggested that other micronutrient deficiencies (e.g. vitamin A, vitamin B12) may have confounded any enhancing effect on iron status (Garcia et al 2003).

A recent study in 122 iron deficient women found iron status improved in women who consumed an iron-fortified fruit juice (containing 18mg iron as microencapsulated iron pyrophosphate and 95mg ascorbic acid) after 4, 8, 12 and 16 weeks. While this study was not investigating the effect of ascorbic acid on iron status per se, it is interesting to note that iron status did not improve in women who consumed the fruit juice only (i.e. ascorbic acid, but no iron) (Blanco-Rojo et al 2011b).

The inconsistent findings in the literature may be explained by various limitations in study designs. These studies have included small sample sizes (range n=11-48) with insufficient power to detect associations (Cook et al 1984, Garcia et al 2003, Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986), interventions of short duration (Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986), a lack of suitable control groups (Cook et al 1984) and the use of participants with normal rather than low iron stores (Cook et al 1984, Kandiah 2002, Malone et al 1986). A measurable effect of dietary interventions on iron status may only be seen in populations with a low iron status (who absorb more iron from the diet (Hallberg et al 1997)). The baseline diet to which

food is added will impact on how much iron is absorbed. Less iron will be absorbed when foods or nutrients are added to a diet with high iron bioavailability compared with adding foods or nutrients to a diet of lower bioavailability (Cook and Monsen 1977). Most dietary intervention studies have not controlled for other factors that may affect iron status including blood loss (Cook et al 1984, Garcia et al 2003, Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986). In addition, it is possible that when participants have low haemoglobin concentrations, absorbed iron is preferentially used for haemoglobin synthesis before iron stores are increased (as indicated by serum ferritin concentrations) and many of the studies were not continued long enough for this to be observed (Hunt et al 1990, Hunt et al 1994, Kandiah 2002, Malone et al 1986). Interventions should be at least 12-16 weeks duration to allow adequate time for red blood cell turnover (red blood cells have a life-span of 90-120 days) (Coad and Conlon 2011). Furthermore, most studies have not reported the iron content of the meals to which ascorbic acid was added (Cook et al 1984, Malone et al 1986) or have only reported total daily iron intake (Garcia et al 2003, Hunt et al 1990, Hunt et al 1994). In the one study that did report the iron content of the meals to which ascorbic acid was added, only 2.24mg of iron was consumed at each meal (Kandiah 2002). Ascorbic acid's mechanistic action for enhancing iron absorption means that ascorbic acid is more likely to improve iron status if added to a meal containing a substantial amount of fortified iron (Garcia et al 2003, Hurrell 2002), especially as ferrous sulphate (Hurrell 2002).

Table 2.7. Interventions in adults using ascorbic acid to improve iron status (Scientific Advisory Committee on Nutrition 2010)

Author, date, country	Participants, Duration of intervention	Dietary intervention	Serum ferritin (µg/L) Baseline/End	Haemoglobin (g/L) Baseline/End	Final outcome
Garcia-Casal et al (2003) Mexico	36 females, 28y (mean), SF<12µg/L 8mth RPC	Maintained normal diet – low intake bioavailable iron Intervention - limeade (25mg aa) at 2 meals, 6d/wk Placebo – limeade (no aa) at 2 meals, 6d/wk	 6.4/9.0 (NS) 6.2/8.7 (NS)	 137/140 (NS) 139/137 (NS)	No significant differences between groups in SF, Hb, sTfR, sTfR:SF ratio
Kandiah (2002) USA	14 females, 20-25y, vegetarian, 1/3 had SF<12µg/L 30d cross-over	Normal diet & 57.7g tofu/meal 3x/d (limitations on aa and iron/serving) - 30d – 83.3mL (101mg aa) orange juice 3x/d with meals - 30d – nothing -	 n/a n/a	 8.1 & 6.4 -5.5 & -6.5[a]	No significant difference in SF between groups; Hb increased significantly with orange juice compared with no orange juice
Hunt et al (1994) USA	25 females, 20-45y, SF 3.5-17.7µg/L 5wk PCDB cross-over	Diet with poor iron bioavailability - 500mg aa 3x/d with meals - Placebo 3x/d with meals OR Typical Western diet - 500mg aa 3x/d with meals - Placebo 3x/d with meals	 -/12.9 (NS) -/11.4 (NS) -/10.6 (NS) -/9.7 (NS)	 -/132 (NS) -/131 (NS) -/132 (NS) -/130 (NS)	No significant increase in SF or Hb with aa compared with placebo

Author, date, country	Participants, Duration of intervention	Dietary intervention	Serum ferritin (µg/L) Baseline/End	Haemoglobin (g/L) Baseline/End	Final outcome
Hunt et al (1990)	11 females, 22-36y,SF<8.5ug/L	13.7mg iron/2000kcal			No significant increase in SF with aa compared with placebo; Hb increased significantly with aa compared with placebo
		500mg aa 3x/d with meals	n/a	n/a	
USA	5.5wks single blinded	Placebo 3x/d with meals	n/a	n/a	
Malone et al (1986)	48 males & females, 17-26y	Self-selected diets			No significant increase in SF with aa compared with placebo
		100mg aa 3x/d	27/31.3 (NS)	n/a	
Ireland	8wk RPCDB	Placebo 3x/d	23/23 (NS)	n/a	
Cook et al (1984)	17 males & females, 20-30y, SF- range	Self-selected diets			No significant increase in SF with aa after 16 weeks or 2 years
		1000mg aa 2x/d with meals	46.3/43.9 (NS)	n/a	
USA	16wk study (n=9 for 20 mths)				

Adapted from 'Iron and Health' Scientific Advisory Committee on Nutrition (Scientific Advisory Committee on Nutrition 2010); [a] *Change in Hb concentrations during first & second experimental period; Aa – Ascorbic acid; Hb – Haemoglobin; n – number; n/a – not available; NS – non significant; PCDB – Placebo controlled double blind; RPC – Randomised placebo controlled; RPCDB – Randomised placebo controlled double blind; SF – Serum ferritin; sTfR – Soluble transferrin receptor*

6.2.2.2 Carotenoids

Carotenoids (a group of non-polar compounds found in plant foods, particularly in green leafy vegetables and yellow, orange and red fruits) also may have the potential to enhance the absorption of fortified iron in foods. Some carotenoids such as α and β-carotene and β-cryptoxanthin are converted into vitamin A (retinol) in the body. However, the effect of carotenoids and vitamin A on iron absorption is controversial. Vitamin A has been shown to increase iron absorption in some (Layrisse et al 1997, Layrisse et al 1998), but not all studies (Walczyk et al 2003). Garcia-Casal et al (1998) found both vitamin A and β-carotene increased iron absorption from rice, wheat and corn.

Carotenoids such as lutein, zeaxanthin and lycopene (which lack provitamin A activity) have been shown to enhance iron absorption in humans when added to a wheat- or corn-based breakfast meal. The increase was similar for lutein and zeaxanthin, while the effect of lycopene was smaller (Garcia-Casal 2006). This effect may be mediated by changes in iron solubility or by the formation of a carotenoid-iron complex (Garcia-Casal 2006), preventing the inhibitory effects of phytic acid and polyphenols on iron absorption (Garcia-Casal et al 1998). No studies have specifically investigated the effect of carotenoids including lutein, zeaxanthin and lycopene on iron status in human beings.

6.3 Conclusion

Iron deficiency may be treated through iron supplementation, iron fortification or interventions to increase the intake and bioavailability of iron in the diet. While ascorbic acid (and carotenoids) enhance iron absorption, the effect of ascorbic acid and caroteinoids on iron status over the long-term is less clear and needs to be further investigated.

7 Dietary assessment methods for investigating the diet-iron status relationship

7.1 Introduction

There are a number of methods available to assess dietary intake, and it is important that this is done accurately. Commonly used methods include food frequency questionnaires, food records, 24-hour recalls and diet histories (Gibson 2005a).

7.2 Dietary assessment methods

7.2.1 Food frequency questionnaires

The FFQ consists of a list of foods and a set of frequency of response categories, to assess how frequently a person consumes certain foods (Gibson 2002). It is designed to assess usual food consumption patterns over a specified, retrospective time period ranging from weeks or months to the previous year (Gibson 2002).

The FFQ may focus on specific foods or groups of foods (e.g. to estimate intakes of iron by only including foods that contribute to dietary intakes of iron), or may include an extensive list of foods to enable estimates of total food intake to be made (Gibson 2002). Focusing on specific foods or food groups saves unnecessary data collection and handling, and minimises participant burden.

The FFQ can minimise error arising from day-to-day variability, and illustrate the habitual dietary intake within a group. It tends to have a high response rate and a relatively low participant burden. It is relatively inexpensive and quick to complete (Gibson 2002). The FFQ is completed with an interviewer or can be self-administered (either on paper or computer). Computerised FFQs eliminate the need for extra data entry (Cade et al 2002).

A FFQ can be quantitative, qualitative or semi-quantitative. Quantitative FFQs require participants to record the serving sizes of foods (e.g. small, medium, large) (Lee and Nieman 2010b). This allows more detail on the quantity of foods and therefore nutrients, but increases participant burden. A semi-quantitative FFQ assesses the frequency of

food intake, while providing standardised portion sizes (usually based on national nutrition survey data). It can be used to assess intake of foods and food groups, as well as intakes of energy and other nutrients (Willett et al 1985). A qualitative FFQ asks for frequency of consumption of foods and food groups only, and can be used to describe dietary patterns (Gibson 2005a).

FFQ data can be used to categorise participants into low, medium and high intakes of specific foods, food components or nutrients. These categories can be compared with outcomes such as prevalence of certain disease (e.g. intake of iron and iron deficiency) (Gibson 2002). The accuracy of the FFQ is often lower than other dietary assessment methods, and depends on the ability of participants to describe their diet (Gibson 2002). The estimation of portion sizes is also subject to error. Participants may overestimate total intake when asked to report frequency of intakes of numerous single foods within a food group. This can be minimised by including summary questions, asking for total daily or weekly intake (Bohlscheid-Thomas et al 1997).

7.2.2 Weighed/estimated food records

Using a food record, participants are asked to record all foods and beverages consumed during a specified time period (usually ranging from one to seven days) (Gibson 2002). Details including food descriptions (brand names, varieties of foods) and preparation and cooking methods are also recorded. For composite dishes, details including the amounts of raw ingredients used, and the final weight of the recipe should be recorded (Gibson 2002). Both week days and weekend days should be represented to account for possible differences across these days (Gibson 2002). Alternatively, across a group it should be ensured that all days of the week are proportionally represented (Gibson 2005c).

For a weighed food record, participants weigh all food items using dietary scales. Results therefore show more accuracy and precision compared with estimated food records (Gibson 2002). However, errors can still occur in misreading scales or recording the dietary information. It is also more time consuming than completing an estimated food record (Gibson 2002), which relies on the participant's ability to estimate quantities correctly. For estimated dietary records, household measures such as measuring cups or measuring spoons can be used to estimate the amounts of foods and beverages

consumed. Portion sizes can be estimated using a ruler for some foods, or counts for others e.g. slices of bread (Gibson 2002), or through photographs of portions of commonly consumed foods provided to participants.

Food records are time consuming and have a higher participant burden than other methods (Gibson 2002). The participant must be motivated, conscientious, numerate and literate (Gibson 2002). Participants may change their usual eating pattern to impress the researcher or to simplify the recording process (Gibson 2005d). Because food intakes are recorded when consumed, errors from memory lapses are less likely to occur (Gibson 2002). Food records are also more expensive than other methods, due to the time taken to code and analyse foods. While recording more days may provide a better picture of what participants eat, it also increases participant burden and may lower compliance (Gibson 2002). Food records are usually used to assess the intake of individuals (Gibson 2002).

7.2.3 24-hour recalls

The objective of the 24-hour recall is to obtain information on a participant's intake of all foods and beverages consumed during the previous day or 24 hours, with the help of a trained interviewer. Advantages of the 24-hour recall method include a low participant burden and good compliance (Gibson 2002). It is also relatively inexpensive and time efficient. It is suitable to use with illiterate participants, and participants may be less likely to alter their dietary intake due to lack of familiarity with how the 24-hour recall is conducted (contains an element of surprise) (Gibson 2002). However, it does rely on participant honesty, memory, motivation and the participant's ability to accurately recall portion sizes. This can be enhanced by using foods models, photographs or household measures to assist in quantifying portion sizes (Gibson 2002). Single 24-hour recalls do not take into account day-to-day variation in an individual's diet, and may omit foods that are consumed infrequently (Gibson 2002). Several 24-hour recalls are often used to estimate usual intake (Gibson 2002). The 24-hour recall is useful for assessing average intakes of a large population, if the sample is representative and days of the week are equally represented (Gibson 2002).

7.2.4 Diet history questionnaires

The diet history questionnaire (DHQ) is an interview-based technique designed to assess usual dietary intake over time. It usually consists of a 24-hour recall of actual food intake (including information on eating patterns at meal times and in between meals), and a food frequency questionnaire (designed to substantiate and clarify information regarding types and amounts of food eaten) (Gibson 2002). The DHQ relies on the participant's memory and ability to estimate portion sizes correctly. It is labour intensive, time consuming, requires a skilled interviewer and is therefore more expensive (Gibson 2002). The DHQ provides an estimate of habitual intake and is often used in the clinical setting (Bingham 2007).

7.2.5 Other dietary assessment methods

More recent developments for assessing dietary intake include telephone-based approaches, use of cameras and videos to record the food consumed and any foods leftover, electronic devices (e.g. smart phones) for recording food intake directly, list-based tools and computer assisted interviewing techniques (Gibson 2002). These methods all aim to reduce errors, decrease researcher and participant burden and therefore improve compliance (Gibson 2002).

7.2.6 Errors associated with dietary assessment

There is potential for errors to occur when dietary intake is measured. These errors include interviewer bias, participant memory lapses, the incorrect estimation of foods, and coding and computation errors (Gibson 2005d). Participants may over-report the consumption of healthy foods, and under-report the consumption of less healthy foods (Gibson 2002). Under-reporting of energy intake is a common problem affecting dietary assessment methodology (Black et al 1991). A number of methods may be used to identify low energy reporters, including the Goldberg method (Goldberg et al 1991), which determines under-reporting according to a cut-off ratio based on physical activity levels, number of days recorded and an individual's energy intake and basal metabolic rate. However, once low energy reporters are identified there is a lack of agreement as to whether they should be eliminated from the analyses (Heath et al 2000). For example,

reporting of low energy intakes may also occur in those with plausible energy intakes (Macdiarmid and Blundell 1997). Excluding only women who have implausible intakes may bias the results. The Goldberg method also assumes individuals are in energy balance and not following a weight-loss diet (Heath et al 2000).

7.2.7 Selecting an appropriate dietary assessment method

The dietary assessment method chosen depends on a number of factors including the study objectives, the dietary outcome of interest, the precision required, the participant group (e.g. adult versus child) and available resources. More accurate methods tend to be associated with higher costs and increased participant burden (Gibson 2002).

7.3 Dietary assessment tools designed to assess iron intake and bioavailability

When using a food record, it is recommended that at least 11 days of dietary intake are recorded to ensure an accurate estimate of iron intake due to large intra-participant variability (Willett 1998). This is often not possible, as it places considerable demand on the researcher and participant (Heath et al 2000). FFQs are often used to ease the burden on both the researcher and participant. While several generic FFQs are available to assess iron intake (Palmer and Morgan 2012, Serra-Majem et al 2009b), a number of FFQs with a specific focus on iron have been developed, with their validity (Heath et al 2000, Heath et al 2005, Matthys et al 2004, Pynaert et al 2008, Zhou et al 2005) and reproducibility (Heath et al 2000, Heath et al 2005, Matthys et al 2004) assessed. These have included a computerised iron FFQ aimed at estimating intake of iron and dietary components affecting iron absorption (ascorbic acid, calcium, grams of meat/fish/poultry, tea and coffee) in New Zealand women (Heath et al 2000); an iron intake assessment tool designed to measure iron, calcium and ascorbic acid intake in Belgian women (Matthys et al 2004); a meal-based intake assessment tool to assess total iron and absorption modifiers (ascorbic acid, phytate, calcium, meat/fish/poultry, black tea, zinc) for males living in the United Kingdom (Heath et al 2005) and an iron checklist aimed at assessing dietary iron intake in pregnant and postpartum women (Zhou et al 2005). One study has investigated the ability of a two-day food record to estimate iron, calcium and ascorbic acid intake in young women aged 18 to 39 years living in Belgium (Pynaert et al 2008).

7.4 Analysis of dietary data – nutrients, foods or dietary patterns

Most studies investigating the associations between diet and iron status have focussed on individual nutrients and foods. This has several limitations, some of which are particularly applicable to iron nutrition. These limitations are discussed in section 2.1. In brief, people don't eat foods or nutrients alone, but consume meals containing a variety of foods and nutrients that may interact (Hu 2002, Newby and Tucker 2004) (e.g. ascorbic acid in orange juice and non-haem iron in breakfast cereal); statistically significant associations may occur by chance when several foods and nutrients are investigated in isolation (Farchi et al 1989, Hu 2002); associations between iron status and individual foods and nutrients may be too small to detect, however the cumulative effects of individual foods and nutrients may be more apparent (Hu 2002, Newby and Tucker 2004), the high levels of correlations between some nutrients in foods make it difficult to investigate their effects separately (Hu 2002, Newby and Tucker 2004) and finally the effects of individual foods and nutrients may be confounded by the effects of dietary patterns (Hu 2002).

In addition to these limitations, some foods may contain components yet to be identified, which may have a role in determining iron status. These will be overlooked in the analysis of nutrient contents alone. Analysis of nutrient intake also depends on the use of food composition data, which has its own limitations including natural variation in food composition, limited or partial coverage of food items or nutrients (e.g. polyphenols and phytates are not available in the New Zealand Food Composition database) and inappropriate food composition values within the database due to random and systematic errors (Gibson 2005e).

7.5 Dietary patterns

Dietary pattern (food/eating pattern) analysis is an alternative and complementary approach to studying individual foods and nutrients and their association with iron status (Hu 2002, Newby and Tucker 2004). Dietary patterns refer to how foods are consumed in various characteristic combinations (Newby and Tucker 2004), and considers the whole diet rather than focusing on a single nutrients or foods. Dietary patterns can be established using a theoretical approach whereby foods and nutrients are grouped

according a set criteria for nutritional health (e.g. a dietary index created to rank dietary intake based on the iron bioavailability of the diet). Alternatively an empirical approach is used where foods and nutrients are reduced into a smaller number of variables using statistical techniques such as factor or cluster analysis (Newby and Tucker 2004). Factor analysis reduces data into patterns based upon inter-correlations between dietary items, while cluster analysis reduces data into patterns based upon individual differences in mean intake (Newby and Tucker 2004). The continuous nature of factor analysis whereby participants receive a factor score for all dietary patterns is often seen as advantageous to cluster analysis (Crozier et al 2006), where participants belong to one cluster only (Newby et al 2004). The continuous variables associated with factor analysis may increase statistical power, while grouping of participants via cluster analysis, can lead to reduced power for detecting associations between dietary intake and disease, particularly in small samples (Slattery 2010). Cluster analysis does however provide clear, categorical descriptions of what sub-groups of participants are eating, and therefore can be useful for designing nutrition interventions (Slattery 2010). Reduced rank regression is another empirical approach which determines combinations of dietary intake variables that best explain the variance in a set of disease-specific response variables (Slattery 2010). This approach is useful for hypothesis generation, however food combinations identified in each dietary pattern are highly outcome specific and not necessarily ideal for optimal health. The food combinations elicited often don't reflect actual eating behavior. Reduced rank regression is a relatively new approach and has not yet been shown to be reproducible across datasets (Slattery 2010).

The use of dietary pattern analysis offers several advantages. An individual's health is likely to be associated with the cumulative effects of a whole diet (as determined by dietary patterns), and not just a select few nutrient and foods (Slattery 2010). There is no one single food that prevents chronic disease, and dietary patterns enable a consistent message to be provided across several disease states with multiple and complex aetiologies (Tucker 2010). While dietary pattern analysis offers a number of advantages, limitations also need to be considered. By considering the whole diet, a single nutrient of importance may not be noted (Slattery 2010) (e.g. folic acid in the case of neural tube defects) (Hu 2002). The labelling of dietary patterns is subjective, and may be confusing to the general public. For example, opinions on what constitutes a 'healthy' dietary pattern may vary (Slattery 2010). Finally, differences in gender, ethnicity, culture and

socio-demographic status are likely to impact on the dietary patterns identified. Therefore research should be replicated in different populations (Hu 2002). Further limitations with regard to factor analysis are discussed below. Dietary pattern analysis should not replace food or nutrient analysis, but be used as a complementary approach to these methods (Hu 2002). Dietary patterns can also be used as a co-variate when investigating associations between nutrient intake and disease, to determine whether the effects of those nutrients are independent of overall dietary patterns (Hu 2002).

Two studies have previously used factor analysis to describe associations between dietary patterns and iron status (discussed in section 5.2.2.2 (Broderstad et al 2011, Shi et al 2006)). The FFQs used in studies investigating associations between dietary patterns and iron status have not been validated for foods, nutrients or dietary patterns (Broderstad et al 2011, Shi et al 2006). No studies have been undertaken to assess the validity (or reproducibility) of dietary patterns determined using a FFQ specifically developed to consider foods affecting iron intake and bioavailability.

7.5.1 Factor analysis methodology

Factor analysis includes both common factor analysis and principal component analysis (Hu 2002). Principal component analysis is commonly used to derive dietary patterns. Individuals receive a score for each factor (dietary pattern), which is a continuous variable (Hu 2002).

Despite being data-driven, factor analysis involves many subjective decisions. These impact on the number and type of patterns derived, reported and analysed (Newby and Tucker 2004). Depending on the dietary assessment method used (e.g. FFQ versus food record), there are often several foods available for analysis. Most studies involving dietary pattern analysis have used data from FFQs. As well as reduced participant and researcher burden, FFQs are able to summarise dietary intake over a longer time period (e.g. one month), and therefore may be better able to describe habitual dietary intake (Crozier et al 2008). The number of foods or food groups entered into the factor analysis will affect the resulting factor solution (Newby and Tucker 2004). Most studies have collapsed the original dietary data into a smaller number of input variables, usually food groups, for entry into factor analysis. Researchers need to decide how foods or food

groups will be quantified, for example, frequency (number of servings), weight (grams per day) or daily percent of total energy (Newby and Tucker 2004). Decisions must be made regarding methods of rotation, the dietary patterns to retain in the final solution, which patterns should be reported or analysed, and how the patterns should be named. The factor analysis is usually run for a number of solutions, and the number of patterns retained is chosen based on the interpretability of the dietary pattern, scree plots and eigenvalues >1.0 (Newby and Tucker 2004). Further research is needed to determine how these choices affect the reproducibility of the findings (Hu 2002).

7.5.2 Development of an FFQ to assess dietary patterns

FFQs are commonly used to assess dietary patterns due to their relative ease of use, lower participant and researcher burden and ability to describe long-term dietary intake (Crozier et al 2008). They may be developed using basic principles or adapted from existing questionnaires (Cade et al 2002). When developing a FFQ, decisions need to made regarding which and how many food items to include, how these food items should be grouped, and the order in which food items should appear on the FFQ. Decisions also need to be made regarding the frequency categories used, and whether to include portion sizes. Many of these decisions will be dependent on the objective for which the FFQ is intended (Cade et al 2002). To ensure an FFQ is acceptable and understood it should be pre-tested and validated in the population for which it is intended to be used (Cade et al 2002).

7.5.2.1 Assessing the validity of a FFQ

Food frequency questionnaires should be validated to assess whether the FFQ measures what it is supposed to measure (Cade et al 2002). Validity may be assessed through use of another dietary assessment tool or through biochemical measures (e.g. 24-hour urinary nitrogen excretion as a measure of protein intake) which provide an independent estimate of dietary intake. However, the use of biochemical measures is often expensive, invasive and nutrient specific, meaning only one nutrient can be evaluated at any one time. They are also prone to error (e.g. biochemical assay errors) (Cade et al 2002).

Weighed food records are the method of choice for validating FFQs, as their measurement errors tend to be independent to those of an FFQ (Cade et al 2002). Although 24-hour recalls may be less demanding (and less likely to influence the participant's actual diet), their sources of error tend to reflect those of a FFQ (e.g. they are reliant on memory and estimation of portion size) (Cade et al 2002). Food records should be kept for a sufficient number of days to represent average intake (Cade et al 2002). Increasing the duration of recording of the reference method may improve validity (Potosky et al 1990), and provide a better measure of habitual intake, which is generally more similar to the type of information generated by an FFQ (Cade et al 2002). Based on calculations from two studies, Stram et al (1995) suggested validation studies usually require no more than four to five days of food records per participant.

The validity of the FFQ should be tested on a sub sample of the main study population. The sample size depends on the statistical method being used (Cade et al 2002), however a sample size of more than 100 participants has been suggested as appropriate (Serra-Majem et al 2009a, Serra-Majem et al 2009b, Willett 1998). Ideally the FFQ should be completed prior to assessment of the reference method (to minimise any effect the reference method has on drawing the participant's attention to their own diet), and both the FFQ and reference method should assess diet over the same time period (Cade et al 2002). For example, a FFQ assessing intake over the past year might be administered twice (one year apart), and compared with food records collected within that year (Cade et al 2002).

Good agreement between two dietary assessment methods does not always indicate validity. Agreement may simply indicate errors in both methods (Gibson 2002). It is therefore vital that an appropriate reference method is selected (Cade et al 2002).

7.5.2.2 Assessing the reproducibility of a FFQ

Reproducibility or reliability is the extent to which a dietary assessment method is capable of producing the same result when used repeatedly in the same circumstance (Cade et al 2002). The reproducibility of FFQs has generally been assessed by administering the FFQs at two different time points (ideally four to eight weeks apart (Block and Hartman

1989)) in the same groups of people, with analysis undertaken to assess the associations between responses (Cade et al 2002).

7.5.2.3 Statistical methods to assess the validity and reproducibility of a FFQ

A number of different statistical methods have been suggested to assess the validity and reproducibility of FFQs. Commonly used methods include correlations, the Bland and Altman method, comparison of group means, classification into categories of consumption, and the weighted Kappa (κ) – statistic (Cade et al 2002).

Most studies have used correlation coefficients to assess the validity and reproducibility of FFQs. Pearson's correlation coefficients are used for parametric data, and Spearman's rank correlation coefficients for non-parametric data. However, correlation coefficients do not measure agreement between dietary assessment methods, only the degree to which dietary assessment methods are related (Cade et al 2002). It is recommended that correlation coefficients are used alongside other methods (Cade et al 2002), such as the Bland and Altman method (Bland and Altman 1986). The Bland and Altman method is able to measure the extent to which an FFQ and reference method agree and can determine if there is any systematic difference between the dietary assessment methods (bias). This method can also identify whether the difference between dietary assessment methods is the same across the range of intakes by plotting the difference between the two methods against the mean of the two methods (Bland and Altman 1986). Comparisons of groups means or medians can be undertaken using paired t-tests for parametric data and the Wilcoxin signed rank test for non-parametric data (Cade et al 2002). Another method of statistical analysis is to divide data from the FFQ being tested and the reference method into three to five categories based on low versus high intakes (Masson et al 2003). This allows an assessment of the percentage of participants classified correctly into the same category, or grossly misclassified into the opposite category. Agreement can be assessed using the weighted κ-statistic (Altman 1991), which overcomes any agreement that occurs by chance. It is recommended that more than one statistical method of analyses be used to demonstrate the robustness of an FFQs validity or reproducibility (Cade et al 2002).

7.5.3 Validation of FFQs designed to assess dietary patterns

Most studies have used FFQ data to determine dietary patterns (Broderstad et al 2011, Kerver et al 2003, Shi et al 2006, Terry et al 2001). Validity may be determined by splitting the study sample and repeating the analysis to identify dietary patterns (Newby and Tucker 2004), or by comparing an FFQ with a food record. Only a handful of studies have investigated the validity of dietary patterns (determined using factor analysis) from an FFQ (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Nanri et al 2012, Togo et al 2003) or a DHQ (Okubo et al 2010) by comparing them with dietary patterns identified using a food record (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Nanri et al 2012, Okubo et al 2010, Togo et al 2003). Validation has typically been assessed using correlation coefficients and the Bland and Altman method (Bland and Altman 1986).

The majority of studies investigating the validity of dietary patterns have identified a 'healthy' or 'prudent' and a 'Western' dietary pattern (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Nanri et al 2012, Okubo et al 2010) (see Table 2.8). A 'healthy' dietary factor tends to include a high intake of vegetables, fruit, legumes, whole grains, and fish and other seafood, while 'Western' patterns are characterised by higher intakes of processed meat, red meat, butter, high fat dairy products, eggs and refined grains.

Within these studies, factor loadings have been similar, but not identical across the dietary patterns identified from the FFQs and food records (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Lau et al 2008, Nanri et al 2012, Okubo et al 2010). These differences have been explained by random statistical variations (Hu et al 1999, Khani et al 2004, Okubo et al 2010), methodological differences (Hu et al 1999, Khani et al 2004, Okubo et al 2010, Willett 1998), different assessment periods for the two dietary assessment methods (Okubo et al 2010), or over or underestimation of foods in the FFQ compared with the food record (Okubo et al 2010).

Uncorrected correlation coefficients between FFQs and food records have ranged from 0.34 to 0.64 for 'prudent' and 'Western' patterns among 127 US male health professionals (Hu et al 1999), 0.41 to 0.73 for 'healthy', 'Western' and 'drinker' patterns among 111

Swedish women (Khani et al 2004), 0.34 to 0.61 for 'green', 'sweet' and 'traditional' patterns among 879 Danish men and 927 Danish women (Togo et al 2003), 0.35 to 0.67 for 'prudent' and 'Western' patterns among 585 UK pregnant women (Crozier et al 2008), 0.27 to 0.43 for 'Western' and 'healthy' patterns in 783 Australian adolescents (Ambrosini et al 2011), and 0.36 to 0.62 for 'healthy' and 'Western' dietary patterns in 184 Japanese men and women, and in women only, 0.44 for a 'Japanese' dietary pattern (Okubo et al 2010). Most recently, correlations ranged from 0.32 to 0.63 in 498 Japanese women and men following 'prudent', Western' and 'traditional' dietary patterns (Nanri et al 2012) (Table 2.8). Other minor dietary patterns have been identified in some of these studies (Hu et al 1999, Khani et al 2004). However, these have been inconsistent across dietary assessment methods and explain only a small amount of the variance.

Three studies have used Bland and Altman plots and limits of agreement (LOA) to compare the validity of dietary patterns and have found similar results (Crozier et al 2008, Lau et al 2008, Okubo et al 2010). In pregnant women, the 95% LOA between a FFQ and FR were -1.58 to 1.58 for a 'prudent' and -2.22 to 2.22 for a 'Western' dietary pattern (Crozier et al 2008). 95% LOAs were between ±1.83 for 'healthy' and between ±2.22 for 'Western' dietary patterns in Japanese men and women (Okubo et al 2010). In Australian adolescents, the 95% LOA between a FFQ and FR were -1.69 to 1.75 for a 'healthy' dietary pattern and -1.89 to 1.82 for a 'Western' dietary pattern (Ambrosini et al 2011).

In all of these studies, limitations include the assumption of the food record as the gold standard of measuring dietary intake (Crozier et al 2008), and the subjectivity associated with the determination of dietary patterns using factor analysis (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Okubo et al 2010, Shi et al 2006).

7.5.4 Reproducibility of FFQs designed to assess dietary patterns

Only three of the above studies have considered the reproducibility of dietary patterns obtained by using the same FFQ undertaken on two occasions (all one year apart) (Hu et al 1999, Khani et al 2004, Nanri et al 2012). Hu et al (1999) found correlations between 2 FFQs to be 0.70 for a 'prudent' dietary pattern and 0.67 for a 'Western' dietary pattern. Correlation coefficients between two FFQs in Swedish women were 0.63, 0.68 and 0.73 for 'healthy', 'Western' and 'drinker' patterns respectively (Khani et al 2004). In a

Japanese population, correlations between FFQs were 0.55 (prudent), 0.71 (Western) and 0.68 (traditional) for women; and 0.56 (prudent), 0.55 (Western) and 0.77 (traditional) for men (Table 2.8).

Table 2.8. Studies investigating the validity and reproducibility of dietary patterns identified using factor analysis from an FFQ against a food record

Author and year	Population	Dietary assessment tool used	Validated against	Dietary patterns identified	Results: validity	Results: reproducibility
Nanri et al (2012)	254 females, 244 males, 40-69y Japan	FFQ – 147 food items reduced to 48 food items administered twice (1y apart)	2 or 4x 7 day FR	Prudent Western Traditional	Correlation coefficients between FFQ & DR: 0.47 (Prudent), 0.32 (Western), 0.49 (Traditional) for males; 0.36 (Prudent), 0.56 (Western), 0.63 (Traditional) for females	Correlation coefficients between two FFQs: 0.55 (Prudent), 0.71 (Western), 0.68 (Traditional) for women; 0.56 (Prudent), 0.55 (Western), 0.77 (Traditional) for men
Ambrosini et al (2011)	783 adolescents, 14y Australia	FFQ – 212 items reduced to 38 food groups	3 day FR	Healthy Western	Correlation coefficients between FFQ & DR: 0.43 (Healthy), 0.27 (Western) 95% LOA: -1.69 to 1.75 (Healthy), -1.89 to 1.82 (Western)	N/A
Okubo et al (2010)	92 females, 92 males, 31-76y Japan	DHQ – 150 items reduced to 33 food groups	4x 4 day weighed FR	Healthy, Western, Japanese (females) Healthy, Western	Correlation coefficients between DHQ1 & DR: 0.57 (Healthy), 0.36 (Western), 0.44 (Japanese) for females; 0.62 (Healthy), 0.56 (Western) for males 95% LOA: ±1.81 (Healthy); ±2.22 (Western), ±2.08 (Japanese) for	N/A

Author and year	Population	Dietary assessment tool used	Validated against	Dietary patterns identified	Results: validity	Results: reproducibility
				(males)	females; ±1.83 (Healthy), ±1.71 (Western) for males	
Crozier et al (2008)	585 females in early pregnancy, >16y United Kingdom	FFQ – 100 items reduced to 49 food groups	4 day FR	Prudent Western	Correlation coefficients between FFQ & DR: 0.67 (Prudent), 0.35 (Western) 95% LOA: ±1.58 (Prudent), ±2.22 (Western)	N/A
Khani et al (2004)	111 females (validity), 197 females (reproducibility), 40-74y Sweden	FFQ - 60 items reduced to 26 food groups administered twice (1y apart)	4x 7 day weighed FR	Healthy Western Drinker	Correlation coefficients between FFQ & DR: 0.47 (Healthy), 0.41 (Western), 0.73 (Drinker)	Correlation coefficients between two FFQs: 0.63 (Healthy), 0.68 (Western), 0.73 (Drinker)
Togo et al (2003)	879 males, 927 females Denmark	FFQ – 26 items reduced to 21 food groups	7 day weighed FR	Green, Sweet, Traditional (males) Green,	Correlation coefficients between FFQ & DR: 0.61 (Green), 0.57 (Sweet traditional) for females; 0.61 (Green), 0.55 (Sweet), 0.34 (Traditional) for males	N/A

Author and year	Population	Dietary assessment tool used	Validated against	Dietary patterns identified	Results: validity	Results: reproducibility
				Sweet-traditional (females)		
Hu et al (1999)	127 males United States of America	FFQ – 131 items reduced to 40 food groups administered twice (1y apart)	2x 7 day weighed FR	Prudent Western	Correlation coefficients between 2 FFQs & DR: 0.34-0.51 (Healthy), 0.41-0.64 (Western)	Correlation coefficients between two FFQs: 0.70 (Prudent), 0.67 (Western)

DHQ – Diet history questionnaire; FFQ – Food frequency questionnaire; FR – Food record; LOA – Limits of agreement

7.6 Conclusion

There are a number of methods available to assess dietary intake. The use of dietary patterns is a relatively recent area of research and has particular relevance to iron nutrition. The majority of studies have used FFQs to identify dietary patterns. FFQs should be tested for their validity and reproducibility prior to being used (Cade et al 2004). However, only a handful of studies have investigated the validity and reproducibility of an FFQ used to identify dietary patterns, and no FFQs have focussed on iron-related dietary patterns.

8 Summary of the literature review

This literature review covered a number of areas focusing on the causes, consequences and solutions to iron deficiency. Iron deficiency is the most common nutrient deficiency worldwide and young women are at particular risk. It is not clear whether self-perceived health, well-being and fatigue are associated with non-anaemic iron deficiency. Previous studies have been limited by a number of factors including the use of a wide range of biochemical indices and cut-off points to determine iron deficiency, the use of non-validated questionnaires, and participant knowledge of iron status prior to completing these questionnaires. Further research is therefore needed to investigate associations between non-anaemic iron deficiency and self-perceived health, well-being and fatigue. A number of dietary factors impact on iron absorption. With the exception of meat, results of studies investigating associations between dietary intake and iron status have been somewhat inconsistent. Many of these studies have had a number of limitations, and few studies have considered how and when foods and nutrients are consumed. Dietary patterns and practices consider the whole diet, and may help to address some of the limitations associated with investigating individual foods and nutrients. Dietary patterns are commonly identified using FFQs, and these FFQs should be tested for their validity and reproducibility prior to use. Dietary patterns as determinants of iron status should also be considered in the context of non-dietary factors (e.g. blood loss and ethnicity), that are likely to impact on iron status. Finally, iron deficiency may be treated through a range of initiatives including iron supplementation, iron fortification or interventions to increase the intake and bioavailability of iron in the diet. A dietary approach offers a number of

advantages to addressing iron deficiency. Ascorbic acid and carotenoids both enhance iron absorption, however, the long-term effect of consuming ascorbic acid and carotenoids on iron status needs to be investigated. In conclusion, a large amount of research has been undertaken in the area of iron nutrition. Despite this, questions remain regarding aspects related to the causes, consequences of and possible solutions to iron deficiency, and these require further investigation.

9 References

Aisen P (2004). Transferrin receptor 1. *Int J Biochem Cell Biol* **36:** 2137-2143.

Alexander D, Ball MJ, Mann J (1994). Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. *Eur J Clin Nutr* **48:** 538-546.

Altman DG (1991). *Practical Statistics for Medical Research*. Chapman and Hall: London.

Ambrosini GL, O'Sullivan TA, de Klerk NH, Mori TA, Beilin LJ, Oddy WH (2011). Relative validity of adolescent dietary patterns: a comparison of a FFQ and 3 d food record. *Br J Nutr* **105:** 625-633.

Ando K, Morita S, Higashi T, Fukuhara S, Watanabe S, Park J *et al* (2006). Health-related quality of life among Japanese women with iron-deficiency anemia. *Qual Life Res* **15:** 1559-1563.

Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M *et al* (2009). Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutr* **12:** 1373-1383.

Bach Kristensen M, Tetens I, Alstrup Jorgensen AB, Dal Thomsen A, Milman N, Hels O *et al* (2005). A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread. *Eur J Nutr* **44:** 334-340.

Badami KG, Taylor K (2008). Iron status and risk-profiling in New Zealand blood donors. *NZ Med J* **121:** 50-60.

Baech SB, Hansen M, Bukhave K, Kristensen L, Jensen M, Sorenson SS *et al* (2003). Increasing the cooking temperature of meat does not affect nonheme iron absorption from a phytate rich meal in women. *J Nutr* **133:** 94-97.

Bairati I, Herbeth B, Spyckerelle Y, Didelot-Barthelemy L, Galan P, Hercberg S *et al* (1989). Dietary intake and other determinants of iron and folate status in female adolescents. *J Clin Biochem Nutr* **7:** 143-151.

Baker SJ, DeMaeyer EM (1979). Nutritional anaemia: its understanding and control with special reference to the work of the World Health Organisation. *Am J Clin Nutr* **32:** 368-417.

Ball MJ, Bartlett MA (1999). Dietary intake and iron status of Australian vegetarian women. *Am J Clin Nutr* **70:** 353-358.

Ballin A, Berar M, Rubinstein U, Kleter Y, Hershkovitz A, Meytes D (1992). Iron state in female adolescents. *Am J Dis Child* **146:** 803-805.

Baynes RD, Macfarlane BJ, Bothwell TH, Siegenberg D, Bezwoda WR, Schmidt U *et al* (1990). The promotive effect of soy sauce on iron absorption in human subjects. *Eur J Clin Nutr* **44:** 419-424.

Baynes RD (1996). Assessment of iron status. *Clin Biochem* **29:** 209-215.

Beard J, Tobin B (2000). Iron status and exercise. *Am J Clin Nutr* **72:** 594S-597S.

Bendich A (2001). Calcium supplementation and iron status of females. *Nutrition* **17:** 46-51.

Beutler E, Larsh SE, Gurney CW (1959). Iron therapy in chronically fatigued, non anaemic women: A double-blind study. *Ann Intern Med* **52:** 378-394.

Bingham S (2007). Dietary assessment. In: Mann J, Truswell AS (eds). *Essentials of Human Nutrition*, 3rd edn. Oxford University Press: New York. pp 416-428.

Bjorn-Rasmussen E (1974). Iron absorption from wheat bread. Influence of various amounts of bran. *Nutr Metab* **16:** 101-110.

Black AE, Goldberg GR, Jebb SA, Livingstone MBE, Cole TJ, Prentice AM (1991). Critical-evaluation of energy-intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* **45:** 583-599.

Blanck HM, Cogswell ME, Gillespie C, Reyes M (2005). Iron supplement use and iron status among US adults: results from the third National Health and Nutrition Survey. *Am J Clin Nutr* **82:** 1024-1031.

Blanco-Rojo R, Baeza-Richer C, Lopez-Parra AM, Perez-Granados AM, Brichs A, Bertoncini S *et al* (2011a). Four variants in transferrin and HFE genes as potential markers of iron deficiency anaemia risk: an association study in menstruating women. *Nutr Metab* **8:** 69.

Blanco-Rojo R, Perez-Granados AM, Toxqui L, Gonzalez-Vizcayno C, Delgado MA, Vaquero MP (2011b). Efficacy of a microencapsulated iron pyrophosphate-fortified fruit juice: a randomised, double-blind, placebo-controlled study in Spanish iron-deficient women. *Br J Nutr* **105:** 1652-1659.

Bland JM, Altman DG (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1:** 307-310.

Block G, Hartman AM (1989). Issues in reproducibility and validity of dietary studies. *Am J Clin Nutr* **50:** 1133-1138.

Bodnar LM, Cogswell ME, McDonald T (2005). Have we forgotten the significance of postpartum iron deficiency? *Am J Obstet Gynecol* **193:** 36-44.

Bodwell CE, Miles CW, Morris E, Prather ES, Mertz W, Canary JJ (1987). Long-term consumption of beef extended with soy protein by men, women and children: II. Effects on iron status. *Plant Foods Hum Nutr* **37:** 361-376.

Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J (1997). Reproducibility and relative validity of energy and macronutrient intake of a food frequency questionnaire developed for the German part of the EPIC project. *Int J Epidemiol* **26:** S71-S81.

Bothwell TH, Charlton RW, Cook JD, Finch CA (1979). *Iron metabolism in man*. Blackwell Scientific Publications: Oxford.

Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, Crawford DHG *et al* (2003). Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. *Lancet* **361:** 669-673.

Broderstad AR, Melhus M, Lund E (2007). Iron status in a multiethnic population (age 36-80 yr) in northern Norway: the SAMINOR study. *Eur J Haematol* **79:** 447-454.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Brussard JH, Brants HAM, Bouman M, Lowik MRH (1997). Iron intake and iron status among adults in the Netherlands. *Eur J Clin Nutr* **51:** S51-S58.

Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL *et al* (2011). Iron deficiency in blood donors: analysis of enrollment data from the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion* **51:** 511-522.

Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL *et al* (2012). Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion* **52:** 702-711.

Cade J, Thompson R, Burley V, Warm D (2002). Development, validation and utilisation of food-frequency questionnaires - a review. *Public Health Nutr* **5:** 567-587.

Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM (2004). Food-frequency questionnaires: a review of their design, validation and utilisation. *Nutr Res Rev* **17:** 5-22.

Cade JE, Moreton JA, O'Hara B, Greenwood DC, Moor J, Burley VJ *et al* (2005). Diet and genetic factors associated with iron status in middle-aged women. *Am J Clin Nutr* **82:** 813-820.

Casabellata G, Di Santolo M, Banfi G, Stel G, Gonano F, Cauci S (2007). Evaluation of iron deficiency in young women in relation to oral contraceptive use. *Contraception* **76:** 200-207.

Cepeda-Lopez AC, Osendarp SJM, Melse-Boonstra A, Aeberli I, Gonzalez-Salazar F, Feskens E *et al* (2011). Sharply higher rates of iron deficiency in obese Mexican women and children are predicted by obesity-related inflammation rather than by differences in dietary iron intake. *Am J Clin Nutr* **93:** 975-983.

Cheng HL, Bryant C, Cook R, O'Connor H, Rooney K, Steinbeck K (2012). The relationship between obesity and hypoferraemia in adults: a systematic review. *Obes Rev* **13:** 150-161.

Coad J, Conlon C (2011). Iron deficiency in women: assessment, causes and consequences *Curr Opin Clin Nutr Metab Care* **14:** 625-634.

Cogswell ME, Looker AC, Pfeiffer CM, Cook JD, Lacher DA, Beard JL *et al* (2009). Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003-2006. *Am J Clin Nutr* **89:** 1334-1342.

Collins JF, Franck CA, Kowdley KV, Ghishan FK (2005). Identification of differentially expressed genes in response to dietary iron deprivation in rat duodenum. *Am J Physiol Gastrointest Liver Physiol* **288:** G964-G971.

Commonwealth Department of Health and Ageing Australia, Ministry Of Health New Zealand, National Health and Medical Research Council (2006). *Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes*. Commonwealth of Australia: Canberra.

Contopoulos-Ioannidis DG, Karvouni A, Kouri I, Ioannidis JPA (2009). Reporting and interpretation of SF-36 outcomes in randomised trials: systematic review. *Br Med J* **338:** 1-6.

Cook JD, Monsen ER (1975). Food iron absorption. I. Use of a semisynthetic diet to study absorption of nonheme iron. *Am J Clin Nutr* **28:** 1289-1295.

Cook JD, Monsen ER (1977). Vitamin C, the common cold and iron absorption. *Am J Clin Nutr* **30:** 235-241.

Cook JD, Morck TA, Lynch SR (1981). The inhibitory effect of soy products on nonheme iron absorption in man. *Am J Clin Nutr* **34:** 2622-2629.

Cook JD, Reusser ME (1983). Iron fortification: an update. *Am J Clin Nutr* **38:** 648-659.

Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS (1984). The effect of high ascorbic acid supplementation on body iron stores. *Blood* **64:** 721-726.

Cook JD, Reddy MB, Hurrell RF (1995). The effect of red and white wines on nonheme iron absorption in humans. *Am J Clin Nutr* **61:** 800-804.

Cook JD, Reddy MB (2001). Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *Am J Clin Nutr* **73:** 93-98.

Cook JD, Boy E, Flowers C, Daroca MD (2005). The influence of high-altitude living on body iron. *Blood* **106:** 1441-1446.

Crozier SR, Robinson SM, Borland SE, Inskip H, SWS Study Group (2006). Dietary patterns in the Southampton Women's Survey. *Eur J Clin Nutr* **60:** 1391-1399.

Crozier SR, Inskip HM, Godfrey KM, Robinson SM (2008). Dietary patterns in pregnant women: a comparison of food-frequency questionnaires and 4d prospective diaries. *Br J Nutr* **99:** 869-875.

Deegan H, Bates HM, McCargar LJ (2005). Assessment of iron status in adolescents: Dietary, biochemical and lifestyle determinants. *J Adolesc Health* **37:** 75.

Department of Health (1991). *Dietary reference values for food energy and nutrients in the United Kingdom. Report of the panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy.* HMSO: London.

Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP (2003). The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78:** 436-440.

Disler PB, Lynch SR, Charlton JD, Torrance JD, Bothwell TH, Walker RB *et al* (1975). The effect of tea on iron absorption. *Gut* **16:** 193-200.

Dittner AJ, Wessely SC, Brown RG (2004). The assessment of fatigue - A practical guide for clinicians and researchers. *J Psychosom Res* **56:** 157-170.

Donovan A, Roy CN, Andrews NC (2005). The ins and outs of iron homeostasis. *Physiology* **21:** 115-123.

Duport N, Preziosi P, Boutron-Ruault MC, Bertrais S, Galan P, Favier A *et al* (2003). Consequences of iron depletion on health in menstruating women. *Eur J Clin Nutr* **57:** 1169-1175.

Elwood PC, Hughes D (1970). Clinical trial of iron therapy on psychomotor function in anaemic women. *Br Med J* **3:** 254-255.

Fairweather-Tait SJ (1995). Iron Absorption. In: British Nutrition Foundation (ed). *Iron: Nutritional and physiological significance*. Chapman and Hall: London. pp 3-12.

Fairweather-Tait SJ, Wortley GM, Teucher B, Dainty J (2000). Iron absorption from a breakfast cereal: effects of EDTA compounds and ascorbic acid. *Int J Vitam Nutr Res* **70:** 117-122.

Farchi G, Mariotti S, Menotti A, Seccareccia F, Torsello S, Fidanza F (1989). Diet and 20-y mortality in two rural population groups of middle-aged men in Italy *Am J Clin Nutr* **50:** 1095-1103.

Fawcett JP, Brooke M, Beresford CH (1998). Iron deficiency and anaemia in a longitudinal study of New Zealanders at ages 11 and 21 years. *NZ Med J* **111:** 400-402.

Fogelholm M (1995). Indicators of vitamin and mineral status in athlete's blood: a review. *Int J Sport Nutr* **5:** 267-284.

Food and Agricultural Organization of the United Nations/World Health Organization (2004). Iron. *Vitamin and Mineral Requirements in Human Nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand*, 2nd edn. World Health Organization: Geneva. pp 246-278.

Food and Agriculture Organization of the United Nations (1996). *Food Fortification: Technology and Quality Control (FAO Food and Nutrition Paper 60): Report of a FAO technical meeting*. Food and Agriculture Organization of the United Nations: Rome.

Food and Nutrition Board: Institute of Medicine (2001). Iron. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. National Academy Press: Washington, DC. pp 290-393.

Fordy J, Benton D (1994). Does iron status influence psychological functioning? *J Hum Nutr Diet* **7:** 127-133.

Fraser IS (2010). Non-contraceptive health benefits of intrauterine hormonal systems. *Contraception* **82:** 396-403.

Frazer DM, Vulpe CD, McKie AT, Wilkins SJ, Trinder D, Cleghorn GJ *et al* (2001). Cloning and gastrointestinal expression of rat hephaestin: relationship to other iron transport proteins. *Am J Physiol Gastrointest Liver Physiol* **281:** G931-G939.

Frith-Terhune AL, Cogswell ME, Kettel Khan L, Will JC, Ramakrishnan U (2000). Iron deficiency anaemia: higher prevalence in Mexican American than in non-Hispanic white

females in the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* **72:** 963-968.

Galan P, Hercberg S, Soustre Y, Dop MC, Dupin H (1985). Factors affecting iron stores in French female students. *Hum Nutr Clin Nutr* **39:** 279-287.

Galan P, Yoon HC, Preziosi P, Viteri F, Valeix P, Fieux B *et al* (1998). Determining factors in the iron status of adult women in the SU.VI.MAX study. *Eur J Clin Nutr* **52:** 383-388.

Galloway R, McGuire J (1994). Determinants of compliance with iron supplementation - supplies, side-effects, or psychology. *Soc Sci Med* **39:** 381-390.

Ganz T (2005). Hepcidin - a regulator of intestinal iron absorption and iron recycling by macrophages. *Best Pract Res Clin Haematol* **18:** 171-182.

Garcia-Casal MN, Macfarlane BJ, Layrisse M, Solano L, Baron MA, Arguello F *et al* (1998). Vitamin A and B-Carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *J Nutr* **128:** 646-650.

Garcia-Casal MN (2006). Carotenoids increase iron absorption from cereal-based food in the human. *Nutr Res* **26:** 340-344.

Garcia OP, Diaz M, Rosado JL, Allen LH (2003). Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. *Am J Clin Nutr* **78:** 267-273.

Gibson RS, Donovan UM, Heath ALM (1997). Dietary strategies to improve the iron and zinc nutriture of young women following a vegetarian diet. *Plant Foods Hum Nutr* **51:** 1-16.

Gibson RS (2002). Dietary Assessment. In: Mann J, Truswell AS (eds). *Essentials of Human Nutrition*, 2nd edn. Oxford University Press: New York. pp 449-466.

Gibson RS (2005a). Measuring food consumption of individuals. In: Gibson RS (ed). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York. pp 41-64.

Gibson RS (2005b). Assessment of iron status. In: Gibson RS (ed). *Principles of Nutrition Assessment*, 2nd edn. Oxford University Press: New York. pp 446-469.

Gibson RS (2005c). Reproducibility in dietary assessment. In: Gibson RS (ed). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York. pp 129-148.

Gibson RS (2005d). Measurement errors in dietary assessment. In: Gibson RS (ed). *Principles of Nutrition Assessment*, 2nd edn. Oxford University Press: New York. pp 105-128.

Gibson RS (2005e). Assessment of nutrient intakes from food consumption data. In: Gibson RS (ed). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York. pp 65-104.

Gibson S, Ashwell M (2003). The association between red and processed meat consumption and iron intakes and status among British adults. *Public Health Nutr* **6:** 341-350.

Gillooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR *et al* (1983). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br J Nutr* **49:** 331-342.

Gitlin D, Cruchaud A (1962). On the kinetics of iron absorption in mice. *J Clin Invest* **41:** 344-350.

Gleerup A, Rossander-Hulten L, Hallberg L (1993). Duration of the inhibitory effect of calcium on non-haem iron absorption in man. *Eur J Clin Nutr* **47:** 875-879.

Gleerup A, Rossander-Hulten L, Gramatkovski E, Hallberg L (1995). Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* **61:** 97-104.

Goddard AF, James MW, McIntyre AS, Scott BB, British Soc G (2011). Guidelines for the management of iron deficiency anaemia. *Gut* **60:** 1309-1316.

Goldberg G, Black A, Jebb S, Cole T, Murgatroyed P, Coward W *et al* (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45:** 569-581.

Greenberg AS, Obin MS (2006). Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* **83:** 461S-465S.

Grondin M-A, Ruivard M, Perreve A, Derumeaux-Burel H, Perthus I, Roblin J *et al* (2008). Prevalence of iron deficiency and health-related quality of life among female students. *J Am Coll Nutr* **27:** 337-341.

Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR (1999). Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. *Am J Clin Nutr* **70:** 586S-593S.

Hallberg L, Bjorn-Rasmussen E, Howard L, Rossander L (1979). Dietary heme iron absorption: A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption. *Scand J Gastroenterol* **14:** 769-779.

Hallberg L (1981). Bioavailability of dietary iron in man. *Annu Rev Nutr* **1:** 123-147.

Hallberg L, Rossander L (1982). Effect of different drinks on the absorption of non-heme iron from composite meals. *Hum Nutr: Clin Nutr* **36A:** 116-123.

Hallberg L, Brune M, Rossander L (1986). Effect of ascorbic acid on iron absorption from different types of meals: Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum Nutr: Clin Nutr* **40A:** 97-113.

Hallberg L, Brune M, Rossander L (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* **49:** 140-144.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Hallberg L, Hulten L, Gramatkovski E (1997). Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* **66:** 347-356.

Hallberg L (1998). Does calcium interfere with iron absorption? *Am J Clin Nutr* **68:** 3-4.

Hallberg L (2002). Advantages and disadvantages of an iron-rich diet. *Eur J Clin Nutr* **56:** S12-S18.

Hanson LN, Engelman HM, Lee Alekel D, Schalinske KL, Kohut ML, Reddy MB (2006). Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. *Am J Clin Nutr* **84:** 774-780.

Harman SK, Parnell WR (1998). The nutritional health of New Zealand vegetarian and non-vegetarian Seventh-day Adventists: selected vitamin, mineral and lipid levels. *NZ Med J* **111:** 91-94.

Harvey LJ, Armah CN, Dainty JR, Foxall RJ, Lewis DJ, Langford NJ *et al* (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *Br J Nutr* **94:** 557-564.

Heath ALM, Skeaff CM, Gibson RS (2000). The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. *Eur J Clin Nutr* **54:** 592-599.

Heath ALM, Skeaff CM, O'Brien SM, Williams SM, Gibson RS (2001a). Can dietary treatment of non-anemic iron deficiency improve iron status? *J Am Coll Nutr* **20:** 477-484.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001b). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Heath ALM, Roe MA, Oyston SL, Fairweather-Tait SJ (2005). Meal-based intake assessment tool: relative validity when determining dietary intake of Fe and Zn and selected absorption modifiers in UK men. *Br J Nutr* **93:** 403-416.

Heath ALM, Roe MA, Oyston SL, Gray AR, Williams SM, Fairweather-Tait SJ (2008). Blood loss is a stronger predictor of iron status in men than C282Y heterozygosity or diet. *J Am Coll Nutr* **27:** 158-167.

Helman AD, Darnton-Hall I (1987). Vitamin and iron status in *new* vegetarians. *Am J Clin Nutr* **45:** 785-789.

Hendricks KM, Herbold N, Fung T (2004). Diet and other lifestyle behaviors in young college women. *Nutr Res* **24:** 981-991.

Herbert V (1987). The 1986 Herman Award Lecture. Nutrition science as a continually unfolding story: the folate and vitamin B-12 paradigm. *Am J Clin Nutr* **46:** 387-402.

Hercberg S, Preziosi P, Galan P (2001). Iron deficiency in Europe. *Public Health Nutr* **4:** 537-545.

Horwath CC (1991). Dietary intake and nutritional status among university undergraduates. *Nutr Res* **11:** 395-404.

Houston MS, Summers SL, Soltesz KS (1997). Lifestyle and dietary practices influencing iron status in university women. *Nutr Res* **17:** 9-22.

Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al* (1999). Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* **69:** 243-249.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Hua NW, Stoohs RA, Faachini FS (2001). Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. *Br J Nutr* **86:** 515-519.

Hulthen L, Gramtkovski E, Gleerup A, Hallberg L (1995). Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. *Eur J Clin Nutr* **49:** 794-808.

Hulthen L, Lindstedt G, Lundberg P-A, Hallberg L (1998). Effect of mild infection on serum ferritin concentration - clinical and epidemiological implications. *Eur J Clin Nutr* **52:** 376-379.

Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH (1990). Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *Am J Clin Nutr* **51:** 649-655.

Hunt JR, Gallagher SK, Johnson LK (1994). Effect of ascorbic acid on apparent iron absorption by women with low iron stores. *Am J Clin Nutr* **59:** 1381-1385.

Hunt JR, Gallagher SK, Johnson LK, Lykken GI (1995). High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. *Am J Clin Nutr* **62:** 621-632.

Hunt JR, Roughead ZK (1999). Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. *Am J Clin Nutr* **69:** 944-952.

Hunt JR (2002). Moving toward a plant-based diet: are iron and zinc at risk? *Nutr Rev* **60:** 127-134.

Hurrell RF, Furniss DE, Burri J, Whittaker P, Lynch SR, Cook JD (1989). Iron fortification of infant cereals: a proposal for the use of ferrous fumerate or ferrous succinate. *Am J Clin Nutr* **49:** 1274-1282.

Hurrell RF (1997). Preventing iron deficiency through food fortification. *Nutr Rev* **55:** 210-222.

Hurrell RF, Reddy M, Cook JD (1999). Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br J Nutr* **81:** 289-295.

Hurrell RF (2002). Fortification: Overcoming technical and practical barriers. *J Nutr* **132:** 806S-812S.

Hurrell RF, Lynch S, Bothwell T, Cori H, Glahn RP, Hertrampf E *et al* (2004). Enhancing the absorption of fortification iron: A SUSTAIN task force report. *Int J Vitam Nutr Res* **74:** 387-401.

Hyder SMZ, Persson LA, Chowdhury AMR, Ekstrom EC (2002). Do side-effects reduce compliance to iron supplementation? A study of daily- and weekly-dose regimens in pregnancy. *J Health Popul Nutr* **20:** 175-179.

Ilich-Ernst JZ, McKenna AA, Badenhop NE, Clairmont AC, Andon MB, Nahhas RW *et al* (1998). Iron status, menarche, and calcium supplementation in adolescent girls. *Am J Clin Nutr* **68:** 880-887.

Ioannou GN, Dominitz JA, Weiss NS, Heagerty PJ, Kowdley KV (2004). The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia. *Gastroenterology* **126:** 1293-1301.

Kalkwarf HJ, Harrast SD (1998). Effects of calcium supplementation and lactation on iron status. *Am J Clin Nutr* **67:** 1244-1249.

Kallich J, McDermott A, Xu X, Fayers P, Cella D (2006). The relationship between patient knowledge of hemoglobin levels and health-related quality of life. *Qual Life Res* **15:** 57-68.

Kandiah J (2002). Impact of tofu or tofu + orange juice on hematological indices of lacto-ovo vegetarian females. *Plant Foods Hum Nutr* **57:** 197-204.

Karl JP, Lieberman HR, Cable SJ, Williams KW, Glickman EL, Young AJ *et al* (2009). Poor iron status is not associated with overweight or overfat in non-obese pre-menopausal women. *J Am Coll Nutr* **28:** 37-42.

Kerver JM, Yang EJ, Bianchi L, Song WO (2003). Dietary patterns associated with risk factors for cardiovascular disease in healthy US adults. *Am J Clin Nutr* **78:** 1103-1110.

Khani BR, Ye WM, Terry P, Wolk A (2004). Reproducibility and validity of major dietary patterns among Swedish women assessed with a food-frequency questionnaire. *J Nutr* **134:** 1541-1545.

Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakulinen T (1994). Body iron stores and risk of cancer. *Int J Cancer* **56:** 379-382.

Krayenbuehl PA, Battegay E, Breymann C, Furrer J, Schulthess G (2011). Intravenous iron for the treatment of fatigue in nonanemic, premenopausal women with low serum ferritin concentration. *Blood* **118:** 3222-3227.

Lau C, Glumer C, Toft U, Tetens I, Carstensen B, Jorgensen T *et al* (2008). Identification and reproducibility of dietary patterns in a Danish cohort: the Inter99 study. *Br J Nutr* **99:** 1089-1098.

Layrisse M, Garcia-Casal MN, Solano L, Baron MA, Arguello F, Llovera D *et al* (1997). The role of Vitamin A on the inhibitors of nonheme iron absorption: Preliminary results. *J Nutr Biochem* **8:** 61-67.

Layrisse M, Garcia-Casal MN, Solano L, Baron MA, Arguello F, Llovera D *et al* (1998). Vitamin A reduces the inhibition of iron absorption by phytates and polyphenols. *Food Nutr Bull* **19:** 3-5.

Lecube A, Hernandez C, Pelegri D, Simo R (2008). Factors accounting for high ferritin levels in obesity. *Int J Obes* **32:** 1665-1669.

Lee PL, Halloran C, Trevino R, Felitti V, Beutler E (2001). Human transferrin G277S mutation: a risk factor for iron deficiency anaemia. *Br J Haematol* **115:** 329-333.

Lee RD, Nieman DC (2010a). Biochemical assessment of nutritional status. In: Lee RD, Nieman DC (eds). *Nutritional Assessment*, 5th edn. McGraw-Hill: New York. pp 311-345.

Lee RD, Nieman DC (2010b). Measuring diet. In: Lee RD, Nieman DC (eds). *Nutritional Assessment*. McGraw-Hill: New York. pp 68-105.

Leggett BA, Brown NN, Bryant SJ, Duplock L, Powell LW, Halliday JW (1990). Factors affecting the concentrations of ferritin in serum in a healthy Australian population. *Clin Chem* **36:** 1350-1355.

Lim WJ, Hong S, Nelesen R, Dimsdale JE (2005). The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. *Arch Intern Med* **165:** 910-915.

Lin XM, Zhang J, Zou ZY, Long Z, Tian W (2008). Evaluation of serum transferrin receptor for iron deficiency in women of child-bearing age. *Br J Nutr* **100:** 1104-1108.

Looker AC, Dallman PR, Carroll MD (1997). Prevalence of iron deficiency in the United States. *JAMA,* **277:** 973-976.

Lyle RM, Weaver CM, Sedlock DA, Rajaram S, Martin B, Melby CL (1992). Iron status in exercising women: the effect of oral iron therapy vs increased consumption of muscle foods. *Am J Clin Nutr* **56:** 1049-1055.

Lynch S (2011a). Improving the assessment of iron status. *Am J Clin Nutr* **93:** 1188-1189.

Lynch SR, Dassenko SA, Morck TA, Beard JL, Cook JD (1985). Soy protein products and heme iron absorption in humans. *Am J Clin Nutr* **41:** 13-20.

Lynch SR, Stoltzfus RJ (2003). Iron and ascorbic acid: Proposed fortification levels and recommended iron compounds. *J Nutr* **133:** 2978S-2984S.

Lynch SR (2011b). Why Nutritional Iron Deficiency Persists as a Worldwide Problem. *J Nutr* **141:** 763S-768S.

Macdiarmid JI, Blundell JE (1997). Dietary under-reporting: What people say about recording their food intake. *Eur J Clin Nutr* **51:** 199-200.

MacPhail AP, Bothwell TH, Torrance JD, Derman DP, Bezwoda WR, Charlton RW *et al* (1981). Factors affecting the absorption of iron from Fe(III)EDTA. *Br J Nutr* **45:** 215-227.

MacPhail AP (2007). Iron. In: Mann J, Truswell AS (eds). *Essentials of human nutrition*, 3rd edn. Oxford University Press: Oxford. pp 125-137.

Malczewska J, Raczynski G, Stupnicki R (2000). Iron status in female endurance athletes and in non-athletes. *Int J Sport Nutr* **10:** 260-276.

Malone H, Kevany J, Scott J, O'Broin S, O'Connor G (1986). Ascorbic acid supplementation: it's effects on body iron stores and white blood cells. *Irish J Med Sci* **155:** 74-79.

Mansson J, Johansson G, Wiklund M, Baigi A, Marklund B (2005). Symptom panorama in upper secondary school students and symptoms related to iron deficiency - Screening with laboratory tests, questionnaire and interventional treatment with iron. *Scand J Prim Health Care* **23:** 28-33.

Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, Wei L *et al* (2003). Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr* **6:** 313-321.

Matthys C, Pynaert I, Roe M, Fairweather-Tait SJ, Heath ALM, De Henauw S (2004). Validity and reproducibility of a computerised tool for assessing the iron, calcium and vitamin C intake of Belgian women. *Eur J Clin Nutr* **58:** 1297-1305.

McClung JP, Karl JP (2009). Iron deficiency and obesity: the contribution of inflammation and diminished iron absorption. *Nutr Rev* **67:** 100-104.

McClung JP, Karl JP, Cable SJ, Williams KW, Nindl BC, Young AJ *et al* (2009). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *Am J Clin Nutr* **90:** 124-131.

McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E *et al* (2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* **291:** 1755-1759.

McLean E, Cogswell M, Egli I, Woidyla D, de Benoist B (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* **12:** 444-454.

Mennen L, Hirvonen T, Arnault N, Bertrais S, Galan P, Hercberg S (2007). Consumption of black, green and herbal tea and iron status in French adults. *Eur J Clin Nutr* **61:** 1174-1179.

Menzie CM, Yanoff LB, Denkinger BI, McHugh T, Sebring NG, Calis KA *et al* (2008). Obesity-related hypoferremia is not explained by differences in reported intake of heme and nonheme, iron or intake of dietary factors that can affect iron absorption. *J Am Diet Assoc* **108:** 145-148.

Minehane A, Fairweather-Tait S (1998). Effect of calcium supplementation on daily nonheme iron absorption and long term iron status. *Am J Clin Nutr* **68:** 96-102.

Ministry of Health (2008). *A Portrait of Health: Key results of the 2006/2007 New Zealand Health Survey*. Ministry of Health: Wellington.

Molgaard C, Kastel P, Michaelsen KF (2005). Long-term calcium supplementation does not affect the iron status of 12-14-y-old girls. *Am J Clin Nutr* **82:** 98-102.

Monsen ER (1988). Iron nutrition and absorption: dietary factors which impact iron bioavailability. *J Am Diet Assoc* **88:** 786-790.

Morris ER, Bodwell CE, Miles CW, Mertz W, Prather ES, Canary JJ (1987). Long-term consumption of beef extended with soy protein by children, women and men: III. Iron absorption by adult men. *Plant Foods Hum Nutr* **37:** 377-389.

Morrow JJ, Dagg JH, Goldberg A (1968). A controlled trial of iron therapy in sideropenia. *Scott Med J* **13:** 78-83.

Muir A, Hopfer U (1985). Regional specificity of iron uptake by small intestinal brush-border membranes from normal and iron-deficient mice. *Am J Physiol* **248:** G376-G379.

Nairz M, Schroll A, Sonnweber T, Weiss G (2010). The struggle for iron - a metal at the host-pathogen interface. *Cell Microbiol* **12:** 1691-1702.

Nanri A, Shimazu T, Ishihara J, Takachi R, Mizoue T, Inoue M *et al* (2012). Reproducibility and validity of dietary patterns assessed by a food frequency questionnaire used in the 5-year follow-up survey of the Japan public health center-based prospective study. *J Epidemiol* **22:** 205-215.

Navas-Carretero S, Perez-Granados AM, Schoppen S, Sarria B, Carbajal A, Vaquero MP (2009). Iron status biomarkers in iron deficient women consuming oily fish versus red meat diet. *J Physiol Biochem* **65:** 165-174.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM *et al* (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* **306:** 2090-2093.

New Zealand Blood Service (2010). *All about donating blood*. New Zealand Blood Service: Auckland.

Newby PK, Muller D, Tucker KL (2004). Associations of empirically derived eating patterns with plasma lipid biomarkers: a comparison of factor and cluster analysis methods. *Am J Clin Nutr* **80:** 759-767.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Oke OL (1969). Oxalic acid in plants and in nutrition. *World Rev Nutr Diet* **10:** 262-303.

Okubo H, Murakami K, Sasaki S, Kim MK, Hirota N, Notsu A *et al* (2010). Relative validity of dietary patterns derived from a self-administered diet history questionnaire using factor analysis among Japanese adults. *Public Health Nutr* **13:** 1080-1089.

Palmer MA, Morgan CL (2012). How well are we validating food frequency questionnaires that measure dietary iron intakes of Australian and New Zealand adults? *Nutrition & Dietetics* **69:** 159-166.

Patterson A, Brown W, Powers J, Roberts D (2000). Iron deficiency, general health and fatigue: Results from the Australian longitudinal study on women's health. *Qual Life Res* **9:** 491-497.

Patterson AJ, Brown WJ, Roberts DCK (2001a). Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. *J Am Coll Nutr* **20:** 337-342.

Patterson AJ, Brown WJ, Roberts DCK, Seldon MR (2001b). Dietary treatment of iron deficiency in women of childbearing age. *Am J Clin Nutr* **74:** 650-656.

Pena-Rosas JP, Viteri FE (2009). Effects and safety of preventive oral iron or iron plus folic acid supplementation for women during pregnancy. *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd.

Péneau S, Dauchet L, Vergnaud A-C, Estaquio C, Kesse-Guyot E, Bertrais S *et al* (2008). Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. *Am J Clin Nutr* **87:** 1298-1305.

Pinhas-Hamiel O, Newfield RS, Koren I, Agmon A, Lilos P, Phillip M (2003). Greater prevalence of iron deficiency in overweight and obese children and adolescents. *Int J Obes* **27:** 416-418.

Piper B, Lindsey A, Dodd M, Ferketich S, Paul S, Weller S (1989). Development of an instrument to measure the subjective dimension of fatigue. In: Funk S, Tournquist E, Champagne M, Copp L, Weise R (eds). *Key Aspects of Comfort: Management of Pain, Fatigue and Nausea*. Springer: New York. pp 199-208.

Potosky AL, Block G, Hartman AM (1990). The apparent validity of diet questionnaires is influenced by number of diet-record days used for comparison *J Am Diet Assoc* **90:** 810-813.

Preziosi P, Hercberg S, Galan P, Devanley M, Cherouvrier F, Dupin H (1994). Iron status of a healthy French population: factors determining biochemical markers. *Ann Nutr Metab* **38:** 192-202.

Pynaert I (2007). *Public health aspects of iron intake and iron status*. Ghent University: Ghent.

Pynaert I, Matthys C, De Bacquer D, De Backer G, De Henauw S (2008). Evaluation of a 2-day food record to determine iron, calcium and vitamin C intake in young Belgian women. *Eur J Clin Nutr* **62:** 104-110.

Pynaert I, De Bacquer D, Matthys C, Delanphe J, Temmerman M, De Backer G *et al* (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutr* **12:** 1775-1782.

Ramakrishnan U, Frith-Terhune A, Cogswell M, Kettel Khan L (2002). Dietary intake does not account for differences in low iron stores among Mexican American and Non-Hispanic white women: Third national health and nutrition examination survey, 1988-1994. *J Nutr* **132:** 996-1001.

Rangan AM, Aitkin I, Blight GD, Binns CW (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pac J Clin Nutr* **6:** 291-295.

Rangan AM, Blight GD, Winns CW (1998). Iron status and non-specific symptoms of female students. *J Am Coll Nutr* **17:** 351-355.

Razagui IB, Barlow PJ, Izmeth MGA, Taylor KDA (1991). Iron status in a group of long-stay mentally handicapped menstruating women: some dietary considerations. *Eur J Clin Nutr* **45:** 331-340.

Reddy MB, Hurrell RF, Cook JD (2000). Estimation of nonheme-iron bioavailability from meal composition. *Am J Clin Nutr* **71:** 937-943.

Reddy S, Sanders TAB (1990). Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores. *Br J Nutr* **64:** 331-338.

Ridsdale L, Evans A, Jerrett W, Mandalia S, Osler K, Vora H (1993). Patients with fatigue in general practice - a prospective study. *Br Med J* **307:** 103-106.

Roe MA, Heath ALM, Oyston SL, Macrow C, Hoogewerff JA, Foxall R *et al* (2005). Iron absorption in male C282Y heterozygotes. *Am J Clin Nutr* **81:** 814-821.

Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L (1991). Competitive inhibition of iron absorption by manganese and zinc in humans. *Am J Clin Nutr* **54:** 152-156.

Russell D, Parnell WR, Wilson N, Faed J, Ferguson E, Herbison P *et al* (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*. Ministry of Health: Wellington.

Ruston D, Hoare J, Henderson L, Gregory J, Bates CJ, Prentice A *et al* (2004). *National Diet and Nutrition Survey: Adults aged 19 to 64 years. vol. 4: Nutritional status (anthropometry and blood analytes), blood pressure and physical activity*. The Stationery Office: London.

Sarria B, Navas-Carretero S, Lopez-Parra AM, Perez-Granados AM, Arroyo-Pardo E, Roe MA *et al* (2007). The G277S transferrin mutation does not affect iron absorption in iron deficient women. *Eur J Nutr* **46:** 57-60.

Schaaf D, Scragg R, Metcalf P, Grant C, Buchanan J (2000). Prevalence of iron deficiency in Auckland high school students. *NZ Med J* **113:** 347-350.

Scientific Advisory Committee on Nutrition (2010). *Iron and Health*. The Stationery Office: London.

Seck BC, Jackson RT (2008). Determinants of compliance with iron supplementation among pregnant women in Senegal. *Public Health Nutr* **11:** 596-605.

Seltzer CC, Mayer J (1963). Serum iron and iron-binding capacity in adolescents. Comparison of obese and nonobese subjects. *Am J Clin Nutr* **13:** 354-361.

Serra-Majem L, Andersen LF, Henrique-Sanchez P, Doreste-Alonso J, Sanchez-Villegas A, Ortiz-Andrelluchi A *et al* (2009a). Evaluating the quality of dietary intake validation studies. *Br J Nutr* **102:** S3-S9.

Serra-Majem L, Pfrimer K, Doreste-Alonso J, Ribas-Barba L, Sanchez-Villegas A, Ortiz-Andrellucchi A *et al* (2009b). Dietary assessment methods for intakes of iron, calcium, selenium, zinc and iodine. *Br J Nutr* **102:** S38-S55.

Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N *et al* (2005). Identification of an intestinal heme transporter. *Cell* **122:** 789-801.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG *et al* (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* **53:** 537-541.

Slattery ML (2010). Analysis of dietary patterns in epidemiological research. *Appl Physiol Nutr Metab* **35:** 207-210.

Snetselaar L, Stumbo P, Chenard C, Ahrens L, Smith K, Zimmerman B (2004). Adolescents eating diets rich in either lean beef or lean poultry and fish reduced fat and saturated fat intake and those eating beef maintained serum ferritin status. *J Am Diet Assoc* **104:** 424-428.

Sokoll LJ, Dawson-Hughes B (1992). Calcium supplementation and plasma ferritin concentrations in premenopausal women. *Am J Clin Nutr* **56:** 1045-1048.

Solomons NW (1986). Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *J Nutr* **116:** 927-935.

Stein KD, Martin SC, Hann DM, Jacobsen PB (1998). A multidimensional measure of fatigue for use with cancer patients. *Cancer Pract* **6:** 143-152.

Stein KD, Jacobsen PB, Blanchard CM, Thors C (2004). Further validation of the multidimensional fatigue symptom inventory-short form. *J Pain Symptom Manage* **27:** 14-24.

Stram DO, Longnecker MP, Shames L, Kolonel LN, Wilkens LR, Pike MC *et al* (1995). Cost-efficient design of a diet validation study. *Am J Epidemiol* **142:** 353-362.

Swain JH, Alekel DL, Dent SB, Peterson CT, Reddy MB (2002). Iron indexes and total antioxidant status in response to soy protein intake in perimenopausal women. *Am J Clin Nutr* **76:** 165-171.

Takkunen H, Seppanen R (1975). Iron deficiency and dietary factors in Finland. *Am J Clin Nutr* **28:** 1141-1147.

Temme EHM, Van Hoydonck PGA (2002). Tea consumption and iron status. *Eur J Clin Nutr* **56:** 379-386.

Terry P, Hu FB, Hansen H, Wolk A (2001). Prospective study of major dietary patterns and colorectal cancer risk in women. *Am J Epidemiol* **154:** 1143-1149.

Tetens I, Bendtsen KM, Henriksen M, Ersboll AK, Milman N (2007). The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores. *Eur J Nutr* **46:** 439-445.

Teucher B, Olivares M, Cori H (2003). Enhancers of iron absorption: Ascorbic acid and other organic acids. *Int J Vitam Nutr Res* **74:** 403-419.

Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP (2010). Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr* **92:** 546-555.

Togo P, Heitmann BL, Sorensen TIA, Osler M (2003). Consistency of food intake factors by different dietary assessment methods and population groups. *Br J Nutr* **90:** 667-678.

Tucker KL (2010). Dietary patterns, approaches, and multicultural perspective. *Appl Physiol Nutr Metab* **35:** 211-218.

Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulten L, Pleehachinda R, Suwanik R *et al* (1991). Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. *Am J Clin Nutr* **53:** 554-557.

Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Guzman G, Holterman AXL *et al* (2010). Elevated systemic hepcidin and iron depletion in obese premenopausal females. *Obesity* **18:** 1449-1456.

United Nations Children's Fund/World Health Organization (1999). *Prevention and Control of Iron Deficiency Anaemia in Women and Children: Report of the UNICEF/WHO Regional Consultation, Geneva, Switzerland*. United Nations Children's Fund.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

van de Vijver LPL, Kardinaal AFM, Charzewska J, Rotily M, Charles P, Maggiolini M *et al* (1999). Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *J Nutr* **129:** 963-968.

Verdon F, Burnand B, Stubi CLF, Bonard C, Graff M, Michaud A *et al* (2003). Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *Br Med J* **326:** 1124-1127.

Walczyk T, Davidsson L, Rossander-Hulthen L, Halberg L, Hurrell RF (2003). No enhancing effect of Vitamin A on iron absorption in humans. *Am J Clin Nutr* **77:** 144-149.

Waldvogel S, Pedrazzini B, Vaucher P, Bize R, Cornuz J, Tissot JD *et al* (2012). Clinical evaluation of iron treatment efficiency among non-anemic but iron-deficient female blood donors: a randomized controlled trial. *BMC Medicine* **10**.

Wang JA, Pantopoulos K (2011). Regulation of cellular iron metabolism. *Biochem J* **434:** 365-381.

Ware JE, Kosinski M, Bjorner JB, Turner-Bowker DM, Gandek B, Maruish ME (2007). *User's Manual for the SF-36v2™ Health Survey*, 2nd edn. QualityMetric Incorporated: Lincoln, Rhode Island.

Whitehead L (2009). The Measurement of Fatigue in Chronic Illness: A Systematic Review of Unidimensional and Multidimensional Fatigue Measures. *J Pain Symptom Manage* **37:** 107-128.

Whitfield JB, Zhu G, Heath AC, Powell LW, Martin NG (2001). Effects of alcohol consumption on indices of iron stores and of iron stores on alcohol intake markers. *Alcohol Clin Exp Res* **25:** 1037-1045.

Whitfield JB, Treloar S, Zhu G, Powell LW, Martin NG (2003). Relative importance of female specific and non-female specific effects on variation in iron stores between women. *Br J Haematol* **120:** 860-866.

Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal (2002). Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. *J Nutr* **132:** 3061-3066.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J *et al* (1985). Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* **122:** 51-65.

Willett WC (1998). Nature of variation in diet. In: Willett WC (ed). *Nutritional Epidemiology*, 2nd edn. Oxford University Press: New York. pp 33-49.

World Health Organization, Centers for Disease Control and Prevention Atlanta (2008). *Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia*. World Health Organization: Geneva.

World Health Organization/Centres for Disease Prevention and Control (2007). *Assessing the iron status of populations. Second edition including literature reviews*. World Health Organization: Geneva.

World Health Organization/Food and Agriculture Organization of the United Nations (2006). *Guidelines on food fortification with micronutrients*. World Health Organization: Geneva.

Worthington-Roberts BS, Breskin MW, Monsen ER (1988). Iron status of premenopausal women in a university community and its relationship to habitual dietary sources of protein. *Am J Clin Nutr* **47:** 275-279.

Yanoff LB, Menzie CM, Denkinger B, Sebring NG, McHugh T, Remaley AT *et al* (2007). Inflammation and iron deficiency in the hypoferremia of obesity. *Int J Obes* **31:** 1412-1419.

Young MF, Glahn RP, Ariza-Nieto M, Inglis J, Olbina G, Westerman M *et al* (2009). Serum hepcidin is significantly associated with iron absorption from food and supplemental sources in healthy young women. *Am J Clin Nutr* **89:** 533-538.

Zhou SJ, Schilling MJ, Makrides M (2005). Evaluation of an iron specific checklist for the assessment of dietary iron intake in pregnant and postpartum women. *Nutrition* **21:** 908-913.

Zhou Y, Alekel L, Dixon PM, Messina M, Reddy MB (2011). The effect of soy food intake on mineral status in premenopausal women. *J Womens Health* **20:** 771-780.

Zimmerman MB, Winichagoon P, Gowachirapant S, Hess SY, Harrington M, Chavasit V *et al* (2005). Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. *Am J Clin Nutr* **82:** 1276-1282.

Zimmermann MB, Chaouki N, Hurrell RF (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. *Am J Clin Nutr* **81:** 115-121.

Zimmermann MB, Hurrell RF (2007). Nutritional iron deficiency. *Lancet* **370:** 511-520.

Zimmermann MB, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I *et al* (2008). Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *Int J Obes* **32:** 1098-1104.

Zimmermann MB, Harrington M, Villalpando S, Hurrell RF (2010). Nonheme-iron absorption in first-degree relatives is highly correlated: a stable-isotope study in mother-child pairs. *Am J Clin Nutr* **91:** 802-807.

CHAPTER 3

The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women in New Zealand

In order to determine dietary patterns associated with suboptimal iron status, a dietary assessment tool for assessing iron-related dietary patterns was developed (iron food frequency questionnaire (FeFFQ)). It is important that the validity and reproducibility of dietary assessment tools are tested prior to being used. The aim of this study was to investigate the relative validity and reproducibility of a FeFFQ specifically designed to identify iron-related dietary patterns in premenopausal women.

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1 Abstract

Background: Using food frequency data to identify dietary patterns is a newly emerging approach to assessing the relationship between dietary intake and iron status. Food frequency questionnaires should however be assessed for their validity and reproducibility prior to use.

Objective: This study aimed to investigate the relative validity and reproducibility of an iron food frequency questionnaire (FeFFQ) specifically designed to identify iron-related dietary patterns.

Methods: Participants (a convenience sample of women (n=115) aged 18-44 years living in Auckland, New Zealand) completed the FeFFQ at baseline (FeFFQ1) and one month later (FeFFQ2) to assess reproducibility. A four-day weighed diet record (4DDR) was completed between these assessments to determine validity. Foods appearing in the 4DDR were classified into the same 144 food groupings as the FeFFQ. Factor analysis was used to determine dietary patterns from FeFFQ1, FeFFQ2 and the 4DDR. Agreement between diet pattern scores was compared using correlation coefficients, Bland and Altman analysis, cross-classification and the weighted κ-statistic.

Results: A 'healthy' and a 'sandwich & drinks' dietary pattern were identified from all three dietary assessments. Correlation coefficients between FeFFQ1 and the 4DDR diet pattern scores (validity) were 0.34 for the 'healthy', and 0.62 for the 'sandwich & drinks' pattern (both $P<0.001$). Correlation coefficients between the two FeFFQs (reproducibility) were 0.76 for both the 'healthy' and 'sandwich & drinks' pattern ($P<0.001$). The FeFFQ1 correctly classified over 50% of participants into the correct tertile and less than 10% into the opposite tertile for both the 'healthy' and 'sandwich & drinks' diet pattern scores when compared with the 4DDR and FeFFQ2.

Conclusions: The FeFFQ appears to be a reproducible and relatively valid method for identifying dietary patterns, and could be used to investigate the relationship between dietary patterns and iron status.

2 Introduction

Anaemia, caused by iron deficiency, is a global health concern (McLean et al 2008), and young women are at particular risk (University of Otago and Ministry of Health 2011, World Health Organization and Centers for Disease Control and Prevention Atlanta 2008). Most research investigating the causes of iron deficiency has focused on the effects of individual foods or nutrients which has several limitations (Hu 2002, Newby and Tucker 2004). Individuals do not eat nutrients in isolation, but in a variety of combinations that may interact (Hu 2002, Newby and Tucker 2004), for example, ascorbic acid enhances non-haem iron absorption (Cook and Monsen 1977). The effect of single nutrients or foods may not be large enough to detect alone (Hu 2002, Newby and Tucker 2004), or conversely, statistically significant associations may occur by chance when a number of nutrients and foods are analyzed independently (Hu 2002). Finally, dietary patterns may confound the effect of single nutrient analysis (Hu 2002). For example, in a 'healthy' dietary pattern, the inhibiting effect of phytic acid (found in whole grain breads and cereals) on iron absorption may be reduced if ascorbic acid containing fruit and vegetables are also consumed at the same time (Siegenberg et al 1991).

Dietary pattern analysis considers the whole diet and how nutrients and foods are consumed together (Hu 2002, Newby and Tucker 2004), and is increasingly being used to investigate relationships between diet and disease (Kerver et al 2003, Newby et al 2004, Newby and Tucker 2004, Okubo et al 2006, Terry et al 2001). A food frequency questionnaire (FFQ) aimed at identifying iron-related dietary patterns will be of particular use as a tool for determining how combinations of foods and beverages impact on iron status, informing future clinical and public health practice in the area of iron deficiency prevention. Dietary patterns can be derived using statistical methods such as factor or cluster analysis (Hu 2002, Newby and Tucker 2004). The majority of studies have used FFQs to establish dietary patterns (Broderstad et al 2011, Kerver et al 2003, Shi et al 2006, Terry et al 2001). While FFQs have several advantages over diet records, including their low respondent and researcher burden (Gibson 2005), they should be validated prior to use to ensure their appropriateness in establishing dietary patterns (Cade et al 2004). However, only a handful of studies have investigated the validity of dietary patterns determined using factor analysis based on a FFQ by comparing it with the 'gold standard' diet record (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004,

Okubo et al 2010, Togo et al 2003), and only two of these studies have considered the reproducibility of dietary patterns obtained using an FFQ by comparing it with the same FFQ completed at a later date (Hu et al 1999, Khani et al 2004).

Neither of the studies that have considered the effect of dietary patterns on iron status (Broderstad et al 2011, Shi et al 2006) have reported the validity of their FFQ for determining dietary patterns.

The aim of this study was to investigate the relative validity and reproducibility of an iron food frequency questionnaire (FeFFQ) specifically designed to identify iron-related dietary patterns.

3 Methods

3.1 Study population

This research was conducted in Auckland, New Zealand. Inclusion criteria were: female, 18-44 years, and free of chronic disease. Exclusion criteria were: current smoking, pregnancy, breastfeeding, or planning to become pregnant in the next six months. For validation studies of dietary assessment methods, a sample size of more than 100 participants is recommended (Serra-Majem et al 2009, Willett 1998). In this study, a convenience sample of 116 women was recruited through a magazine advertisement and invitations sent to women on the Human Nutrition Research Unit (HNRU) database of potential study volunteers.

3.2 Study procedure

Participants visited the HNRU at Massey University, Auckland, on two occasions one month apart. Demographic data (age, ethnicity, medical history) were obtained at the initial visit. Body weight and height were measured in duplicate at the initial visit using the International Society for the Advancement of Kinanthropometry guidelines (Marfell-Jones et al 2006). Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. At both appointments, participants completed a FeFFQ. Between visits, participants were asked

to maintain their normal daily routine (e.g. eating patterns, physical activity, alcohol consumption) and to complete a four-day diet record (4DDR).

The Massey University Human Ethics Committee: (Southern A) approved the study protocol (Reference No 08/63) and all participants provided written informed consent.

3.3 Dietary assessment

3.3.1 Iron food frequency questionnaire

All participants completed a self-administered computerised FeFFQ at both visits (FeFFQ1 and FeFFQ2) (Appendix 5). The electronic format assisted in ensuring complete data capture. The FeFFQ was developed by the researchers to determine consumption patterns over the previous month of foods that may affect iron intake and absorption. A list of food items was developed based on foods used in the New Zealand 1997 National Nutrition Survey FFQ (Russell et al 1999) and New Zealand food composition data (Athar et al 2006).

Foods were grouped according to their iron, ascorbic acid and calcium content (low, medium, high and very high per common standard measure (Athar et al 2006)) because these nutrients affect iron intake and bioavailability (Cook and Monsen 1977, Hallberg et al 1991). For example, prawns and shrimps were grouped separately from scallops, crabsticks, crab, squid and crayfish due to their higher iron content (Athar et al 2006). Vitamin A and fibre were also considered due to their potential effects on iron absorption (Cook et al 1983, Layrisse et al 1997) (see Table 3.1).

Table 3.1. Classifications used to group foods for the FeFFQ based on nutrient levels (per common standard measure)

	Low	Medium	High	Very high
Iron (mg)	<2	2-3.9	≥4-5.9	≥6
Ascorbic acid (mg)	<20	20-49.9	≥50	-
Calcium (mg)	<50	50-99.9	≥100	-
Fibre (g)	<2	2-4.9	≥5	-
Vitamin A (µg)	-	-	≥1000	-

The foods were also grouped according to their cognitive similarities (e.g. berry fruits grouped together) and frequency of consumption (e.g. apples grouped separately due to a high frequency of consumption (Russell et al 1999)). The final FeFFQ inquired only about frequency of consumption, and did not specify portion size.

The FeFFQ contained 144 food groupings (each corresponding to a single question) divided into the following 16 food categories: meat/chicken; prepared meat; fish/seafood; eggs; nuts; legumes; dairy products; fruit; vegetables; breakfast cereals/porridge; grains/cereals; breads; cakes/biscuits/crackers; miscellaneous foods/drinks; alcoholic beverages; and non-alcoholic beverages.

Participants were asked how often they consumed each food grouping in the past month. Options for frequency of intake included: never, less than once per month, one to three times per month, once per week, two to three times per week, four to six times per week, once per day, two to three times per day, four or more times per day.

In analysing responses to the FeFFQ, one month was considered to equal four weeks. All possible responses were converted into frequencies of intake per week for each participant (range (never eaten) to 28 times (eaten four or more times per day)). These weekly values were converted to frequency of intake over four days in order to align with the 4DDR by multiplying by four (per four days) and dividing by seven (per day). The midpoint of each frequency of intake response was used as the level of consumption. For example, one to three times per month was converted to two times per month. This equated to 0.5 times/week, or 0.29 times/four days (0.5 x 4/7).

3.3.2 Diet record

A 4-day weighed diet record was chosen to validate the FFQ as their measurement error tends to be independent to those of an FFQ. For example, unlike a 24-hour recall, completion of a weighed diet record is not dependent on memory or estimation of portion size Cade et al (2002). Participants completed a 4DDR following their initial visit. It was ensured that all days of the week were equally represented across participants (Gibson 2005). Participants were provided with electronic food scales, household measuring cups and spoons, a food diary, and received detailed written and verbal instructions from a registered dietitian on how to complete the diet record, including a photographic portion guide. Participants were required to weigh all food and beverages, except when the situation did not allow, for example when eating out, when they were asked to estimate portion size. The diet records were returned at the second visit, and a dietitian reviewed the diet record with each participant to clarify answers, and to check for missing or incomplete information, including the ingredients in mixed dishes where recipes were not provided.

To enable comparison with the FeFFQ, all items on the diet record were manually categorised by a dietitian into one of the 144 food groupings in the FeFFQ. On each occasion a food was eaten, it was counted as being consumed once, regardless of the amount eaten. Water was an exception, where several participants provided a total water consumption amount for the whole day, rather than identifying when water was consumed. In this case, 200-250ml water was considered as a single occasion. When two items from the same food grouping were eaten at the same meal (e.g. leeks and onions) these were recorded as the food grouping being eaten twice; however, when two types of onion were eaten at the same meal (e.g. brown onion and red onion), they were recorded as the food grouping being eaten once. For mixed dishes and where participants provided recipes, the dishes were separated into main ingredients that could be assigned to a FeFFQ grouping. For example, a homemade pasta sauce containing leek, mushrooms and chicken was assigned to each of these three categories.

A few food items did not match a food grouping within the FeFFQ. These food items included salt, pepper, herbs and spices, various other condiments, flour, artificial

sweeteners, sweets, and some vegetables (egg plant, choko, alfalfa sprouts). These items were excluded from the analysis.

All diet records were entered into the Foodworks Professional diet analysis program (version 6.00, 2009, Xyris Software, Queensland). Intake was assessed using the Goldberg method (Goldberg et al 1991). Under-reporting was identified if the energy intake: basal metabolic rate ratio was <1.06 (cut-off value specific for a sedentary individual (physical activity level=1.55)), based on four days of dietary intake (Goldberg et al 1991). The Schofield equation (Schofield 1985) was used to calculate basal metabolic rate.

3.3.3 Identification of the 30 most frequently consumed food groupings (the "30 food groupings")

The 30 most frequently consumed food groupings were determined from FeFFQ1, and compared to the 30 most frequently consumed food groupings in the 4DDR and FeFFQ2.

3.3.4 Identification of dietary patterns from the FeFFQs and the diet record

Three separate factor analyses were conducted using the 30 food groupings identified from FeFFQ1, one each for: FeFFQ1, 4DDR (to assess validity), and FeFFQ2 (to assess reproducibility). The FeFFQ1 food groupings were used rather than FeFFQ2 to avoid any influence that recording of food for the diet record may have had on responses to the FeFFQ (Gibney et al 2004). As our focus was on whether a single FeFFQ captured valid dietary patterns, the same 30 food groupings were used across all dietary assessment methods after testing their similarity. This also ensured the consistency of food groupings entered into the factor analysis.

Factor analysis combines food groupings in the dataset based on the extent to which food groupings are correlated with one another (Newby and Tucker 2004). Principal components analysis and the orthogonal varimax rotation were used to facilitate interpretability of factors. Factors were retained based on examination of the scree plot, by having an eigenvalue of at least one and a meaningful dietary pattern. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's Test *P* values (to

demonstrate the presence of relationships between variables in the factor analysis) were determined (Field 2005).

In addition, a single diet pattern score (estimated using the regression method in the PASW statistical software program) was created (from the factor loadings on each food grouping) for each individual for each dietary pattern that emerged from the data (Field 2005).

3.4 Statistical analysis

Statistical analysis, including principal components analysis, was performed using PASW software (SPSS Inc 2009). Due to the large number of statistical comparisons being made, a *P*-value threshold of 0.01 was used to determine statistically significant tests. Two-sided tests were used in all analyses.

Firstly, the relative validity and reproducibility of the FeFFQ for determining frequency of intake of individual food groupings were determined using paired t-tests to assess differences in frequency of intake. Effect size was calculated for significant differences between dietary assessments to obtain an objective measure of the effect's importance. The following formula was used: effect size $r = \sqrt{t^2}/ (t^2 + df)$ (where t = t-statistic produced by paired t-test and df=degrees of freedom). An effect size of 0.1 indicates a small effect, 0.3 a medium effect and ≥0.5 a large effect (Field 2005). Spearman rank correlation coefficients were used to assess associations between frequency intakes. Relative validity was determined by comparing frequency of intakes from FeFFQ1 and the 4DDR. Reproducibility was determined by comparing frequency of intakes from FeFFQ1 and FeFFQ2.

Secondly, the relative validity and reproducibility of dietary patterns derived from FeFFQ1 were examined by calculating Pearson correlation coefficients between diet pattern scores obtained from FeFFQ1 and the 4DDR (relative validity), and FeFFQ1 and FeFFQ2 (reproducibility).

Thirdly, agreement between diet pattern scores across the range of intakes was determined using the Bland and Altman method (Bland and Altman 1986). Plots were

drawn of the average of the diet pattern scores between dietary patterns identified from FeFFQ1 and FeFFQ2 or the diet record (x-axis) versus the average difference of diet pattern scores from FeFFQ1 and FeFFQ2 or the diet record (y-axis).

Finally, the FeFFQ and the 4DDR diet pattern scores were divided into tertiles to assess whether the dietary assessment methods classified participants into the same third or the opposite third of intake. Masson's et al (2003) criteria were used to assess levels of agreement (>50% participants correctly classified in the same third) and misclassification (<10% of participants classified into opposite third) between dietary assessment methods. The level of agreement between the two assessment methods was determined using the weighted κ-statistic (Altman 1991). A weighting of one was used for participants classified into the same third by each dietary assessment method; 0.5 for adjacent thirds; and zero for opposite thirds. Values of κ greater than 0.80 indicate very good agreement, between 0.61 and 0.80 good agreement, 0.41-0.60 moderate agreement, 0.21-0.40 fair agreement and <0.20 poor agreement (Altman 1991).

4 Results

4.1 Participant characteristics

A total of 116 women completed the study. However, results from the computerised FeFFQ2 were unavailable for one woman due to technical problems, so her results were excluded from the analysis. Fourteen (12.2%) participants were identified as under-reporters based on their reported energy intake on the 4DDR, however no women were excluded on the basis of under-reporting. The women had a median (25th, 75th percentile) age of 33 (27, 40) years and BMI of 22.5 (20.9, 25.5)kg/m^2. The majority of women were of European ethnicity (85.2%), 7.0% were of Asian ethnicity, 5.2% of Māori ethnicity and 2.6% of other ethnicity.

4.2 Frequency of intake of food groupings

The 30 most frequently consumed food groupings from FeFFQ1 are listed in Table 3.2. The mean intake of the 30 food groupings ranged from 12.95 (water) to 1.07 (almonds)

times per four days for FeFFQ1. The 30 most frequently consumed food groupings found in the 4DDR and FeFFQ2 differed only slightly from FeFFQ1. Within the 4DDR, the 30 most frequently consumed food groupings were consumed a minimum of 1.03 times per four days, and included “potatoes”, “milk chocolate” and “zucchinis/cucumber/gherkins/marrows”, instead of “porridge”, “crackers” and “almonds” in FeFFQ1 (in 4DDR ranked 33, 37 and 42 respectively). For FeFFQ2, the 30 most frequently consumed food groupings were consumed at least 1.00 times per four days, with “citrus fruits”, “feijoas/persimmons/tamarillos” and “potato” instead of “fruit and vegetable juice”, “almonds” and “beef” in FeFFQ1 (in FeFFQ2 ranked 31, 32 and 34 respectively).

The mean frequency of intake of each of the 30 food groupings over four days from FeFFQ1, FeFFQ2 and the 4DDR can be seen in Table 3.2. For most food groupings the frequency of intake was not significantly different between the FeFFQs and the 4DDR. Using paired t-tests, seven food groupings were overestimated by FeFFQ1 in comparison to the 4DDR ($P<0.01$): sugar, cooking oil, apples, yoghurt, bananas, crackers and almonds. Two food groupings were underestimated by FeFFQ1: cheese and white bread ($P<0.01$). Mean differences in frequencies of intake of these foods ranged from 0.47 per four days for almonds to 1.80 per four days for sugar. However, the effect size was large ($r\geq0.5$) for white bread only. Lettuce and beef were overestimated in FeFFQ1 compared with FeFFQ2 ($P<0.01$). Mean differences were 0.20 per four days for beef and 0.46 per four days for lettuce, with small and medium effect sizes respectively. Spearman correlation coefficients ranged from 0.33 (cooking oil) to 0.84 (milk added to drinks) for comparisons between FeFFQ1 and the 4DDR, and from 0.61 (eggs and egg based dishes) to 0.93 (coffee) for comparisons between FeFFQ1 and FeFFQ2.

Table 3.2. Intake of the 30 food groupings over four days as assessed by the FeFFQ1, FeFFQ2 and 4DDR (n=115)

	Frequency of intake over 4 days (mean±SD)			Mean difference ±SD		Correlations[1]	
Food groupings	**FeFFQ1**	**FeFFQ2**	**4DDR**	**FeFFQ1 vs 4DDR**	**FeFFQ1 vs FeFFQ2**	**FeFFQ1 vs 4DDR**	**FeFFQ1 vs FeFFQ2**
Water	12.95±4.18	12.31±4.67	12.21±4.89	0.75±4.60	0.65±3.25*	0.54	0.72
Milk added to drinks	5.83±5.65	5.83±5.59	5.43±5.37	0.40±3.10	0.00±3.36	0.84	0.87
Sugar	3.46±4.45	2.82±3.82	1.66±2.90	1.80±4.03***[2]	0.64±3.59	0.47	0.76
Coffee	3.18±4.32	3.40±4.38	2.80±3.58	0.38±2.56	-0.22±2.68	0.81	0.93
Cooking oil	3.11±2.05	2.85±1.87	2.10±1.67	1.00±1.95***[2]	0.26±1.54	0.33	0.69
Brown bread[4]	2.75±2.83	2.56±2.91	2.57±2.08	0.18±2.39	0.19±2.61	0.55	0.70
Herbal/fruit tea	2.57±4.10	2.62±4.44	2.39±3.76	0.18±2.68	-0.05±2.56	0.77	0.85
Butter or margarine	2.53±3.03	2.78±3.49	2.38±2.31	0.15±2.41	-0.24±2.34	0.63	0.86
Milk added to food	2.35±2.28	2.31±2.10	2.17±2.01	0.19±2.24	0.04±2.16	0.64	0.74
Black tea	2.30±4.34	2.58±4.59	2.61±4.26	-0.31±3.64	-0.28±3.29	0.64	0.83
Apples	2.28±2.24	2.10±2.28	1.68±1.98	0.60±1.92**	0.17±1.54	0.61	0.74
Tomatoes	2.16±2.03	1.77±1.55	2.36±1.94	-0.20±2.24	0.39±1.84*	0.45	0.72
Onions, leeks, celery	2.03±1.78	1.98±1.76	2.26±1.91	-0.23±2.08	0.05±1.19	0.36	0.80
Carrots	1.89±1.52	1.83±1.33	1.95±1.58	-0.05±1.65	0.07±0.94	0.36	0.76
Yoghurt	1.86±2.21	1.60±1.96	1.37±1.65	0.49±1.97**	0.26±1.53	0.55	0.80
Lettuce	1.84±1.62	1.38±1.64	1.53±1.35	0.31±1.67*	0.46±1.45**[2]	0.38	0.73
Cheese	1.83±1.83	1.64±1.54	2.71±2.15	-0.89±1.99***[2]	0.19±1.15	0.50	0.69

	Frequency of intake over 4 days (mean±SD)			Mean difference ±SD		Correlations[1]	
Food groupings	**FeFFQ1**	**FeFFQ2**	**4DDR**	**FeFFQ1 vs 4DDR**	**FeFFQ1 vs FeFFQ2**	**FeFFQ1 vs 4DDR**	**FeFFQ1 vs FeFFQ2**
Bananas	1.73±1.88	1.62±1.68	1.19±1.64	0.54±1.64**[2]	0.11±1.00	0.65	0.81
Crackers	1.39±2.12	1.21±1.95	0.79±1.16	0.60±1.96**	0.18±1.40	0.41	0.68
Capsicum, peppers	1.37±1.04	1.25±1.30	1.28±1.44	0.10±1.27	0.13±1.04	0.49	0.75
Chicken, turkey or duck	1.35±1.63	1.24±1.19	1.75±1.54	-0.40±1.67*	0.11±0.86	0.52	0.76
Porridge, oats	1.21±1.39	1.20±1.28	0.94±1.39	0.27±1.12*	0.02±1.09	0.62	0.69
Eggs/ egg based dishes	1.21±1.18	1.03±0.78	1.03±1.09	0.18±1.10	0.19±0.88*	0.51	0.61
Broccoli	1.20±0.98	1.40±1.27	1.03±1.20	0.16±1.12	-0.20±0.97*	0.51	0.72
White bread	1.11±1.23	1.11±1.35	2.43±1.96	-1.31±2.00***[3]	0.00±1.30	0.36	0.63
Fruit and vegetable juices	1.11±1.92	0.97±1.97	1.11±1.92	0.00±1.76	0.14±1.74	0.57	0.74
White rice	1.09±1.25	1.04±1.25	1.32±1.40	-0.23±1.14*	0.05±0.58	0.52	0.76
Soft/fizzy drinks	1.09±2.14	1.00±2.13	1.31±2.13	-0.23±1.26	0.09±1.15	0.67	0.78
Beef	1.08±0.84	0.88±0.66	1.22±1.26	-0.14±1.26	0.20±0.68**	0.43	0.72
Almonds	1.07±1.52	0.93±1.39	0.60±1.32	0.47±1.50**[2]	0.15±0.91	0.51	0.77
Average correlations						0.54	0.75

*[1]Spearman correlation coefficients; all P<0.001; [2]Medium effect size (>0.3-0.49); [3]Large effect size (>0.5); [4]Brown bread=whole meal, whole wheat, whole grain bread; *P<0.05, **P<0.01, ***P<0.001; SD - standard deviation*

4.3 Dietary pattern analysis

The factor analysis identified two major dietary patterns that were similar for each of the three sources of dietary data. The KMO measure of sampling adequacy was 0.511 for FeFFQ1, 0.511 for the 4DDR and 0.552 for the FeFFQ2 (>0.5 acceptable) (Field 2005), and Bartlett's Test *P* values were all <0.001 (<0.001 acceptable). The dietary patterns were: a 'healthy' dietary pattern, which consisted of tomatoes, lettuce, capsicum, broccoli, carrots, onions, apples, almonds, yoghurt, brown bread, crackers, porridge, herbal tea, and water; and a 'sandwich & drinks' pattern consisting of brown bread, butter, cheese, beef, coffee, black tea, and milk added to drinks. The two dietary patterns obtained from each of the FeFFQs and diet records explained approximately twenty percent of the variance in the food intake scores (Table 3.3).

Table 3.3. Factor loadings for the two major dietary patterns identified in FeFFQ1, FeFFQ2 and the 4DDR (n=115)

	Pattern 1 – 'Healthy'			**Pattern 2 – 'Sandwich & drinks'**		
	FeFFQ1[123]	**FeFFQ2**	**4DDR**	**FeFFQ1**	**FeFFQ2**	**4DDR**
Tomatoes	0.61	0.40	0.36	0.29	0.24	
Lettuce	0.58	0.30	0.27			0.25
Almonds	0.56	0.60	0.49	-0.19		
Yoghurt	0.54	0.48	0.26	-0.19	-0.20	-0.27
Capsicum	0.52	0.26	0.17			-0.33
Broccoli	0.51	0.70	0.46			
Crackers	0.50	0.63	0.31			-0.22
Apples	0.44	0.26	0.54			
Chicken	0.41	0.69	-0.31	-0.18		
Cheese	0.39	0.58		0.29	0.32	0.33
Carrots	0.39	0.35	0.49			
Porridge	0.35	0.29	0.48			
Herbal tea	0.32	0.26	0.30			
Soft drinks	0.29	0.54	-0.37	-0.24		
Onions	0.26	0.16	0.27	0.21		
Bananas	0.24		0.29			
White bread	0.24	0.22	-0.37		0.21	0.18

	Pattern 1 – 'Healthy'			Pattern 2 – 'Sandwich & drinks'		
	FeFFQ1[123]	**FeFFQ2**	**4DDR**	**FeFFQ1**	**FeFFQ2**	**4DDR**
White rice	-0.23		-0.34		-0.17	-0.46
Eggs		0.24			0.17	
Sugar			-0.15		0.35	0.53
Milk added to drinks			0.21	0.62	0.73	0.74
Butter				0.61	0.71	0.41
Brown bread	0.17	0.23	0.48	0.61	0.43	0.20
Coffee				0.54	0.53	0.58
Water	0.23	0.19	0.30	-0.52	-0.29	-0.48
Black tea			0.26	0.42	0.40	0.45
Milk added to food			0.41	0.29	0.17	
Fruit and vegetable juices				-0.26	-0.26	
Beef			0.31	0.22	0.26	0.23
Cooking oil					0.16	-0.30
Variance[4] (%)	11.45	11.75	10.12	8.55	8.15	9.05

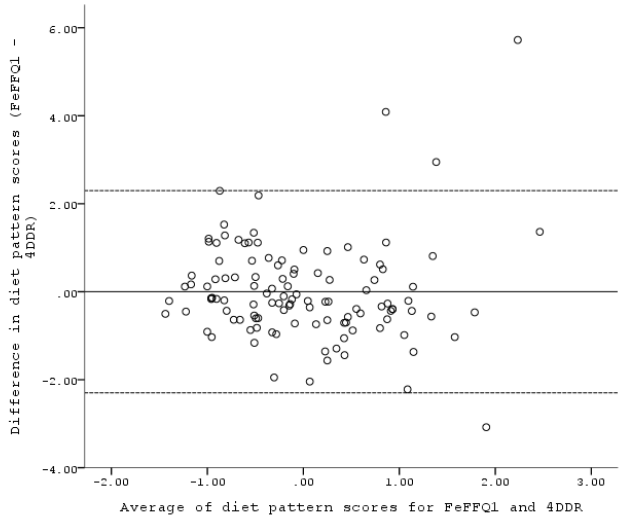
[1]Sorted by loadings on FeFFQ1 factors; [2]Absolute values < 0.15 excluded to enable ease in interpretation; [3]For food groupings: Positive loadings are positively associated and negative loadings are negatively associated with the dietary pattern; higher loadings mean a greater contribution to the dietary pattern; [4]Total variance of food intake scores explained by the 'healthy' and 'sandwich & drinks' dietary patterns: FeFFQ1=20%; FeFFQ2=19.9%; 4DDR=19.17%

4.4 Validity and reproducibility of the two dietary patterns

Pearson correlation coefficients between the diet pattern scores of the FeFFQ1 and 4DDR suggested reasonable relative validity: 0.34 for the 'healthy' dietary pattern and 0.62 for the 'sandwich & drinks' dietary pattern (P<0.001). Correlations between FeFFQ1 and FeFFQ2 suggested good reproducibility: 0.76 for the 'healthy' dietary pattern and 0.76 for the 'sandwich & drinks' dietary pattern (P<0.001).

Visual inspection of Bland and Altman plots suggested the difference between diet pattern scores for FeFFQ1 and the 4DDR and FeFFQ2 for the 'healthy' and 'sandwich & drinks' dietary patterns increased as average diet pattern scores increased (see Figure 3.1 and 3.2).

(a)



(b)

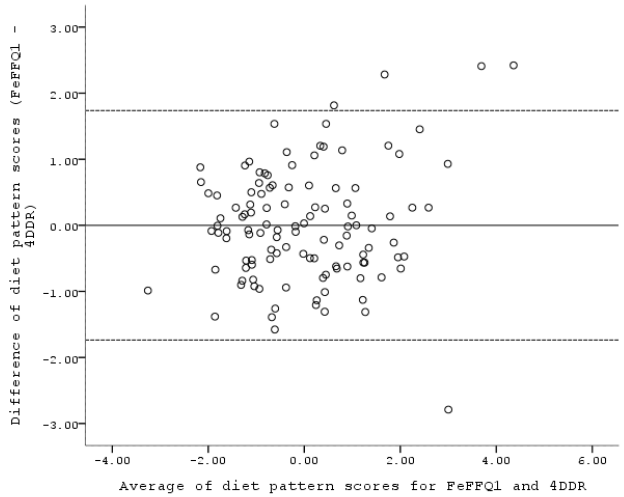
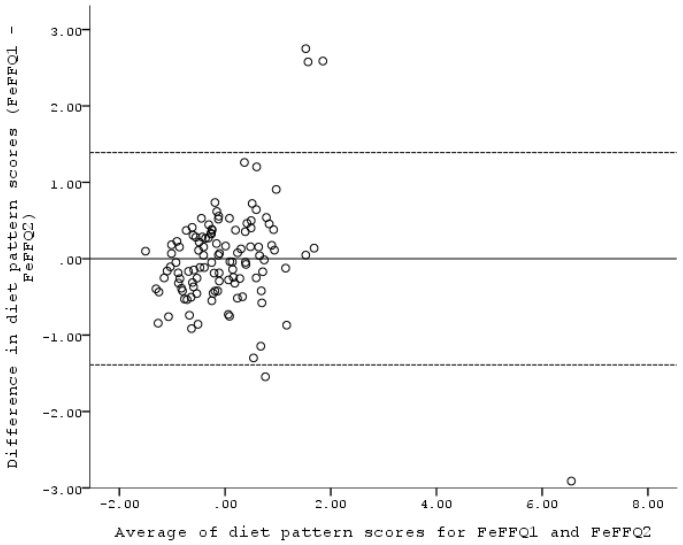


Figure 3.1. Bland and Altman plots for validity: agreement between dietary pattern scores for (a) 'Healthy' dietary pattern – Iron food frequency questionnaire (FeFFQ) 1 versus four-day diet record (4DDR), (b) 'Sandwich & drinks' dietary pattern - FeFFQ1 versus 4DDR. The solid line represents the mean difference and the dashed lines represent the limits of agreement (mean difference ± 2 standard deviations (SD)).

(a)



(b)

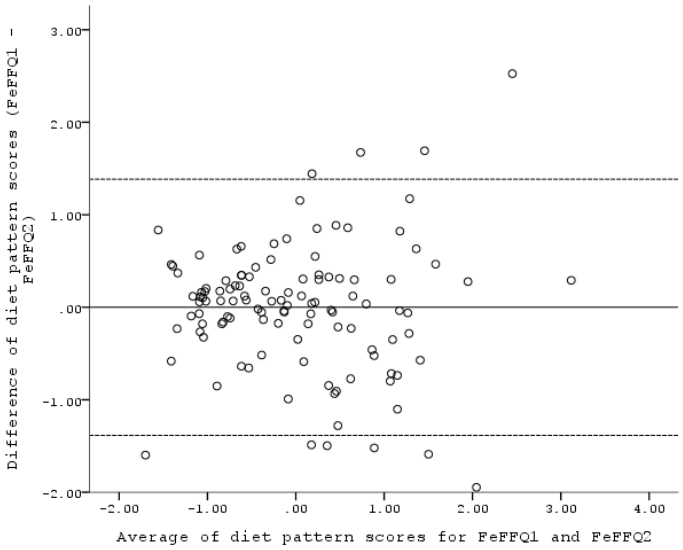


Figure 3.2. Bland and Altman plots for reproducibility: agreement between dietary pattern scores for (a) 'Healthy' dietary pattern - Iron food frequency questionnaire (FeFFQ) 1 versus FeFFQ2, (b) 'Sandwich & drinks' dietary pattern - FeFFQ1 versus FeFFQ2. The solid line represents the mean difference and the dashed lines represent the limits of agreement (mean difference ± 2 standard deviations (SD)).

Cross-classification of the diet pattern scores found more than 50% of participants were classified in the same third and less than 10% were misclassified into the opposite third for both the 'healthy' and 'sandwich & drinks' diet pattern scores between the FeFFQ1 and the 4DDR (Masson et al 2003), and between FeFFQ1 and FeFFQ2 (see Table 3.4). The weighted κ-statistic between FeFFQ1 and the 4DDR diet pattern scores were fair ('healthy') and moderate ('sandwich & drinks'); and between FeFFQ1 and FeFFQ2 were moderate ('healthy') and good ('sandwich & drinks').

Table 3.4. Cross-classification and weighted κ-coefficient for the diet pattern scores for the two dietary patterns derived from the FeFFQs and 4DDR

	Proportion (%) classified in		**Weighted κ-coefficient**
	Same third	**Opposite third**	
'Healthy' FeFFQ1 vs 4DDR	52.17	7.83	0.37
'Sandwich & drinks' FeFFQ1 vs 4DDR	54.78	5.22	0.43
'Healthy' FeFFQ1 vs FeFFQ2	62.61	0.87	0.57
'Sandwich & drinks' FeFFQ1 vs FeFFQ2	70.43	1.74	0.65

5 Discussion

Two dietary patterns ('healthy' and 'sandwich & drinks') were identified in this population of 115 healthy young women using the FeFFQ, 4DDR and a repeat administration of the FeFFQ. The FeFFQ appears to be a reproducible and reasonably valid method for determining frequency of intake of food groupings and identifying iron-related dietary patterns, as determined using a cascade of statistical testing.

Correlation coefficients for the diet pattern scores between FeFFQ1 and the 4DDR suggested reasonable validity (0.34 for 'healthy' and 0.62 for 'sandwich & drinks') for the FeFFQ. Previous studies validating dietary patterns determined using factor analysis from a diet history questionnaire (Okubo et al 2010) or a FFQ (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Togo et al 2003) against diet records have found similar uncorrected correlation coefficients to those observed in our study. Uncorrected correlation coefficients have ranged from 0.34 to 0.67 for 'healthy/prudent' patterns (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Okubo et al

2010), from 0.27 to 0.64 for 'Western' patterns (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Okubo et al 2010), from 0.34 to 0.69 for 'Japanese' (Okubo et al 2010), 'sweet', 'traditional' and 'green' (Togo et al 2003) dietary patterns, and up to 0.73 for a 'drinker' dietary pattern (Khani et al 2004).

The reproducibility of our FeFFQ (0.76 for both dietary patterns) was high compared with other studies comparing two FFQs undertaken one year apart. In these studies reproducibility coefficients ranged from 0.63 to 0.73 for 'healthy', 'Western' and 'drinker' dietary patterns (Hu et al 1999, Khani et al 2004). The higher level of reproducibility in our study is possibly due to the one month time interval between administration of FeFFQ1 and FeFFQ2. When determining reproducibility, four to eight weeks are recommended between the first and second administration of the dietary assessment method (Block and Hartman 1989).

Correlation coefficients can be misleading, as they measure the relation between two methods, rather than the agreement between them (Bland and Altman 1986, Lau et al 2008). The Bland and Altman method may be a better measure of how two dietary assessment methods compare (Bland and Altman 1986). The difference between diet pattern scores for FeFFQ1 and the 4DDR and FeFFQ2 for both dietary patterns appeared to increase as average diet pattern scores increased. This observed divergence indicates an increase in measurement error for individuals with higher dietary pattern scores which should be confirmed in a larger sample.

When diet pattern scores from the FeFFQs and 4DDR were classified into tertiles, more than 50% of participants were correctly classified into the same category and less than 10% were misclassified into opposite categories, which is in line with recommendations (Masson et al 2003). The weighted κ-statistic was used to overcome agreement that occurred by chance (Masson et al 2003). Relative validity was fair to moderate for the 'healthy' and 'sandwich & drinks' diet patterns scores respectively and both diet patterns showed moderate to good reproducibility. However, the use of the weighted κ-statistic is controversial (Maclure and Willett 1987), with the magnitude of the weighted κ-statistic dependent on the number of categories used (Masson et al 2003) and the weightings applied (Cohen 1968).

More than one approach should be used when validating FFQs (Cade et al 2004). Alongside the range of statistical methods used, a further strength of this study was the high compliance of participants. All women completed the study, and only one woman was excluded due to technical issues. We tried to minimise under-reporting by reviewing diet records with each participant to clarify missing or incomplete foods. Using Goldberg cut-offs (Goldberg et al 1991), it was estimated that 12.2 percent of women under-reported their energy intake on the diet record. As the FeFFQ was not designed to measure energy intake (and therefore is unable to identify under-reporters), we were not able to determine whether the same women under-reported when completing the FeFFQ. Low energy reporting may have also occurred amongst those with plausible energy intakes and excluding only women with implausible intakes may have biased the results (Macdiarmid and Blundell 1997). Finally, the Goldberg cut-offs are appropriate only for individuals in energy balance. It is possible that some women were dieting and the diet records actually reflect true energy intake (Heath et al 2000). For these reasons, under-reporters were not excluded from our analyses.

Limitations of our study include the assumption of the diet record as the gold standard of measuring dietary intake (Crozier et al 2008). Measurement error can occur when participants keep diet records due to changes in eating behaviors and mistakes in recording (Khani et al 2004, Okubo et al 2010, Willett 1998), which may affect the assessment of true dietary intake. The diet record required participants to weigh foods, whereas the FeFFQ did not quantify amounts. Selection of the 30 most frequently consumed foods without quantifying amounts gave relatively equal weighting to all foods regardless of serving size and therefore nutrient content. While quantification of foods in the FeFFQ may have resulted in greater accuracy, it would have increased participant burden and it is therefore not uncommon for dietary patterns to be determined on the basis of frequency of consumption rather than amount (Khani et al 2004). The analysis of dietary patterns using the FeFFQ and factor analysis also has limitations. When developing the FeFFQ subjective decisions were made regarding how many foods to include, and how the foods were grouped. The 30 most frequently consumed food groupings were entered in the factor analysis. This is a different approach to that used by other studies, which tend to collapse all the foods consumed into larger food groups (Ambrosini et al 2011, Crozier et al 2008, Hu et al 1999, Khani et al 2004, Okubo et al 2010, Shi et al 2006). Our food groupings were considered likely to have an impact on

iron status only if they were consumed at least once every four days (approximately twice per week). The 30 food groupings fitting this criterion accounted for 62.6% of the total number of food groupings consumed by all women from the FeFFQ1. For most people, the consumption of 15-20 different foods per week is achievable and optimum health occurs when 30 or more different foods are consumed per week (Hodgson et al 1994). The use of 30 food groupings is consistent with this suggestion. When conducting factor analysis, subjective decisions are made, including the number of factors to extract, the method of rotation, and the names assigned to dietary patterns (Ambrosini et al 2011, Hu 2002, Newby and Tucker 2004, Okubo et al 2010). Despite these decisions being made in a considered and *a priori* fashion, it is possible that results regarding validity and reproducibility may have differed, had other decisions been made.

All study participants were volunteers and likely to be health conscious, which may have increased the validity of the FeFFQ (Khani et al 2004). The majority of women were of European ethnicity. Dietary patterns may differ between ethnicities (Hu et al 1999) and have been shown to vary between genders in some (Okubo et al 2010, Togo et al 2003), but not all (Newby and Tucker 2004) studies. It would be useful to repeat this study in other groups to be able to use the FeFFQ in a wider population group. However, future research using the FeFFQ is likely to be undertaken in groups at high risk of iron deficiency (including young women such as in this sample) and the positive results of this study show that the overall method and approach works.

In conclusion, food frequency questionnaires provide a useful way to assess the dietary intake of large groups due to their low respondent and investigator burden (Gibson 2005). Unlike individual nutrients and foods, dietary patterns take into account the whole diet (Ambrosini et al 2011, Hu et al 1999, Newby and Tucker 2004). This is likely to be particularly useful for studies investigating the relationship between diet and iron status because iron absorption is strongly influenced by other food components in the diet. As far as we are aware, this is the first study to determine the validity and reproducibility of an FFQ designed specifically to identify iron-related dietary patterns. The FeFFQ appears to be a reproducible and reasonably valid method of assessing frequency of food grouping intakes and iron-related dietary patterns, and could be used to investigate the relationship between dietary patterns and iron status in young women.

6 References

Altman DG (1991). *Practical Statistics for Medical Research*. Chapman and Hall: London.

Ambrosini GL, O'Sullivan TA, de Klerk NH, Mori TA, Beilin LJ, Oddy WH (2011). Relative validity of adolescent dietary patterns: a comparison of a FFQ and 3 d food record. *Br J Nutr* **105:** 625-633.

Athar N, McLaughlin J, Taylor G, Mishra S (2006). *The Concise New Zealand Food Composition Tables*, 7th edn. New Zealand Institute for Crop & Food Research Ltd and Ministry of Health: Palmerston North.

Bland JM, Altman DG (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1:** 307-310.

Block G, Hartman AM (1989). Issues in reproducibility and validity of dietary studies. *Am J Clin Nutr* **50:** 1133-1138.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Cade J, Thompson R, Burley V, Warm D (2002). Development, validation and utilisation of food-frequency questionnaires - a review. *Public Health Nutr* **5:** 567-587.

Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM (2004). Food-frequency questionnaires: a review of their design, validation and utilisation. *Nutr Res Rev* **17:** 5-22.

Cohen J (1968). Weighted Kappa - Nominal scale agreement with provision for scaled disagreement or partial credit *Psychol Bull* **70:** 213-220.

Cook JD, Monsen ER (1977). Vitamin C, the common cold and iron absorption. *Am J Clin Nutr* **30:** 235-241.

Cook JD, Noble NL, Morck TA, Lynch SR, Petersburg SJ (1983). Effect of fiber on nonheme iron absorption. *Gastroenterology* **85:** 1354-1358.

Crozier SR, Inskip HM, Godfrey KM, Robinson SM (2008). Dietary patterns in pregnant women: a comparison of food-frequency questionnaires and 4d prospective diaries. *Br J Nutr* **99:** 869-875.

Field A (2005). *Discovering Statistics Using SPSS*, 2nd edn. SAGE Publications Ltd: London.

Gibney MJ, Margetts BM, Kearney JM, Arab L (2004). *The Nutrition Society Textbook Series: Public Health Nutrition*. Blackwell Publishing: Oxford.

Gibson RS (2005). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York.

Goldberg G, Black A, Jebb S, Cole T, Murgatroyed P, Coward W *et al* (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45:** 569-581.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Heath ALM, Skeaff CM, Gibson RS (2000). The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. *Eur J Clin Nutr* **54:** 592-599.

Hodgson JM, Hsu-Hage B, Wahlqvist M (1994). Food variety as a quantitative descriptor of food intake. *Ecol Food Nutr* **32:** 137-148.

Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al* (1999). Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* **69:** 243-249.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Kerver JM, Yang EJ, Bianchi L, Song WO (2003). Dietary patterns associated with risk factors for cardiovascular disease in healthy US adults. *Am J Clin Nutr* **78:** 1103-1110.

Khani BR, Ye WM, Terry P, Wolk A (2004). Reproducibility and validity of major dietary patterns among Swedish women assessed with a food-frequency questionnaire. *J Nutr* **134:** 1541-1545.

Lau C, Glumer C, Toft U, Tetens I, Carstensen B, Jorgensen T *et al* (2008). Identification and reproducibility of dietary patterns in a Danish cohort: the Inter99 study. *Br J Nutr* **99:** 1089-1098.

Layrisse M, Garcia-Casal MN, Solano L, Baron MA, Arguello F, Llovera D *et al* (1997). The role of Vitamin A on the inhibitors of nonheme iron absorption: Preliminary results. *J Nutr Biochem* **8:** 61-67.

Macdiarmid JI, Blundell JE (1997). Dietary under-reporting: What people say about recording their food intake. *Eur J Clin Nutr* **51:** 199-200.

Maclure M, Willett WC (1987). Misinterpretation and misuse of the Kappa-Statistic *Am J Epidemiol* **126:** 161-169.

Marfell-Jones M, Olds T, Stewart A, Carter L (2006). *International standards for anthropometric assessment*. ISAK: Potchefstroom.

Masson LF, McNeill G, Tomany JO, Simpson JA, Peace HS, Wei L *et al* (2003). Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr* **6:** 313-321.

McLean E, Cogswell M, Egli I, Woidyla D, de Benoist B (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* **12:** 444-454.

Newby PK, Muller D, Hallfrisch J, Andres R, Tucker KL (2004). Food patterns measured by factor analysis and anthropometric changes in adults. *Am J Clin Nutr* **80:** 504-513.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Okubo H, Sasaki S, Horiguchi H, Oguma E, Miyamoto K, Hosoi Y *et al* (2006). Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. *Am J Clin Nutr* **83:** 1185-1192.

Okubo H, Murakami K, Sasaki S, Kim MK, Hirota N, Notsu A *et al* (2010). Relative validity of dietary patterns derived from a self-administered diet history questionnaire using factor analysis among Japanese adults. *Public Health Nutr* **13:** 1080-1089.

Russell D, Parnell WR, Wilson N, Faed J, Ferguson E, Herbison P *et al* (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*. Ministry of Health: Wellington.

Schofield WN (1985). Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* **39:** 5-41.

Serra-Majem L, Andersen LF, Henrique-Sanchez P, Doreste-Alonso J, Sanchez-Villegas A, Ortiz-Andrelluchi A *et al* (2009). Evaluating the quality of dietary intake validation studies. *Br J Nutr* **102:** S3-S9.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG *et al* (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* **53:** 537-541.

SPSS Inc (2009). *PASW Statistics 18, Release Version 18.0.0*. SPSS Inc: Chicago.

Terry P, Hu FB, Hansen H, Wolk A (2001). Prospective study of major dietary patterns and colorectal cancer risk in women. *Am J Epidemiol* **154:** 1143-1149.

Togo P, Heitmann BL, Sorensen TIA, Osler M (2003). Consistency of food intake factors by different dietary assessment methods and population groups. *Br J Nutr* **90:** 667-678.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

Willett WC (1998). Nature of variation in diet. In: Willett WC (ed). *Nutritional Epidemiology*, 2nd edn. Oxford University Press: New York. pp 33-49.

World Health Organization, Centers for Disease Control and Prevention Atlanta (2008). *Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia*. World Health Organization: Geneva.

CHAPTER 4

Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand

Most studies investigating determinants of suboptimal iron status have focused on individual foods and nutrients. This has several limitations, as people do not eat foods or nutrients alone but in various combinations. These limitations are particularly relevant to iron nutrition, where a number of foods and nutrients affect iron absorption (e.g. ascorbic acid enhances non-haem iron absorption). Assessing how foods are eaten in combination through the use of dietary patterns and practices may overcome some of these limitations. This study investigated the dietary patterns and practices of premenopausal women in relation to their risk of suboptimal iron status.

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1 Abstract

Objective: To investigate associations between dietary patterns and suboptimal iron status in premenopausal women living in Auckland, New Zealand.

Methods: Premenopausal women (n=375; 18-44 years) were included in this cross-sectional analysis. Suboptimal iron status was defined as serum ferritin <20µg/L. Participants completed a 144-item iron food frequency questionnaire (FeFFQ) and a questionnaire on dietary practices to assess dietary intake over the past month. Factor analysis was used to determine dietary patterns from the FeFFQ. Logistic regression was used to determine associations between these dietary patterns and iron status.

Results: Seven dietary patterns were identified: refined carbohydrate & fat; Asian; healthy snacks; meat & vegetable; high tea & coffee; bread & crackers; and milk & yoghurt. Logistic regression suggested that following a 'meat & vegetable' dietary pattern reduced the risk of suboptimal iron status by 41% (95% CI: 18%, 58%; *P*=0.002) and following a 'milk & yoghurt' pattern increased the risk of suboptimal iron status by 50% (95% CI: 15%, 96%; *P*=0.003).

Conclusions: These results suggest that dietary patterns characterised by either a low intake of meat and vegetables, or a high intake of milk and yoghurt, are associated with an increased risk of suboptimal iron status. Dietary pattern analysis is a novel and potentially powerful tool for investigating the relationship between diet and iron status.

2 Introduction

Iron deficiency is common in developed and developing countries (McLean et al 2008), and is associated with a number of health effects including reduced physical performance, deficits in cognitive function, and poor pregnancy outcome (Food and Nutrition Board: Institute of Medicine 2001). Young premenopausal women living in New Zealand are at risk of iron deficiency (University of Otago and Ministry of Health 2011), and for some this is due to diet (Heath et al 2001).

Iron bioavailability is more important than iron content in determining the amount of iron absorbed from a meal (Hallberg and Hulthen 2000). Ascorbic acid (Diaz et al 2003) and an as yet unidentified factor in meat, fish and poultry enhance (Hurrell et al 2006), while phytic acid (Hurrell et al 2003), polyphenols (Hurrell et al 1999) and calcium (Benkhedda et al 2010) inhibit non-haem iron absorption. Several cross-sectional studies which have included premenopausal women have investigated the association between individual nutrients (iron, ascorbic acid and calcium) or foods and beverages (meat, fruit, vegetables, dairy products, tea and coffee) and iron status (Brussard et al 1997, Galan et al 1998, Heath et al 2001, Pynaert et al 2009, Rangan et al 1997, Ruston et al 2003). However, the results have been somewhat inconsistent. For example, in most (Brussard et al 1997, Galan et al 1998, Heath et al 2001, Rangan et al 1997) but not all (Pynaert et al 2009, Ruston et al 2003) cross-sectional studies meat consumption has been associated with an increased iron status. An inverse association between calcium intake and iron status (Galan et al 1998, Rangan et al 1997) has been demonstrated in some studies, while other studies have observed no effect (Brussard et al 1997, Heath et al 2001, Pynaert et al 2009).

The focus on individual nutrients and foods in cross-sectional studies has several limitations, as described by Hu (2002) and Newby and Tucker (2004), a number of which are relevant to iron nutrition. People do not eat nutrients alone, but consume meals containing many combinations of foods and nutrients that may interact (Hu 2002, Newby and Tucker 2004) – for example, non-haem iron is influenced by enhancers and inhibitors of iron absorption consumed in the same meal. Statistically significant associations may occur by chance when several foods and nutrients are analysed separately (Hu 2002), and single nutrient analysis may be confounded by the effect of dietary patterns (Hu

2002). For example, although phytic acid in whole grains inhibits iron absorption, this effect may be negated or blunted if sufficient ascorbic acid is consumed at the same time (Siegenberg et al 1991), for instance if whole grains are consumed with fruits and vegetables high in ascorbic acid.

Dietary pattern analysis using factor or cluster analysis offers an alternative approach to studying individual foods and nutrients and their association with iron status (Hu 2002, Newby and Tucker 2004). Only two studies have explored the association between dietary patterns and iron status (Broderstad et al 2011, Shi et al 2006). In China, 'traditional' and 'sweet tooth' dietary patterns were positively associated, and a 'healthy' dietary pattern was negatively associated with anaemia (Shi et al 2006). In Norway, high serum ferritin concentrations were associated with a 'reindeer meat' dietary pattern (Broderstad et al 2011).

Dietary pattern analysis describes combinations of foods consumed in the diet as a whole. However, dietary factors impacting on iron bioavailability are more likely to affect non-haem iron absorption if consumed at the same time (Gleerup et al 1993, Gleerup et al 1995) so it is also important to investigate dietary practices such as the regular consumption of particular foods together in the same meal. One study found women (n=15) with a serum ferritin <12µg/L had a higher intake of tea and lower intake of ascorbic acid at meals (Razagui et al 1991), suggesting that dietary practices may impact on iron status.

Combinations of foods and beverages may be more strongly related to iron status than individual foods and nutrients. This study aimed to investigate the dietary patterns and practices of premenopausal women living in New Zealand in relation to their risk of suboptimal iron status.

3 Methods

3.1 Study design and participants

This cross-sectional study was undertaken in Auckland, New Zealand. Participants visited the Human Nutrition Research Unit (HNRU) at Massey University's Albany campus on one occasion during which demographic data (age, ethnicity) was collected, anthropometric measurements were made and dietary intake was assessed.

A total of 404 women, aged 18-44 years, who were participating in two separate studies were included in this analysis: one investigating iron status in female university students (n=276) (Beck et al 2012a) and the other screening women from the general population for participation in a randomised controlled trial investigating the effect of a dietary intervention on iron status (n=128) (Beck et al 2011). For the analysis conducted in this study, exclusion criteria were: current pregnancy, pregnancy in the past year, current breastfeeding; and any known health problems likely to influence iron status including inflammatory bowel disease, coeliac disease; and history of gastric ulcers, red blood cell disorders, menorrhagia, haemorrhoids, haematuria or malaria. Women who regularly consumed iron supplements (20mg elemental iron or more at least 3-4 times per week) within the three month period prior to the study were also excluded from the analysis.

The procedures followed were in accordance with the ethical standards of the Massey University Human Ethics Committee: (Southern A), Reference No 07/73 and 08/20, and all participants gave written informed consent.

3.2 Blood sampling and biochemical analysis to determine iron status

A venipuncture blood sample was taken at the HNRU or at Diagnostic MedLab, Auckland between 7am and 5pm. Four millilitres of blood was collected into an EDTA (Ethylene Diamine Tetraacetic Acid) tube for analysis of haemoglobin (Hb) and 3.5mL of blood was collected into a SST (Serum Separator Tube) for the analysis of serum ferritin (SF) and C-reactive protein (CRP). Haemoglobin, SF and CRP were analysed at Diagnostic MedLab, an International Accreditation New Zealand laboratory. Serum ferritin was analysed using

the immunoturbidimetric test (Roche Diagnostics, Indianapolis) (Cat. No. 11661400) and CRP using the particle enhanced immunoturbidimetric assay (Roche Diagnostics, Indianapolis) (Cat. No. 03002039). Haemoglobin was analysed using the SLS-Hb (sodium lauryl sulphate-Hb) method using an automated haematology analyser XE-2100 (Sysmex Corporation, Auckland, NZ).

Participants were divided into those with sufficient iron stores (SF≥20µg/L, Hb≥120g/L) versus participants with suboptimal iron status (SF<20µg/L, Hb < or ≥120g/L) (Gibson 2005). Participants who had anaemia without iron deficiency (SF≥20µg/L, Hb<120g/L) were excluded from the analysis.

3.3 Anthropometric measurements

Height and weight were measured in duplicate at baseline by a trained researcher using the International Society for the Advancement of Kinanthropometry protocols (Marfell-Jones et al 2006). Body mass index (BMI), was calculated as weight (kg)/height $(m)^2$.

3.4 Dietary assessment

Participants completed two questionnaires to assess dietary intake: an iron food frequency questionnaire (FeFFQ) to determine dietary patterns (Appendix 5), and a questionnaire to assess dietary practices (Appendix 6). The FeFFQ was developed to assess intake of foods containing iron or affecting iron bioavailability. The dietary practices questionnaire considered the various combinations in which foods and beverages were consumed.

3.4.1 Determination of dietary patterns

The FeFFQ considered food consumption over the past month. It collected information on frequency of consumption (nine options) for 144 food groupings. Portion size was not specified within the FeFFQ. An extensive list of food groupings was developed based on the foods in the New Zealand 1997 National Nutrition Survey food frequency questionnaire (Russell et al 1999) and food composition data from the New Zealand Food

Composition Tables (Athar et al 2006). Foods were grouped according to their similarities (e.g. stone fruits were grouped together), frequency of consumption (e.g. bananas were kept as a separate item because they are consumed frequently (Russell et al 1999)) and nutrient content of iron, ascorbic acid, and calcium per common standard measure (Athar et al 2006). These nutrients were chosen due to their potential impact on iron intake and bioavailability (e.g. red cabbage was grouped separately to other cabbages due to its higher ascorbic acid and calcium content).

The final FeFFQ contained 144 food groupings (each corresponding to a single question) categorised into 16 food categories: meat/chicken; prepared meat; fish/seafood; eggs; nuts; legumes; dairy products; fruit; vegetables; breakfast cereals/porridge; grains/cereals; breads; cakes/biscuits/crackers; miscellaneous foods/drinks; alcoholic beverages; and non-alcoholic beverages.

Responses from the FeFFQ were converted into nine frequencies of intake per week for each food grouping for each participant, ranging from zero to 28 times eaten per week. The average weekly consumption of each food grouping was determined, and the 30 most frequently consumed food groupings (as number of times consumed per week) were identified for use in the factor analysis. The 30 most frequently consumed food groupings were selected as they were consumed at least two times per week on average. Overall they accounted for 61.8% of the total number of food groupings from the FeFFQ consumed per week.

3.4.2 Determination of dietary practices

A separate questionnaire was developed to investigate dietary practices and to determine whether particular combinations of foods and beverages consumed at main meals and snacks affected iron status. In the dietary practices questionnaire, participants were given a list of foods: breakfast cereals, porridge, bread/toast, noodles/rice, milk products, fruit, vegetables, meats, baked beans/eggs, starchy foods (e.g. pasta, rice, potato), legumes, nuts and eggs, and asked to indicate which foods were a part of their usual breakfast, lunch and evening meals. Beverages consumed with these meals or within one hour of a meal or snack were also investigated: fruit/vegetable juice, milk, soy or chocolate-based drinks, coffee and tea. Habitual food and beverage intake between meals was

investigated and in addition to the food items listed above, typical snack choices were investigated: biscuits/cakes, crackers, potato crisps, cereal/muesli bars, and chocolate/sweets.

3.5 Statistical analysis

Statistical analysis was performed using Predictive Analytics SoftWare (PASW) Statistics 18 (SPSS Inc 2009). Two-sided tests were used for all analyses.

3.5.1 Determination of dietary patterns from the FeFFQ using factor analysis

Dietary patterns from the FeFFQ were identified using factor analysis (Field 2005). Factor analysis aggregates food groupings based on the degree to which food groupings in the dataset are correlated with one another (Newby and Tucker 2004). Principal components analysis and the orthogonal varimax rotation were used to facilitate interpretability of factors. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.607 (>0.5 acceptable) and Bartlett's Test *P* value was <0.001 (<0.001acceptable) demonstrating the presence of relationships between variables in the factor analysis.

To decide which factors to retain, factor solutions ranging from two to 10 were run. Factors with an eigenvalue >1 were considered. Both the scree plot and the factors themselves were examined to see which factors had the most meaningful dietary patterns. Labelling of dietary patterns was based on the interpretation of foods with high factor loadings for each dietary pattern (equivalent to simple correlations between the food groupings and dietary patterns). Only foods positively associated with a factor loading of ≥0.3 were included.

Seven factors were retained and considered as the major dietary patterns of these participants. A dietary pattern score was created for each individual for each dietary pattern. The regression method in PASW was used to estimate these scores. These dietary pattern scores were used in subsequent statistical analysis to examine the relationship between dietary patterns and iron status.

3.5.2 Dietary patterns and suboptimal iron status

Participants were divided into those with sufficient iron stores versus participants with suboptimal iron status. It was calculated, retrospectively, using G*Power 3 (Faul et al 2009) that a sample size of 375 (final sample used in the analysis) provided 87 and 99% power to detect significant differences (α=0.05) and odds ratios (OR) of 1.5 and 0.5, respectively between participants with sufficient iron stores and suboptimal iron status. Dietary pattern scores were divided into quintiles. Non-normally distributed variables were transformed into approximately normal distributions by logarithmic transformations and again tested for normality. Differences between groups were tested using the independent t-test for parametric data, the Mann-Whitney test for non-parametric data, and the Chi-square test to investigate categorical variables.

Stepwise multiple logistic regression analysis was performed to determine which dietary patterns contributed independently to suboptimal iron status (dependent variable) while considering the effects of age, BMI, ethnicity (European versus Asian; European versus other) and other dietary patterns. Dietary patterns, age and BMI were entered into the model as continuous variables and ethnicity as a categorical variable. The entry criterion was set at $P<0.05$ for forward stepwise multiple logistic regression analysis. All assumptions for multicollinearity were met.

3.5.3 Dietary practices and suboptimal iron status

Chi square analysis was used to investigate dietary practices, including foods and beverages consumed at main meals and snacks for participants who had sufficient iron stores versus participants with suboptimal iron status. For participants consuming potential sources of iron at main meals (e.g. meat), we investigated whether foods or beverages affecting iron bioavailability were consumed at the same time (e.g. milk products). The *P*-value for analysing dietary practices was set at $P<0.01$ due to the multiple comparisons that were made.

4 Results

4.1 Iron status and characteristics of participants

Of the 404 participants who took part in this study, data for 29 were excluded: blood results were unavailable for three participants, four did not complete the dietary questionnaires, four participants with a CRP>10mg/L (Zimmermann and Hurrell 2007) (marker of inflammation) were removed from the analysis as serum ferritin is an acute phase protein and is artificially increased during infection or inflammation (Umbreit 2005), 16 participants had anaemia without iron deficiency (SF≥20µg/L, haemoglobin <120g/L), and two participants with SF>200µg/L (Qaseem et al 2005) were excluded from the analysis due to possible associations with haemochromatosis (Wrede et al 2006). Of the remaining 375 participants included in the analysis, 70 (18.7%) had suboptimal iron status (SF<20µg/L, Hb < or ≥120g/L). Of these, 20 (28.6%) had iron deficiency anaemia (SF<20µg/L, Hb<120g/L). Table 4.1 shows the characteristics of the study participants. Participants with suboptimal iron status were more likely to be older (*P*=0.033) and of Asian ethnicity (*P*=0.002).

Table 4.1. Characteristics of study participants with and without suboptimal iron status

Characteristic	Participants with sufficient iron stores[1] (n=305)	Participants with suboptimal iron status[2] (n=70)	*P*-value for difference
Age (years)	25 (20, 35)	29 (21, 40)	0.033
BMI (kg/m^2)	22.7 (20.8, 24.9)	22.3 (21.1, 24.4)	0.711
SF (µg/L)	46 (34, 63)	13 (9, 17)	<0.001
Hb (g/L)	133 (128, 139)	126 (119, 132)	<0.001
Ethnicity n (%)			
European	239 (78.6)	43 (61.4)	
Asian	36 (11.8)	20 (28.6)	
Other	29 (9.5)	7 (10.0)	0.002

[1]SF≥20 µg/L and Hb ≥120 g/L; [2]SF<20 µg/L and Hb <120 or ≥120g/L; All results expressed as median (25, 75 percentile) or n (%); Hb – haemoglobin; BMI – Body mass index; SF - serum ferritin

4.2 Dietary patterns

The 30 most frequently consumed food groupings are listed in Table 4.2 with the factor loadings for each dietary pattern (factor loadings of 0.3 or higher are in bold). Seven dietary patterns were determined using factor analysis: refined carbohydrate & fat; Asian; healthy snacks; meat & vegetables; high tea & coffee; bread & crackers; milk & yoghurt. The seven patterns identified explained 44.3% of the variance in the intake scores.

Table 4.2. Factor loadings for each food grouping for the seven dietary patterns identified

	Factor components and factor loadings							**Frequency of consumption per week[1]**
Food groupings as contained in the FeFFQ	**DP 1: Refined carbohydrate & fat**	**DP 2: Asian**	**DP 3: Healthy snacks**	**DP 4: Meat & vegetables**	**DP 5: High tea & coffee**	**DP 6: Bread & crackers**	**DP 7: Milk & yoghurt**	
Butter or margarine	**0.62**	-0.10	-0.09	-0.05	0.23	**0.33**	-0.15	5.1 (4.6, 5.7)
Potatoes	**0.59**	0.02	-0.06	0.25	-0.12	0.01	0.26	2.5 (2.3, 2.7)
Jam	**0.52**	-0.04	0.20	-0.06	0.06	0.04	-0.09	2.2 (1.9, 2.6)
White bread and rolls	**0.52**	0.09	-0.26	-0.05	-0.05	0.19	-0.08	2.3 (2.0, 2.7)
Sugar	**0.51**	0.14	-0.06	0.02	0.09	-0.06	0.24	7.7 (6.9, 8.6)
Onions, leeks, celery	-0.01	**0.80**	0.00	0.10	0.08	-0.05	-0.03	3.3 (3.0, 3.6)
Tomatoes	-0.07	**0.77**	0.11	-0.05	0.06	0.24	0.13	3.4 (3.0, 3.7)
Cooking oil	**0.44**	**0.64**	0.02	0.00	-0.10	-0.18	-0.02	5.3 (4.9, 5.6)
Apples	-0.14	0.02	**0.68**	0.04	-0.05	-0.05	0.01	3.2 (2.9, 3.5)
Bananas	0.04	0.04	**0.58**	0.12	0.05	0.06	0.15	3.1 (2.8, 3.4)
Citrus fruits	0.13	0.15	**0.55**	-0.07	-0.22	-0.10	-0.03	2.8 (2.5, 3.1)
Herbal tea, fruit tea	-0.09	-0.06	**0.39**	-0.03	0.10	0.09	-0.16	2.7 (2.2, 3.2)
Chicken, turkey, duck	0.18	-0.09	-0.09	**0.64**	0.04	-0.17	-0.07	2.2 (2.0, 2.3)
Broccoli	-0.09	0.03	0.05	**0.63**	-0.05	0.10	0.13	2.2 (2.0, 2.4)
Carrots	-0.01	0.16	0.28	**0.54**	0.12	0.04	0.08	3.3 (3.0, 3.6)
Capsicum, peppers	-0.33	**0.36**	0.04	**0.46**	0.13	0.20	-0.05	2.0 (1.8, 2.2)

	Factor components and factor loadings							Frequency of consumption per week[1]
Food groupings as contained in the FeFFQ	**DP 1: Refined carbohydrate & fat**	**DP 2: Asian**	**DP 3: Healthy snacks**	**DP 4: Meat & vegetables**	**DP 5: High tea & coffee**	**DP 6: Bread & crackers**	**DP 7: Milk & yoghurt**	
Lettuce	-0.14	**0.30**	0.06	**0.45**	-0.09	**0.45**	-0.04	3.1 (2.8, 3.5)
Beef	0.28	-0.22	-0.12	**0.41**	-0.00	-0.16	-0.09	2.0 (1.8, 2.2)
Milk added to drinks[2]	0.21	0.03	-0.10	0.04	**0.77**	0.08	0.15	11.0 (10.0, 11.9)
Coffee	0.05	0.08	0.01	0.04	**0.65**	0.06	0.02	6.7 (5.9, 7.6)
Black tea	0.03	-0.01	0.14	-0.09	**0.55**	-0.02	-0.23	2.7 (2.1, 3.3)
Fruit/vegetable juices	0.13	0.02	0.03	-0.05	-0.29	0.05	-0.09	2.0 (1.7, 2.2)
White rice	0.14	**0.32**	0.07	0.02	-0.05	-0.64	-0.19	2.5 (2.1, 2.8)
Cheese	0.28	0.16	-0.05	-0.07	-0.01	**0.59**	0.01	3.0 (2.7, 3.3)
Brown bread and rolls	0.24	0.03	**0.38**	0.00	0.13	**0.46**	-0.18	5.4 (4.8, 5.9)
Crackers	0.21	0.29	0.15	0.13	-0.10	**0.38**	-0.11	2.2 (1.9, 2.5)
Milk added to food[2]	0.05	-0.10	0.01	-0.02	0.25	0.12	**0.69**	4.5 (4.0, 4.9)
Milk as a drink[2]	0.16	0.03	-0.13	0.02	-0.18	-0.20	**0.52**	2.0 (1.6, 2.4)
Yoghurt	-0.19	0.11	**0.35**	0.03	0.06	0.04	**0.43**	3.0 (2.7, 3.3)
Water	-0.17	0.11	0.23	0.17	-0.20	0.12	0.25	21.8 (20.9, 22.6)
Variance in the intake scores (%)	7.9	7.5	6.3	6.2	5.9	5.9	4.7	

[1]Frequency of consumption reported as means (95% CI); [2]Milk = cow's milk, FeFFQ - iron food frequency questionnaire; DP - dietary pattern

4.3 Dietary patterns and iron status

Table 4.3 compares the characteristics of participants with dietary pattern scores in the highest (quintile five) and lowest (quintile one) quintiles for each dietary pattern. Participants in the highest quintile for the 'meat & vegetable' dietary pattern, had significantly higher serum ferritin and haemoglobin concentrations, and were less likely to have suboptimal iron status than those in quintile one. In contrast, participants in the highest quintile for the 'milk & yoghurt' dietary pattern had a significantly lower serum ferritin concentration and greater levels of suboptimal iron status than those in quintile one. Two other patterns were also significantly associated with iron status. Participants in the highest quintile for the 'high tea and coffee' dietary pattern had significantly lower serum ferritin concentrations. In contrast, participants in the highest quintile for the 'bread & crackers' dietary pattern were less likely to have suboptimal iron status than participants in quintile one. There were no significant differences in iron status between quintiles one and five for the 'refined carbohydrate & fat', 'healthy snacks' and 'Asian' dietary patterns.

Table 4.3. Characteristics of participants with dietary pattern scores in the lowest (quintile 1) and highest (quintile 5) quintiles for the seven dietary patterns

	DP 1: Refined carbohydrate & fat		**DP 2: Asian**		**DP 3: Healthy snacks**		**DP 4. Meat & vegetables**	
	Quintile 1	**Quintile 5**	**Quintile 1**	**Quintile 5**	**Quintile 1**	**Quintile 5**	**Quintile 1**	**Quintile 5**
Age (years)	26 (21, 35)	25 (20, 37)	22 (19, 32)	26 (21, 33)*	22 (19, 31)	27 (21, 37)*	25 (20, 34)	25 (20, 35)
BMI (kg/m^2)	22 (21, 24)	23 (21, 27)	23 (21, 25)	23 (20, 26)	23 (20, 25)	22 (21, 25)	23 (21, 24)	23 (21, 27)
SF (µg/L)	40 (28, 51)	36 (21, 57)	36 (21, 57)	43 (26, 60)	34 (18, 58)	38 (21, 47)	29 (18, 42)	48 (37, 64)***
Hb (g/L)	132 (127, 138)	132(126, 137)	132 (127, 139)	133(126, 138)	131 (125, 137)	130 (126, 137)	129 (123, 133)	133 (128, 140)***
Suboptimal iron status (%)	11 (14.7)	16 (21.3)	16 (21.3)	14 (18.7)	19 (25.3)	16 (21.3)	21 (28)	5 (6.7)***
Ethnicity n (%)								
European	58 (78.4)	51 (68.0)	59 (79.7)	42 (56.0)***	58 (77.3)	47 (63.5)	54 (72)	61 (81.3)
Asian	8 (10.8)	16 (21.3)	8 (10.8)	26 (34.7)	8 (10.7)	16 (21.6)	16 (21.3)	7 (9.3)
Other	8 (10.8)	8 (10.7)	7 (9.5)	7 (9.3)	9 (12.0)	11 (14.9)	5 (6.7)	7 (9.3)

	DP 5: High tea and coffee		**DP 6: Bread & crackers**		**DP 7: Milk & yoghurt**	
	Quintile 1	**Quintile 5**	**Quintile 1**	**Quintile 5**	**Quintile 1**	**Quintile 5**
Age (years)	22 (19, 30)	34 (26, 40)***	26 (21, 34)	26 (20, 38)	28 (22, 39)	22 (19, 32)**
BMI (kg/m^2)	22 (21, 24)	23 (22, 26)	23 (20, 24)	23 (21, 27)	23 (21, 25)	22 (20, 24)
SF (µg/L)	44 (32, 74)	38 (19, 58)*	39 (18, 63)	41 (29, 58)	43 (29, 62)	33 (17, 53)*
Hb (g/L)	132 (127, 137)	131 (126, 137)	131 (125, 137)	132 (127, 138)	132 (126, 139)	131 (125, 136)

	DP 5: High tea and coffee		DP 6: Bread & crackers		DP 7: Milk & yoghurt	
	Quintile 1	**Quintile 5**	**Quintile 1**	**Quintile 5**	**Quintile 1**	**Quintile 5**
Suboptimal iron status (%)	11 (14.7)	19 (25.3)	20 (26.7)	8 (10.7)*	9 (12.0)	22 (29.3)*
Ethnicity n (%)						
European	57 (76)	61 (81.3)	29 (39.2)	62 (82.7)***	51 (68.0)	55 (74.3)*
Asian	11 (14.7)	9 (12.0)	36 (48.6)	3 (4.0)	18 (24.0)	15 (20.3)
Other	7 (9.3)	5 (6.7)	9 (12.2)	10 (13.3)	6 (8.0)	4 (5.4)

** P<0.05, ** P<0.01, *** P<0.001; Results expressed as either median (25, 75 percentile) or n (%); for each quintile, n=75; Difference between groups (independent t-test, Mann-Whitney test or chi-square test); DP - dietary pattern; SF - serum ferritin*

When all seven dietary patterns, age, ethnicity and BMI were entered into the stepwise multiple logistic regression analysis, it was found that participants following the 'meat & vegetable' dietary pattern reduced their risk of suboptimal iron status by 41% (*P*=0.002) and following a 'milk & yoghurt' dietary pattern increased the risk of suboptimal iron status by 50% (*P*=0.003) (Table 4.4). Being of Asian ethnicity almost tripled the risk of suboptimal iron status (*P*=0.002), while the risk of suboptimal iron status increased slightly with each year of age (*P*=0.003).

Table 4.4. Multiple logistic regression model for associations between dietary patterns and suboptimal iron status

		95.0% CI for exp (B)		
	Exp(B)[1]	**Lower**	**Upper**	***P*-value**
Dietary pattern 4 Meat & vegetables	.589	.422	.820	0.002
Dietary pattern 7. Milk & yoghurt	1.502	1.153	1.957	0.003
Asian ethnicity	2.961	1.509	5.811	0.002
Age (years)	1.051	1.017	1.086	0.003

[1]Change in the odds of suboptimal iron status occurring for each unit change in the predictor variable. If >1.0, as predictor variable increases, odds of sub optimal iron status increase. If <1.0, as predictor variable increases, odds of suboptimal iron status decreases; Variables included in the model: Age, ethnicity (European versus Asian; European versus other), BMI and all seven dietary patterns; R^2 = .104 (Hosmer & Lemeshow), .095 (Cox & Snell), .154 (Nagelkerke), Model χ^2 = 37.50

The results did not change when the variables that were excluded from the original stepwise multiple regression model due to non-significance (dietary patterns 'refined carbohydrate & fat', 'Asian', 'healthy snacks', 'high tea & coffee' and 'bread & crackers'; BMI; other ethnicity) were added independently to the basic model (including the 'meat & vegetable' dietary pattern, 'milk & yoghurt' dietary pattern, Asian ethnicity, age) using forced entry multiple logistic regression analysis. There was no evidence of an interaction effect between the 'meat & vegetable' and 'milk & yoghurt' dietary patterns. Furthermore, the percentage of participants who scored low (quintile one) versus high (quintile five) on the 'milk & yoghurt' pattern did not differ according to which quintile they belonged to on the 'meat & vegetable' pattern (*P*=0.473), suggesting that participants who consumed higher amounts of milk and yoghurt were doing so independently of their intake of meat and vegetables (data not shown).

4.4 Dietary practices and iron status

Analysis of the dietary practices revealed that participants with sufficient iron stores were more likely to consume milk or milk products between meals or at supper time (52.0%) than were participants with suboptimal iron status (32.9%) (*P*=0.005). There were no other significant differences in dietary practices identified between participants with sufficient versus suboptimal iron status

5 Discussion

Seven dietary patterns were identified in this population of healthy premenopausal New Zealand women. Following a 'meat & vegetable' dietary pattern was associated with a lower risk while a 'milk & yoghurt' dietary pattern was associated with an increased risk of suboptimal iron status.

The 'meat & vegetable' pattern consisted of beef, chicken, capsicum, broccoli, carrots and lettuce. Meat contains both haem and non-haem iron. Haem iron is better absorbed than non-haem iron and its absorption is less likely to be affected by a person's iron status and other dietary factors (Hallberg et al 1997, Hallberg 2002). In addition, beef and chicken contain the meat/fish/poultry factor (MFP factor) which enhances non-haem iron absorption (Hurrell et al 2006). Most cross-sectional studies have observed an increased iron status with a higher meat intake (Brussard et al 1997, Galan et al 1998, Heath et al 2001, Rangan et al 1997), although some have found no association (Pynaert et al 2009, Ruston et al 2003). In Norway, Broderstad et al (2011) found high serum ferritin concentrations to be associated with a 'reindeer meat' dietary pattern. No association was found between a dietary pattern consisting of meat and alcohol in Chinese adults, however the average daily meat consumption was low, and all those with anaemia did not necessarily have iron deficiency anaemia (Shi et al 2006). Capsicum and broccoli are good sources of ascorbic acid (Athar et al 2006), which enhances non-haem iron absorption (Diaz et al 2003).

Participants following a 'milk & yoghurt' dietary pattern (milk added to food, milk as a drink and yoghurt) had an increased risk of suboptimal iron status. Both milk and yoghurt are

high in calcium which inhibits both haem and non-haem iron absorption (Hallberg et al 1991). However, calcium's effect is less clear and weaker than that of other inhibitors of iron absorption. While some cross-sectional studies in premenopausal women have found no association between dairy product or calcium intake and iron status (Brussard et al 1997, Heath et al 2001, Pynaert et al 2009), others have found a negative association (Galan et al 1998, Rangan et al 1997, van de Vijver et al 1999). A dietary pattern consisting of drinks, milk and cake was positively associated with anaemia in Chinese adults (Shi et al 2006). However, long term calcium supplementation does not appear to affect iron status in healthy female adolescents (Molgaard et al 2005) or adults (Minehane and Fairweather-Tait 1998). Statistical analysis suggested that the 'milk & yoghurt' dietary pattern exerted an independent effect on iron status, which was not due to the replacement of meat and vegetables with milk and yoghurt, as has been suggested previously (Heath et al 2001). This was confirmed by a post hoc analysis which showed the effect of the 'milk & yoghurt' dietary pattern was still significant after controlling for frequency of meat, poultry, and fish intake entered as a single continuous variable. However, it was not possible to determine whether there were differences in the absolute intake of meat associated with the 'milk & yoghurt' dietary pattern as serving size information was not collected.

Analysis of dietary practices suggested that participants with sufficient iron stores were more likely to consume milk or milk products between meals than participants with suboptimal iron status. However, the consumption of other foods and beverages in various combinations at meals and in between meals was not associated with iron status, including consumption of milk and milk products with meals. The only study that has observed a difference in iron status when investigating consumption of beverages in relation to meals reported an increased risk when tea was consumed with main meals. However this study was undertaken in 15 institutionalised mentally handicapped women (Razagui et al 1991), whose diets are likely to be more regulated than those of women living independently. In practice, women consume a variety of foods in a variety of combinations across the day. The dietary practices questionnaire was not validated meaning we cannot be sure it measured what it was intended to measure. Furthermore, it may not have been sensitive enough and our sample size may have been too small to account for the effects of all possible combinations of foods eaten. For these reasons, it is difficult to reach a conclusion about how dietary practices may impact on iron status.

This is the first study, to our knowledge, to focus on dietary patterns and iron status in premenopausal women using at least two measures of iron status. Serum ferritin allowed us to determine whether iron deficiency was present and haemoglobin indicated the presence of anaemia (Gibson 2005). In contrast, previous studies investigating the relationship between dietary patterns and iron status did not use haemoglobin and serum ferritin in combination (Broderstad et al 2011, Shi et al 2006). As a result, the severity of iron deficiency is not known in one study (Broderstad et al 2011) and the aetiology of the anaemia in the other study is unknown and is not necessarily iron deficiency (Shi et al 2006).

We used the 30 most frequently consumed foods from the FeFFQ in the factor analysis. Food groupings were considered likely to have an impact on iron status only if they were consumed at least twice per week. It has been suggested that for most people, consuming 15-20 different foods per week should be achievable and that optimal health occurs when 30 or more biologically distinct foods are consumed per week (Hodgson et al 1994). As our FeFFQ was not restricted in the development phase to commonly consumed foods, some foods were consumed infrequently (for example, black pudding was only consumed on average 0.01 times per week), and were therefore likely to have minimal effects on iron status so were not included in the factor analysis. Foods which may contribute positively to iron status that did not appear in the 30 most frequently consumed foods included fish (consumed 1.7 times per week after combining the three categories of fish within the FeFFQ) and breakfast cereal (consumed 1.4 (muesli) to 0.1 times per week (chocolate-based cereals)). Of studies that have investigated the independent effect of fish on iron status (Asakura et al 2009, Broderstad et al 2011, Galan et al 1998), only one has observed a positive relationship between fish consumption and serum ferritin concentrations (Galan et al 1998). Cade et al (2005) found no association between serum ferritin concentrations and intake of fortified breakfast cereals.

In a separate study (Beck et al 2012b), the validity and reproducibility of the FeFFQ was investigated in 115 women, aged 18-44 years using a weighed four day food record and a FeFFQ completed on two occasions, one month apart. The FeFFQ demonstrated good validity (compared to the weighed record) and high reproducibility (compared to the second administration of the FeFFQ) for the frequency of intake of food groupings. The majority of food groupings were comparable, with only six out of 30 foods showing

meaningful differences (i.e. medium effect sizes: r>0.3 (Field 2005)) between the FeFFQ and food record. The average Spearman rank correlation coefficient was 0.54 and ranged from 0.33 to 0.84. High reproducibility was seen between the two administrations of the FeFFQ, with an average Spearman rank correlation coefficient of 0.75 (range 0.61 to 0.93), and a comparable frequency of intake (with the exception of one, all effect sizes were small: r<0.3 (Field 2005)).

The use of factor analysis to determine dietary patterns overcomes some of the limitations inherent in using food composition databases to assess nutrient intake (Gibson 2005), such as the natural variation in food composition, limited coverage of food items (requiring substitutions) or nutrients (for example, phytic acid data are not available in the New Zealand Food Composition database), and inappropriate food composition values within the database due to random and systematic errors (Gibson 2005). The use of factor analysis to determine dietary patterns does however involve several subjective decisions (Hu 2002, Newby and Tucker 2004). These include the number of food groups to enter into the factor analysis, the number of factors to extract, and interpretation of the results, including factor loadings and labelling dietary patterns (Hu 2002, Newby and Tucker 2004).

In conclusion, a 'meat & vegetable' dietary pattern was associated with lower and a 'milk & yoghurt' dietary pattern with a higher risk of suboptimal iron status independent of age, ethnicity or other dietary patterns in this group of healthy premenopausal women. These findings reinforce our knowledge of meat's beneficial effect on iron status, and provide some support for calcium (or another component in milk and yoghurt) having a negative effect on iron status in the context of the whole diet. Further research is needed to explore the relationship between iron status and intake of dairy products, with a focus on how and when these are consumed. However, as causality cannot be established in a cross-sectional study design, it is not known whether these dietary patterns caused suboptimal iron status, or whether women with suboptimal iron status were more likely to consume these dietary patterns. The use of dietary patterns to determine predictors of suboptimal iron status confirms that both enhancers and inhibitors of iron absorption influence iron status. Analysis of dietary patterns is an effective way to assess the impact of diet on iron status, and potentially provides more relevant information than individual nutrients or foods alone.

6 References

Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M *et al* (2009). Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutr* **12:** 1373-1383.

Athar N, McLaughlin J, Taylor G, Mishra S (2006). *The Concise New Zealand Food Composition Tables*, 7th edn. New Zealand Institute for Crop & Food Research Ltd and Ministry of Health: Palmerston North.

Beck K, Conlon CA, Kruger R, Coad J, Stonehouse W (2011). Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. *Br J Nutr* **105:** 101-109.

Beck KL, Conlon CA, Kruger R, Heath ALM, Matthys C, Coad J *et al* (2012a). Iron status and self-perceived health, well-being and fatigue in female university students living in New Zealand. *J Am Coll Nutr* **31:** 45-53.

Beck KL, Kruger R, Conlon C, Heath ALM, Coad J, Matthys C *et al* (2012b). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women in New Zealand. *J Acad Nutr Diet* **112:** 1177-1187.

Benkhedda K, L'Abbe MR, Cockell KA (2010). Effect of calcium on iron absorption in women with marginal iron status. *Br J Nutr* **103:** 742-748.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Brussard JH, Brants HAM, Bouman M, Lowik MRH (1997). Iron intake and iron status among adults in the Netherlands. *Eur J Clin Nutr* **51:** S51-S58.

Cade JE, Moreton JA, O'Hara B, Greenwood DC, Moor J, Burley VJ *et al* (2005). Diet and genetic factors associated with iron status in middle-aged women. *Am J Clin Nutr* **82:** 813-820.

Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP (2003). The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78:** 436-440.

Faul F, Erdfelder E, Buchner A, Lang AG (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Meth* **41:** 1149-1160.

Field A (2005). *Discovering Statistics Using SPSS*, 2nd edn. SAGE Publications Ltd: London.

Food and Nutrition Board: Institute of Medicine (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc: A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary References Intakes, and the Standing Committee on the Scientific Evaluation of Dietary References Intakes*. National Academy Press: Washington, DC.

Galan P, Yoon HC, Preziosi P, Viteri F, Valeix P, Fieux B *et al* (1998). Determining factors in the iron status of adult women in the SU.VI.MAX study. *Eur J Clin Nutr* **52:** 383-388.

Gibson RS (2005). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York.

Gleerup A, Rossander-Hulten L, Hallberg L (1993). Duration of the inhibitory effect of calcium on non-haem iron absorption in man. *Eur J Clin Nutr* **47:** 875-879.

Gleerup A, Rossander-Hulten L, Gramatkovski E, Hallberg L (1995). Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* **61:** 97-104.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Hallberg L, Hulten L, Gramatkovski E (1997). Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* **66:** 347-356.

Hallberg L, Hulthen L (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* **71:** 1147-1160.

Hallberg L (2002). Advantages and disadvantages of an iron-rich diet. *Eur J Clin Nutr* **56:** S12-S18.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Hodgson JM, Hsu-Hage B, Wahlqvist M (1994). Food variety as a quantitative descriptor of food intake. *Ecol Food Nutr* **32:** 137-148.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Hurrell RF, Reddy M, Cook JD (1999). Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br J Nutr* **81:** 289-295.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* **77:** 1213-1219.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2006). Meat protein fractions enhance nonheme iron absorption in humans. *J Nutr* **136:** 2808-2812.

Marfell-Jones M, Olds T, Stewart A, Carter L (2006). *International standards for anthropometric assessment*. ISAK: Potchefstroom.

McLean E, Cogswell M, Egli I, Woidyla D, de Benoist B (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* **12:** 444-454.

Minehane A, Fairweather-Tait S (1998). Effect of calcium supplementation on daily nonheme iron absorption and long term iron status. *Am J Clin Nutr* **68:** 96-102.

Molgaard C, Kastel P, Michaelsen KF (2005). Long-term calcium supplementation does not affect the iron status of 12-14-y-old girls. *Am J Clin Nutr* **82:** 98-102.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Pynaert I, De Bacquer D, Matthys C, Delanphe J, Temmerman M, De Backer G *et al* (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutr* **12:** 1775-1782.

Qaseem A, Aronson M, Fitterman N, Snow V, Weiss KB, Owens DK (2005). Screening or hereditary hemochromatosis: A clinical practice guideline from the American College of Physicians. *Ann Intern Med* **143:** 517-521.

Rangan AM, Aitkin I, Blight GD, Binns CW (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pac J Clin Nutr* **6:** 291-295.

Razagui IB, Barlow PJ, Izmeth MGA, Taylor KDA (1991). Iron status in a group of long-stay mentally handicapped menstruating women: some dietary considerations. *Eur J Clin Nutr* **45:** 331-340.

Russell D, Parnell WR, Wilson N, Faed J, Ferguson E, Herbison P *et al* (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*. Ministry of Health: Wellington.

Ruston D, Hoare J, Henderson L, Gregory J, Bates CJ, Prentice A *et al* (2003). *The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 4. Nutritional status (anthropometry and blood analytes), blood pressure and physical activity*. The Stationery Office: London.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG *et al* (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr* **53:** 537-541.

SPSS Inc (2009). *PASW Statistics 18, Release Version 18.0.0*. SPSS Inc: Chicago.

Umbreit JN (2005). Iron deficiency: A concise review. *Am J Hematol* **78:** 225-231.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

van de Vijver LPL, Kardinaal AFM, Charzewska J, Rotily M, Charles P, Maggiolini M *et al* (1999). Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *J Nutr* **129:** 963-968.

Wrede CE, Buettner R, Bollheimer LC, Scholmerich J, Palitzsch KD, Hellerbrand C (2006). Association between serum ferritin and the insulin resistance syndrome in a representative population. *Eur J Endocrinol* **154:** 333-340.

Zimmermann MB, Hurrell RF (2007). Nutritional iron deficiency. *Lancet* **370:** 511-520.

CHAPTER 5

Blood donation, Asian ethnicity and parity are stronger predictors of suboptimal iron status than dietary patterns in premenopausal women living in Auckland, New Zealand

Having identified dietary patterns associated with suboptimal iron status (Chapter four), it was important to determine their relative importance against other determinants (non-dietary factors) of suboptimal iron status. This study investigated the relative importance of dietary patterns and non-dietary factors (blood loss, ethnicity, medical history) and their associations with suboptimal iron status in premenopausal women.

Beck KL, Conlon C, Kruger R, Heath ALM, Matthys C, Coad J, Stonehouse W. Blood donation, Asian ethnicity and parity are stronger predictors of suboptimal iron status than dietary patterns in premenopausal women living in Auckland, New Zealand.

1 Abstract

Background: Previous research has not considered the effects of dietary patterns as well as non-dietary determinants (e.g. blood loss) on iron status in premenopausal women.

Objective: This cross-sectional study aimed to investigate the relative importance of dietary patterns as determinants of suboptimal iron status in premenopausal women living in Auckland, New Zealand.

Methods: Participants were 375 women aged 18-44 years (305 with sufficient iron status (serum ferritin (SF) 20-200µg/L, haemoglobin ≥120g/L); 70 with suboptimal iron status (SF <20µg/L)). Information on demographics, health, menstruation, blood donation, nose bleeds, and dietary patterns (using a validated iron food frequency questionnaire) was obtained.

Results: Using multiple logistic regression analysis, significant determinants of suboptimal iron status were: blood donation in the past year [odds ratio (OR): 6.74 (95% confidence interval (CI): 3.10, 14.65) *P*<0.001], being Asian [OR: 5.22 (95% CI: 2.42, 11.24) *P*<0.001], having children [OR: 2.72 (95% CI: 1.41, 5.28) *P*=0.003], previous iron deficiency [OR: 2.07 (95% CI: 1.09, 3.94) *P*=0.027], a 'milk & yoghurt' dietary pattern [OR: 1.44 (95% CI: 1.08, 1.91) *P*=0.014], and longer duration of menstrual periods [OR: 1.31 (95% CI: 1.07, 1.61) *P*=0.01]. A 'meat & vegetable' dietary pattern was associated with lower risk [OR: 0.56 (95% CI: 0.39, 0.81) *P*=0.002].

Conclusions: The strongest determinants of suboptimal iron status were blood donation and Asian ethnicity, followed by parity and previous iron deficiency. However, both dietary patterns were stronger determinants than duration of menstrual blood loss and together accounted for 5.7% of the variance in suboptimal iron status. These determinants could be important in identifying and treating women at risk of suboptimal iron status.

2 Introduction

Iron deficiency is associated with a number of health consequences including reduced work performance, impaired cognitive function and poor pregnancy outcomes (Food and Nutrition Board: Institute of Medicine 2001). In the 2008/09 New Zealand Adult Nutrition Survey, 12.1% of women aged 31 to 50 years had iron deficiency and 6.3% had iron deficiency anaemia (University of Otago and Ministry of Health 2011). In the United States, 11.0% of women aged 20-49 years were iron deficient and 4.7% had iron deficiency anaemia (Cogswell et al 2009). Many more women are at risk of iron deficiency, with a New Zealand study finding 23% of women aged 18 to 40 years had a serum ferritin (SF) <20µg/L (University of Otago and Ministry of Health 2011). Suboptimal iron status is likely to be due to a number of factors including dietary intake and blood loss (Heath et al 2001).

Most studies investigating associations between dietary intake and iron status have focussed on the effects of individual nutrients (e.g. iron) and foods (e.g. meat) (Asakura et al 2009, Brussard et al 1997, Cade et al 2005, Galan et al 1998, Harvey et al 2005, Heath et al 2001, Pynaert et al 2009, Ramakrishnan et al 2002, Rangan et al 1997). The results of these studies have not always been consistent. This may be partly explained by the fact that people do not eat foods and nutrients alone, but as meals consisting of a variety of foods and nutrients that may impact on one another (Hu 2002, Newby and Tucker 2004). For example, phytic acid decreases non-haem iron absorption (Hurrell et al 2003). The analysis of dietary patterns may overcome this problem by considering how foods are consumed in combination. In recent years, empirically derived dietary patterns have been used to assess the association between dietary intake and anaemia (Shi et al 2006) and iron status (Beck et al 2012b, Broderstad et al 2011). In Chinese men and women aged 20 years and over, 'traditional' and 'sweet' dietary patterns were positively associated with anaemia, whereas a 'healthy' dietary pattern was inversely associated with anaemia (Shi et al 2006). In Norway, a 'reindeer meat' dietary pattern has been linked to increased serum ferritin concentrations in men and women aged 36 to 79 years (Broderstad et al 2011). Previous investigations of the women in our study suggested those following a 'meat & vegetable' dietary pattern had a reduced risk of suboptimal iron status, while women following a 'milk & yoghurt' dietary pattern had an increased risk (Beck et al 2012b).

As iron status is affected by both dietary and non-dietary determinants, we investigated these in combination to determine the importance of dietary patterns in the context of other potential determinants of suboptimal iron status. This study aimed to investigate the relative importance of dietary patterns and non-dietary determinants associated with suboptimal iron status in premenopausal women living in Auckland, New Zealand.

3 Methods

The methods are described in detail in Beck et al (2012b). In brief, 404 women aged 18 to 44 years living in Auckland, New Zealand, were recruited for this cross-sectional study using a range of recruitment methods including written material (e.g. posters, flyers, email contacts, newspaper articles and advertisements) and announcements at various events (e.g. lectures, mothers groups, cooking classes, sporting events). Exclusion criteria were: current pregnancy or breast feeding, pregnancy in the past year, health problems likely to affect iron status including menorrhagia, consumption of high dose iron supplements (≥20mg elemental iron at least 3-4x/week) in the past three months, elevated iron stores (SF>200µg/L), anaemia without iron deficiency (SF≥20µg/L, haemoglobin (Hb) <120g/L), and C-reactive protein (CRP) >10mg/L as serum ferritin is an acute phase protein and is increased during inflammation and infection (Hulthen et al 1998).

Ethical approval was obtained from the Massey University Human Ethics Committee (Southern A, Reference Numbers 07/73 and 08/20) and written informed consent was obtained from all women before participation in the study.

All participants visited the Human Nutrition Research Unit (HNRU) at Massey University, Auckland. At this appointment participants were interviewed by a researcher, anthropometric measurements were taken, and a validated blood loss questionnaire (Heath et al 1998) and a validated iron food frequency questionnaire (FeFFQ) (Appendix 5) (Beck et al 2012a) were completed.

A venipuncture blood sample was taken for the determination of serum ferritin, haemoglobin and CRP at either the HNRU or at Diagnostic MedLab, Auckland. All samples were analysed by Diagnostic MedLab, Auckland, an International Accreditation New Zealand laboratory, with the methods described in Beck et al

(2012b). Women were categorised as having sufficient iron stores (SF 20-200µg/L, Hb≥120g/L) or suboptimal iron status (SF<20µg/L), including iron deficiency anaemia (SF<20µg/L, Hb<120g/L).

During the interview, questions were asked about demographics (e.g. age, ethnicity), lifestyle (e.g. parity, smoking, use and type of contraception), and medical history (e.g. illness, medication, and supplement use in the past year) (Appendix 7). Height and weight were measured using the International Society for the Advancement of Kinanthropometry standards (Marfell-Jones et al 2006) and Body Mass Index (BMI) was calculated as weight (kg)/height $(m)^2$. As blood loss is known to affect iron status (Heath et al 2001), participants completed a previously validated menstrual blood loss (MBL) questionnaire (Heath et al 1998) and questions on blood donation and nose bleeds. The questionnaire was updated to include details on sanitary items (brand and absorbency) that were not available when the questionnaire was developed, and was completed online.

All participants completed an online FeFFQ (described in detail in Beck et al (2012a)) that was designed to include foods and food groupings containing iron or factors known to affect iron absorption. The mean weekly frequency at which each food item was consumed was determined, and the 30 most frequently consumed food groupings were entered into a factor analysis to determine dietary patterns. Seven dietary patterns were identified using factor analysis, explaining 44.3% of the variance in food intake scores. These were 'refined carbohydrate & fat', 'Asian', 'healthy snacks', 'meat & vegetables', 'high tea & coffee', 'bread & crackers', and 'milk & yoghurt' dietary patterns. These patterns are explained further in Beck et al (2012b). Only the 'meat & vegetable' and 'milk & yoghurt' dietary patterns were associated with risk of suboptimal iron status (Beck et al 2012b) and therefore included in the present analysis.

All statistical analyses were performed using PASW software (SPSS Inc 2009). Two-sided tests were used for all analyses and a *P*-value of <0.05 was considered statistically significant.

Descriptive statistics including geometric mean (95% confidence interval (CI)), median (25, 75 percentile) or frequency summary statistics were used to describe the study population. Non-normally distributed variables were transformed into approximately normal distributions by logarithmic transformations and re-tested for normality. Comparisons between groups were made using the independent t-test for parametric

data, the Mann-Whitney test for non-parametric data, and the Chi-square test to investigate categorical variables.

Simple logistic regression analysis was used to investigate the following potential determinants of suboptimal iron status: age, BMI, ethnicity (European, Asian or other), being born in New Zealand, having children, blood donation in the past year, having nose bleeds, estimated MBL, duration of menstrual periods, using oral contraception, using an intra-uterine device (IUD), being a user of low dose iron supplements or multivitamins in the past year, previous iron deficiency (defined as having low iron stores, iron deficiency or iron deficiency anemia in the past), smoking, and 'meat & vegetable' and 'milk & yoghurt' dietary patterns (Beck et al 2012b). Age, BMI, estimated MBL, duration of menstrual periods, and dietary patterns were entered into the model as continuous variables. All other variables were treated as binary variables. Reference categories were the absence of the determinant, and being of European descent (for ethnicity). No significant interactions were found between potential determinants of suboptimal iron status.

Variables with a univariate *P*-value <0.20 were entered into the multiple logistic regression analysis. This value was chosen as univariate *P*-values ≥0.20 were considered unlikely to contribute to a model containing other potential determinants of suboptimal iron status. As estimated MBL was calculated using duration of menstrual periods, both variables could not be included in the multiple logistic regression analysis. Duration of menstrual period was entered into the model as it had a higher level of significance following simple logistic regression analysis (P=0.006 versus P=0.14) and has been used more often within the literature (Galan et al 1985, Heath et al 2001, Rangan et al 1997, Razagui et al 1991). Multicollinearity existed between age and having children (r=0.75 (P<0.001)), however both variables were included in the model as both could potentially contribute to suboptimal iron status. Forward stepwise multiple logistic regression with the entry criterion set at P<0.05 was used to determine which variables to include in the final multivariate model. Because 70 cases of suboptimal iron status were identified, seven determinants at most (i.e. at most one determinant for each 10 cases and 10 non-cases) could be included in the final model (Field 2005). Residual statistics (namely the standardised residual, Cook's distance, leverage and DFBeta for the constant and determinants) were examined to ensure no cases exerted an undue influence on the final logistic regression model.

4 Results

Of the 404 women recruited, a total of 375 were included in the final analysis (29 were excluded for the following reasons: elevated serum ferritin (n=2), anaemia without iron deficiency (n=16), CRP>10mg/L (n=4), and unavailable dietary data (n=4) or blood results (n=3)). Of those eligible, 305 (81%) had sufficient iron stores, and 70 (19%) had suboptimal iron status, of whom 20 (5%) had iron deficiency anaemia.

Table 5.1 shows the participants' characteristics. Women with suboptimal iron status were more likely to be older, be of Asian ethnicity, have children, have donated blood in the past year, have a longer duration of menstrual periods, have had iron deficiency previously, and were less likely to use oral contraception compared with women who had sufficient iron stores.

Table 5.1. Characteristics of study participants with and without suboptimal iron status[1]

Characteristic	Participants with sufficient iron stores[2] (n=305)	Participants with suboptimal iron status[3] (n=70)
n	305	70
Age (years)	25 (20, 35)	29 (21, 40)*
BMI (kg/m^2)[4,6]	23.2 (22.8, 23.6)	22.8 (22.1, 23.5)
SF (µg/L)	46 (34, 63)	13 (9, 17)***
Hb (g/L)	133 (128, 139)	126 (119, 132)***
Ethnicity		
European	239 (78.6)	43 (61.4)**
Asian	36 (11.8)	20 (28.6)
Other	29 (9.5)	7 (10.0)
Born in New Zealand (n (%))	177 (58.2)	35 (50.0)
Parity (n (%))	80 (26.3)	32 (45.7)**
Blood donation in the past year (n (%))	24 (7.9)	19 (27.1)***
Has nose bleeds (n (%))	30 (9.9)	8 (11.4)
Estimated MBL (blood loss units)	32 (29, 35)	38 (32, 45)
Duration of menstrual period (days)	5 (4, 5)	6 (5, 6)***
Uses oral contraception (n (%))	99 (32.7)	12 (17.1)*
Uses IUD (n (%))	17 (5.6)	2 (2.9)[4]
Uses low dose iron supplements or multivitamins in the past year (n (%))	87 (28.7)	21 (30.0)
Previous iron deficiency (n (%))	116 (38.2)	39 (55.7)*
Smoker (n (%))	21 (6.9)	3 (4.3)[4]

*[1]BMI values are geometric means (95% CI), all other results are median (25, 75 percentile) or n (%), asterisks indicate a difference between groups: *P<0.05, **P<0.01, ***P<0.001 (independent t-test, Mann-Whitney test or chi-square test); [2]SF≥20µg/L and Hb≥120g/L; [3]SF<20µg/L (Hb<120 or ≥120g/L); [4]n/a – expected count less than 5; BMI - Body mass index; Hb - Haemoglobin; IUD - Intra-uterine device; MBL - Menstrual blood loss; SF - Serum ferritin*

Nine variables with a *P*-value of <0.20 on univariate analysis were entered into the forward stepwise multiple logistic regression analysis: age, ethnicity (European versus Asian), having children, blood donation in the past year, duration of menstrual period, uses oral contraception, previous iron deficiency, 'meat & vegetable' dietary pattern, 'milk & yoghurt' dietary pattern. The following variables did not meet the screening criterion so were not included in the multiple logistic regression analysis: BMI, ethnicity (European versus other), born in New Zealand, having nose bleeds,

estimated MBL, uses IUD, uses low dose iron supplements or multivitamins in the past year, and smoking.

Donation of blood in the past year increased the risk of suboptimal iron status almost seven times, being of Asian ethnicity compared to European ethnicity increased the risk of suboptimal iron status more than five times, and having children increased the risk of suboptimal iron status by nearly three times (see Table 5.2). Having previously had iron deficiency doubled the risk of suboptimal iron status, and a longer duration of menstrual periods increased the risk of suboptimal iron status by 31%. Following a 'milk & yoghurt' dietary pattern increased the risk of having suboptimal iron status by 44%, while following a 'meat & vegetable' dietary pattern reduced the risk of having suboptimal iron status by 44%. The two dietary patterns together explained 5.7% of the variance in suboptimal iron status, compared to 17.0% for the non-dietary factors.

Table 5.2. Results of step-wise multiple logistic regression identifying predictors of suboptimal iron status[1, 3]

Variable	Exp(B)[2]	95.0% CI for Exp(B)
Blood donation in the past year	6.74	3.10, 14.65***
Asian ethnicity	5.22	2.42, 11.24***
Parity	2.72	1.41, 5.28**
Previous iron deficiency	2.07	1.09, 3.94*
'Meat & vegetable' dietary pattern	0.56	0.39, 0.81**
'Milk & yoghurt' dietary pattern	1.44	1.08, 1.91*
Duration of menstrual period	1.31	1.07, 1.61*

*[1]Asterisks indicate a contribution to the model: *P<0.05, **P<0.01, ***P<0.001; [2]Change in odds of suboptimal iron status occurring for each unit change in predictor variable. If >1.0, as predictor variable increases, odds of sub optimal iron status increase. If <1.0, as predictor variable increases, odds of suboptimal iron status decreases; [3]R^2 =0.22 (Hosmer & Lemeshow), 0.19 (Cox & Snell), 0.31 (Nagelkerke); Model χ^2 = 77.91*

5 Discussion

To our knowledge, this is the first study to investigate the role of dietary patterns as well as non-dietary factors in predicting iron status in premenopausal women. Donation of blood in the past year, being of Asian ethnicity, having had children, and having had iron deficiency in the past were all stronger determinants of suboptimal iron status than either of the dietary patterns. However, the dietary patterns were important determinants of suboptimal iron status, with the 'milk & yoghurt' pattern increasing risk

by 44%, and the 'meat & vegetable' pattern decreasing risk by 44%. Together, the dietary patterns explained 5.7% of the variance in suboptimal iron status once the non-dietary variables had been taken into account.

Blood donation in the past year was the strongest determinant of suboptimal iron status, increasing the risk of suboptimal iron status by almost seven times. This finding is in agreement with several other studies which have found blood donation to be associated with a lowered iron status in women (Brussard et al 1997, Cade et al 2005, Heath et al 2001, Pynaert et al 2009, Rangan et al 1997), with approximately 1-1.35mg of iron lost from the body per day each time blood is donated assuming six months between donations (Coad and Conlon 2011). However, the effect of blood donation on iron status may be due to a transient effect in the first few months following blood donation (Heath et al 2001). Less frequent donation of blood (currently in New Zealand, individuals can donate blood up to four times per year (New Zealand Blood Service 2010)) or discouragement of blood donation in women at risk of iron deficiency may help to reduce their risk of developing suboptimal iron status.

Being of Asian ethnicity increased the risk of suboptimal iron status by more than five times compared with being of European ethnicity. This group of Asian women was comprised of predominantly Chinese (41.8%) and Indian (29.1%) women. Most studies in premenopausal women have not investigated associations between ethnicity and iron status, possibly due to less ethnically diverse study populations. The reasons for a higher incidence of suboptimal iron status in Asian women are not known, but may be due to genetic (Whitfield et al 2003) or lifestyle factors. Another New Zealand study found Māori, Pacific Island and Asian female high school students had two to four times the risk of iron deficiency and iron deficiency anaemia compared with European high school students (Schaaf et al 2000). In the United States (NHANES III), Mexican American women were more likely to have iron deficiency anaemia than non-Hispanic white women after adjustment for iron supplement use, parity and poverty level (Frith-Terhune et al 2000). In the current study, being of 'other' ethnicity (which included women of Māori and Pacific Island descent) was not found to be significantly associated with suboptimal iron status. While we had good power for detecting large differences between women of different ethnicities, it is possible that a larger sample size would have detected small to moderate differences between other ethnic groups. More research is needed to determine why Asian women appear to be at increased risk of suboptimal iron status.

Having children significantly increased the risk of suboptimal iron status. Looker et al (1997) found an association between increased parity and iron deficiency, while other studies have found no association between having children (Heath et al 2001) or number of children (Broderstad et al 2011, Whitfield et al 2003) and iron status. The effect of age on suboptimal iron status cannot be ruled out, due to the multicollinearity that existed between age and having children. Removing the determinant 'having children' from the model for multiple logistic regression analysis resulted in age becoming a significant determinant of suboptimal iron status. This is presumably because increased age is associated with having children, as other studies in premenopausal women have not found age to be associated with iron deficiency (Heath et al 2001, Pynaert et al 2009, Rangan et al 1997).

Previous iron deficiency was found to double the risk of suboptimal iron status in this study. Heath et al (2001) found no significant differences in the rates of previous low iron status between women with mild iron deficiency and those without. Other studies in premenopausal women have not investigated associations between iron status and previous iron deficiency.

Both the 'milk & yoghurt' and 'meat & vegetable' dietary patterns remained significant determinants of suboptimal iron status after controlling for the non-dietary determinants. Following a 'milk & yoghurt' dietary pattern increased the risk of suboptimal iron status by 44% (OR 1.44), while following a 'meat & vegetable' dietary pattern reduced the risk of suboptimal iron status by 44% (OR 0.56). The odds ratios without controlling for non-nutritional determinants were very similar: 1.50 and 0.59 for the two dietary patterns respectively. Further research is needed to determine how dietary patterns related to iron status can be assessed in clinical practice, and how they can be used to inform food-based dietary guidelines.

A longer duration of bleeding at each menstrual period was associated with suboptimal iron status. Heath et al (2001) found a greater duration and extent of menstrual bleeding to be associated with mild iron deficiency (SF<20 µg/L, Hb≥120 g/L). Most (Galan et al 1985, Rangan et al 1997, Razagui et al 1991), but not all studies (Whitfield et al 2003) have shown a greater duration of menstrual period to be inversely associated with iron status. A reduced iron status has been found to be associated with both self-reported heavy menstrual blood loss (Asakura et al 2009, Whitfield et al 2003) and increased menstrual blood loss determined by direct measurement (Harvey

et al 2005). Asakura et al (2009) found a decreased risk of iron deficiency in women with irregular or no menstrual cycles.

Oral contraceptive agents are known to decrease menstrual blood loss and have been found to be positively associated with serum ferritin concentrations (Galan et al 1998), although other studies have found no significant associations with iron status (Brussard et al 1997, Galan et al 1985, Rangan et al 1997). In the present study, women with sufficient iron stores were more likely to use oral contraceptives than women with suboptimal iron status. However, when considered alongside other potential determinants of iron status, their use was not significantly associated with increased risk of suboptimal iron status. These findings are in agreement with Heath et al (2001) who found that once the extent and duration of menstrual bleeding were controlled for, there was no effect of oral contraceptive use on risk of mild iron deficiency.

This is the first study to investigate possible associations between dietary patterns and a wide range of non-dietary determinants (including blood loss and ethnicity), and iron status in premenopausal women. Unlike previous studies investigating associations between iron status and dietary patterns (Broderstad et al 2011, Shi et al 2006), we used a food frequency questionnaire validated specifically for dietary patterns (Beck et al 2012a), and iron status was determined using both serum ferritin (a measure of iron stores) and haemoglobin (to indicate anaemia) (Gibson 2005). CRP was also measured to ensure serum ferritin was not affected by infection (Hulthen et al 1998). However, as this study relied on a convenience sample of women, caution must be used when applying the results to all premenopausal women. Furthermore, the cross-sectional study design meant causality could not be determined. It is also difficult to compare the results of this study with earlier studies due to inconsistencies in study design including the indices and cut-off points used to define iron deficiency, the populations studied, and the variables investigated.

Donation of blood, being of Asian ethnicity, having had children, and having had iron deficiency in the past were all stronger determinants of suboptimal iron status than either of the dietary patterns in this population of premenopausal women. However, dietary patterns remained important determinants of suboptimal iron status after taking into account these non-dietary variables. Both dietary patterns were stronger predictors than the only significant measure of menstrual blood loss, duration of menstrual periods, and together the dietary patterns explained 5.7% of the variance in

suboptimal iron status. Further research is required on how to assess dietary patterns in clinical practice so these determinants can be used to identify and treat women at risk of suboptimal iron status.

6 References

Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M *et al* (2009). Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutr* **12:** 1373-1383.

Beck KL, Kruger R, Conlon C, Heath ALM, Coad J, Matthys C *et al* (2012a). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women in New Zealand. *J Acad Nutr Diet* **112:** 1177-1187.

Beck KL, Kruger R, Conlon C, Heath ALM, Matthys C, Coad J *et al* (2012b). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. *Eur J Nutr* **52:** 467-476.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Brussard JH, Brants HAM, Bouman M, Lowik MRH (1997). Iron intake and iron status among adults in the Netherlands. *Eur J Clin Nutr* **51:** S51-S58.

Cade JE, Moreton JA, O'Hara B, Greenwood DC, Moor J, Burley VJ *et al* (2005). Diet and genetic factors associated with iron status in middle-aged women. *Am J Clin Nutr* **82:** 813-820.

Coad J, Conlon C (2011). Iron deficiency in women: assessment, causes and consequences *Curr Opin Clin Nutr Metab Care* **14:** 625-634.

Cogswell ME, Looker AC, Pfeiffer CM, Cook JD, Lacher DA, Beard JL *et al* (2009). Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003-2006. *Am J Clin Nutr* **89:** 1334-1342.

Field A (2005). *Discovering Statistics Using SPSS*, 2nd edn. SAGE Publications Ltd: London.

Food and Nutrition Board: Institute of Medicine (2001). Iron. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. National Academy Press: Washington, DC. pp 290-393.

Frith-Terhune AL, Cogswell ME, Kettel Khan L, Will JC, Ramakrishnan U (2000). Iron deficiency anaemia: higher prevalence in Mexican American than in non-Hispanic white females in the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* **72:** 963-968.

Galan P, Hercberg S, Soustre Y, Dop MC, Dupin H (1985). Factors affecting iron stores in French female students. *Hum Nutr Clin Nutr* **39:** 279-287.

Galan P, Yoon HC, Preziosi P, Viteri F, Valeix P, Fieux B *et al* (1998). Determining factors in the iron status of adult women in the SU.VI.MAX study. *Eur J Clin Nutr* **52:** 383-388.

Gibson RS (2005). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York.

Harvey LJ, Armah CN, Dainty JR, Foxall RJ, Lewis DJ, Langford NJ *et al* (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *Br J Nutr* **94:** 557-564.

Heath AL, Skeaff CM, Gibson RS (1998). Validation of a questionnaire method for estimating extent of menstrual blood loss in young adult women. *J Trace Elem Med Biol* **12:** 231-235.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Hulthen L, Lindstedt G, Lundberg P-A, Hallberg L (1998). Effect of mild infection on serum ferritin concentration - clinical and epidemiological implications. *Eur J Clin Nutr* **52:** 376-379.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* **77:** 1213-1219.

Looker AC, Dallman PR, Carroll MD (1997). Prevalence of iron deficiency in the United States. *JAMA,* **277:** 973-976.

Marfell-Jones M, Olds T, Stewart A, Carter L (2006). *International standards for anthropometric assessment*. ISAK: Potchefstroom.

New Zealand Blood Service (2010). *All about donating blood*. New Zealand Blood Service: Auckland.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Pynaert I, De Bacquer D, Matthys C, Delanphe J, Temmerman M, De Backer G *et al* (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutr* **12:** 1775-1782.

Ramakrishnan U, Frith-Terhune A, Cogswell M, Kettel Khan L (2002). Dietary intake does not account for differences in low iron stores among Mexican American and Non-Hispanic white women: Third national health and nutrition examination survey, 1988-1994. *J Nutr* **132:** 996-1001.

Rangan AM, Aitkin I, Blight GD, Binns CW (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pac J Clin Nutr* **6:** 291-295.

Razagui IB, Barlow PJ, Izmeth MGA, Taylor KDA (1991). Iron status in a group of long-stay mentally handicapped menstruating women: some dietary considerations. *Eur J Clin Nutr* **45:** 331-340.

Schaaf D, Scragg R, Metcalf P, Grant C, Buchanan J (2000). Prevalence of iron deficiency in Auckland high school students. *N Z Med J* **113:** 347-350.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

SPSS Inc (2009). *PASW Statistics 18, Release Version 18.0.0*. SPSS Inc: Chicago.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

Whitfield JB, Treloar S, Zhu G, Powell LW, Martin NG (2003). Relative importance of female specific and non-female specific effects on variation in iron stores between women. *Br J Haematol* **120:** 860-866.

CHAPTER 6

Iron status and self-perceived health, well-being and fatigue in female university students living in New Zealand

While iron deficiency anaemia is associated with a number of health consequences, the effects of iron deficiency in the absence of anaemia on self-perceived health, well-being and fatigue remain unclear. Female university students are likely to be vulnerable to both iron deficiency and fatigue. The objective of this study was to investigate the relationship between non-anaemic iron depletion and self-perceived health, well-being and fatigue in a female university student population.

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1 Abstract

Objective: To determine the relationship between iron depletion and self-perceived health, well-being and fatigue in a female university student population living in New Zealand.

Methods: A total of 233 women aged 18-44 years studying at Massey University, Auckland, were included in this cross-sectional analysis. Serum ferritin (SF), haemoglobin (Hb) and C-reactive protein (CRP) were analysed from a venipuncture blood sample. Participants completed the SF-36v2 General Health Survey (SF-36 questionnaire) and the Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) questionnaire; and anthropometric measurements (height and weight) and data on demographics, lifestyle and medical history were obtained. Characteristics of iron sufficient (SF≥20µg/L, Hb≥120g/L) and iron depleted (SF<20µg/L, Hb≥120g/L) participants were compared, and multiple regression analyses were carried out to determine predictors of health, well-being and fatigue using a *P*-value of $P<0.01$ to indicate statistical significance because multiple comparisons were being made.

Results: There were no significant differences in self-perceived health and well-being determined using the SF-36 questionnaire between women who were iron sufficient and women who were iron depleted. Although MFSI-SF physical fatigue was significantly lower in those with iron depletion ($P=0.008$), it was not predicted by current iron status in a multivariate model controlling for factors expected to be associated with iron status and fatigue ($P=0.037$). However, smoking, a history of suboptimal iron status, and having a current medical condition were significant (negative) predictors of MFSI-SF physical fatigue, explaining 22.5% of the variance ($P<0.001$). There were no significant differences in the other measures of fatigue determined using the MFSI-SF between women who were iron sufficient and those who were iron depleted.

Conclusions: Women with iron depletion did not differ significantly from women who were iron sufficient with regard to self-perceived health, well-being or fatigue. Future studies investigating fatigue should control for previous diagnosis of suboptimal iron status, smoking and presence of a medical condition.

2 Introduction

Suboptimal iron status is common in young women with previous studies in New Zealand indicating up to 23% of women are iron depleted (serum ferritin (SF) <20µg/L, haemoglobin (Hb) ≥120g/L) (Heath et al 2001). It has been proposed that even in the absence of anaemia, iron deficiency may be associated with decreased physical work capacity (Zhu and Haas 1997), increased fatigue (Verdon et al 2003) and deficits in cognitive function (Murray-Kolb and Beard 2007), although this remains controversial. These symptoms, or iron deficiency itself, may impact on quality of life, with negative effects on perceived health, well-being and fatigue.

Perceptions of health and well-being are increasingly being recognised as important alongside more traditional measures of health such as disease prevalence. However, the effects of iron depletion in the absence of anaemia on self-perceived health and well-being remain unclear (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Mansson et al 2005, Patterson et al 2000, Patterson et al 2001, Rangan et al 1998). In previous studies investigating the relationship between iron status and quality of life, iron status has been determined using a range of biochemical indicators and cut-off points (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Mansson et al 2005, Patterson et al 2001, Rangan et al 1998) or through self-reporting of previous iron deficiency (Patterson et al 2000). In some studies, participants have known their iron status prior to completing questionnaires on health, well-being, and fatigue (Patterson et al 2000, Patterson et al 2001), which is likely to have influenced their responses.

Fatigue, defined as extreme and persistent tiredness, weakness or exhaustion (mental, physical or both) is a subjective experience (Dittner et al 2004). Fatigue is commonly reported by young women (Ridsdale et al 1993) and female students (Rangan et al 1998), and is often attributed to iron deficiency by both the medical profession and general population (Patterson et al 2000). Some studies have found a link between iron deficiency and fatigue (Patterson et al 2000, Patterson et al 2001, Verdon et al 2003), while others have found no relationship (Grondin et al 2008, Mansson et al 2005, Rangan et al 1998) so that it is not clear whether fatigue is associated with iron deficiency in the absence of anaemia (Verdon et al 2003).

Female university students may be at particular risk of iron deficiency. In one New Zealand study, 10% of first year university nutrition students were diagnosed with iron deficiency (SF<12µg/L) (Horwath 1991). Young women in general are at risk of iron deficiency due to menstrual blood loss and dietary factors such as low intakes of meat and other flesh foods (Heath et al 2001). Balancing the demands of study with part time employment, having limited finances, consuming diets low in energy (Hendricks et al 2004) and in some cases, the transition from home to independent living may lead to a poorer diet and an even greater risk of iron deficiency.

Previous studies have used a range of tools to investigate the link between iron status and health and well-being, and fatigue. The SF-36v2 Health Survey (SF-36 questionnaire) is one of the most extensively validated and used generic instruments for measuring quality of life (Contopoulos-Ioannidis et al 2009). It measures an individual's perception of their health status and functioning (Patterson et al 2000), while the vitality subscale of the SF-36 is recognised as an established measure of fatigue. The SF-36 questionnaire has been used previously to investigate the relationship between iron status and quality of life in young women, but with inconsistent results (Grondin et al 2008, Patterson et al 2000, Patterson et al 2001).

A systematic review by Whitehead (2009) found no single scale met all criteria as an ideal instrument to measure fatigue. Newer instruments such as the Multidimensional Fatigue Symptom Inventory – Short Form (MFSI-SF) consider the multiple characteristics and manifestations of fatigue (Lim et al 2005) and are more informative than scales which measure fatigue severity alone (Stein et al 1998). The MFSI-SF is increasingly being used as a measure of fatigue (Bardwell et al 2006), and demonstrates good psychometric properties (Stein et al 2004, Whitehead 2009). While the MFSI-SF has mainly been used in cancer patients, it may also be useful in healthy populations, as it contains no reference to any medical diagnosis or disease (Dittner et al 2004), nor does it assume the presence of fatigue (Stein et al 2004). It has not been used to investigate the relationship between iron status and fatigue.

The use of varying methodologies and lack of a clear consensus regarding the impact of iron deficiency on self-perceived health, well-being and fatigue suggests further investigation is required.

This study aimed to determine the relationship between iron depletion and self-perceived health, well-being and fatigue in a female university student population living in New Zealand.

3 Methods

This study was part of the Women's Iron Status and Education (WISE) Study. Potential participants were all women aged 18-44 years studying at the Massey University Campus in Auckland. Exclusion criteria were: (a) pregnancy, (b) self report of unusually high levels of fatigue on the day of testing, (c) C-reactive protein (CRP) ≥10mg/L (as SF is an acute phase protein and increases during infection or inflammation) (Hulthen et al 1998), (d) anaemia (Hb<120g/L), (e) SF>200µg/L, and (g) incomplete data or inconsistencies of responses on the SF-36 and MFSI-SF questionnaires.

We calculated that 246 participants were needed to be able to determine the prevalence of depleted iron stores to within 5% based on an expected prevalence of depleted iron stores of 20%, and a 5% significance level. Recruitment strategies (e.g. pamphlets, posters, announcements during lectures) were extensive and all potentially eligible participants were invited to take part through a centrally distributed email and information sheet. An appointment was made for students who agreed to take part. Eligible participants visited the human nutrition research unit at Massey University, Auckland, having fasted and not undertaken physical activity on the morning of the appointment. This two hour appointment included a face-to-face interview to collect demographic information (e.g. age, ethnicity, number of children), lifestyle information (e.g. smoking, use and type of contraception), medical history (e.g. medical conditions, medication and supplement use in the past year), and history of iron deficiency (including details of diagnosis, date and duration) (Appendix 7); a venipuncture blood sample; anthropometric measurements (height, weight, calculated body mass index (BMI)); and two computer-administered questionnaires (SF-36 and MFSF-SF) to assess levels of self-perceived health, well-being and fatigue. Breakfast was provided for participants following collection of the blood sample and prior to completing the online questionnaires. Ethical approval was obtained from the Massey University Human Ethics Committee: (Southern A), Reference No. 07/73. All participants provided written informed consent.

3.1 Blood sampling and biochemical analysis to determine iron status

A venipuncture blood sample was taken by a trained phlebotomist between 7.00am and 10.00am to minimise diurnal variation. Four millilitres of blood were collected into an ethylenediaminetetraacetic acid (EDTA) tube for analysis of Hb and 3.5mL of blood was collected into a serum separator tube (SST) for the analysis of SF and CRP (Becton Dickinson, Plymouth, UK). Samples were refrigerated at 4ºC and sent to Diagnostic MedLab (Auckland) on the same day for analysis.

Serum ferritin was analysed using the immunoturbidimetric test (Roche Diagnostics, Indianapolis) (Cat. No. 11661400) and CRP using the particle enhanced immunoturbidimetric assay (Roche Diagnostics, Indianapolis) (Cat. No. 03002039). Haemoglobin was analysed using the SLS-Hb (sodium lauryl sulphate-Hb) method using an automated hematology analyser XE-2100 (Sysmex Corporation, Auckland, NZ). Diagnostic MedLab is an International Accreditation New Zealand medical testing laboratory.

3.2 Questionnaires on self-perceived health, well-being and fatigue

The SF-36 questionnaire is a subjective tool that focuses on an individual's perceived health and well-being over the previous four weeks. It contains 36 items scored as eight multi-item scales (physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems, and mental health), scored from zero to 100. These scales are combined to produce the physical component summary score and mental component summary score. The physical component score and mental component score are standardised with a score below 50 indicating poorer physical or mental health, and a score above 50 indicating better health than the 1998 United States (US) general population (non-institutionalised males and females, 18-96 years) (Ware et al 2007). The vitality scale includes four items which ask participants to indicate the extent to which they have felt "energetic" versus "tired" and "worn out".

The MFSI-SF is a subjective tool that measures multiple characteristics and manifestations of fatigue during the previous week. The MFSI-SF consists of 30 items which produce five subscales, each derived from six items (Stein et al 2004). Each

scale produces a score ranging from zero to 24. Higher scores on the MFSI-SF subscales other than vigour are indicative of more fatigue. The first four subscales (general fatigue, physical fatigue, emotional fatigue and mental fatigue) are summed and the fifth subscale (vigour) score is subtracted from these to obtain a total fatigue score (ranges from -24 to 96).

3.3 Statistical analysis

Statistical analysis was performed using SPSS for Windows Version 16 (SPSS Inc 2007). The population was described by using mean (95% confidence intervals) for normally distributed data, median (25, 75 percentile) for non-normally distributed data, or frequency summary statistics for categorical data. Normality of distribution was evaluated using the Kolmogorov-Smirnov test, Shapiro-Wilk Test and normality plots.

Women were divided into those who were iron sufficient (SF≥20µg/L, Hb≥120g/L), and those who were iron depleted (SF<20µg/L, Hb≥120g/L). Iron deficiency anaemia was defined as SF<20µg/L and Hb<120g/L.

Comparisons between subjects with iron depletion and subjects who were iron sufficient were made using the independent t-test for parametric data, the Mann-Whitney test for non-parametric data, and the chi-square test for categorical variables. Two-sided tests were used for all variables. Where $P<0.05$ for comparisons between the iron depleted and iron sufficient for the SF-36 and MFSI-SF questionnaire sub scales (i.e. SF-36 physical functioning, MFSI-SF general fatigue, MFSI-SF physical fatigue, MFSI-SF total fatigue), the subscales were further explored using multiple regression analysis.

The independent variables for the multiple regression analyses were: iron depletion, history of suboptimal iron status, age, current medical condition, ethnicity (European, Asian, Other), cigarette smoking, BMI, use of oral contraceptive agent, use of intra-uterine device, having children, and regular use (>3-4x per week in the past three months) of iron or multivitamin supplements. Age and BMI were entered into the model as continuous variables. All other variables were entered as dichotomous variables. The variables were analysed using forced entry multiple regression analysis. The sample size was adequate for the number of predictors included to see a medium size effect (Field 2005). Assumptions for multicollinearity were met. The

residuals were normally distributed and independent for the models which included MFSI-SF general fatigue, MFSI-SF physical fatigue, and MFSI-SF total fatigue. For the model containing SF-36 physical functioning as the dependent variable, the residuals were not normally distributed. However, all outliers had a Cook's distance <1, and the final results were not strongly affected by these outliers. Variance was constant for the MFSI-SF total and general fatigue models. For the SF-36 physical functioning and the MFSI-SF physical fatigue scales, there was mild heteroscedasticity because participants were grouped at the upper and lower end of the scales respectively. Significance levels were set at the stricter level of $P<0.01$ because multiple comparisons were being made.

4 Results

4.1 Participant characteristics

Two hundred and eighty three women enrolled to take part in the WISE Study. Four women were excluded due to pregnancy and three due to having a CRP≥10mg/L. Of the remaining 276 women, a further 43 were excluded from the analysis (self-report of unusually high levels of fatigue on the day of testing (n=2), iron deficiency anaemia (n=8; 2.9%), anaemia without iron deficiency (n=12; 4.3%), SF>200µg/L (n=2; 0.7%), incomplete data from the SF-36 and MFSI-SF questionnaires (n=12) and inconsistencies of responses in the SF-36 questionnaire (n=7)). Of the final 233 women included in investigations, 211 (90.6%) were iron sufficient and 22 (9.4%) were iron depleted.

The characteristics of the participants, including their scores from the SF-36 and MFSI-SF questionnaires can be seen in Table 6.1. The majority (73.8%) of participants were of European descent (Asian, 16.7%; Māori, 6.0%; Pacific Island, 1.3%; Other, 2.1%). Eighteen (7.7%) were smokers, 14.2% had a current medical condition, and 12.4% had children. Of the 39.9% of women using contraception the majority (84.9%), used an oral contraceptive method, and 9.7% used an intra-uterine device. The majority of students (40.4%) were living with their parents, 26.8% with a partner or own family, 23.7% shared accommodation with others, 7.0% lived in university accommodation (e.g. halls of residence, home stay) and only 2.2% lived alone. Thirty three (14.2%) women regularly consumed iron, or multivitamin and mineral, supplements.

Table 6.1. Characteristics of participants and results of the SF-36 and MFSI-SF

Characteristic (n=233)	**Median (25, 75 percentile)[1]**
Age (y)	22 (19, 28)
Body mass index (kg/m^2)	22.2 (20.4, 24.4)
Serum ferritin (µg/L)	44 (32, 66)
Haemoglobin (g/L)[1]	134 (133, 135)
SF-36 Physical component score[2]	54.4 (50.6, 58.0)
SF-36 Mental component score	45.8 (37.3, 51.2)
SF-36 Physical functioning	54.9 (52.8, 57.0)
SF-36 – Role physical	54.4 (47.1, 56.9)
SF-36 – Bodily pain	53.7 (46.1, 55.4)
SF-36 – General health	52.0 (43.4, 57.7)
SF-36 – Vitality[1]	46.8 (45.6, 48.0)
SF-36 – Social functioning	45.9 (40.5, 56.8)
SF-36 – Role emotional	48.1 (36.4, 55.9)
SF-36 – Mental health	50.0 (41.6, 52.8)
MFSI-SF – General	9.0 (5.0, 14.0)
MFSI-SF – Physical	3.0 (1.0, 6.0)
MFSI-SF – Emotional	5.0 (3.0, 8.0)
MFSI-SF – Mental	5.0 (3.0, 8.5)
MFSI-SF – Vigour[1]	11.8 (11.3, 12.4)
MFSI-SF – Total	10.0 (0.0, 25.0)

[1] Hb, SF-36-Vitality and MFSI-SF-Vigour reported as means (95% confidence interval); [2]SF-36 scores are norm based scores; SF-36 - SF-36v2 Health Survey; MFSI-SF - Multidimensional Fatigue Symptom Inventory – Short Form

4.2 Current iron status and self-perceived health and well-being

There were no significant differences for the physical component score, mental component score and other scales of the SF-36 questionnaire between women who were iron sufficient and those who were iron depleted (Table 6.2). Both groups of women had better physical component scores (median = 54.4), but poorer mental component scores (median = 45.8) than the general population (i.e. US non-institutionalised male and females, 18-96 years), who have a norm based score of 50

(Ware et al 2007). Only the SF-36 physical functioning scale met the screening criterion of $P<0.05$ for the comparison between iron sufficient and iron depleted women (Table 6.2) and so was investigated further to determine the extent to which it was associated with iron status.

Table 6.2. Participant characteristics for women with iron sufficiency or iron depletion[1]

	Iron sufficient[2] (n=211)	**Iron depletion[3] (n=22)**	***P*-value**
Age (y)	23 (20, 28)	21 (18, 24)	0.056
Body mass index (kg/m^2)	22.3 (20.4, 24.6)	21.2 (20.2, 22.7)	0.065
Serum ferritin (µg/L)	47 (35, 69)	13 (9, 16)	<0.001
Haemoglobin (g/L)[4]	135 (134, 136)	131 (127, 134)	0.027
Cigarette smoker (n (%))	16 (7.6)	2 (9.1)	n/a
Medical condition (n (%))	32 (15.2)	1 (4.5)	n/a
Ethnicity(n(%)):			
European	160 (75.8)	12 (54.5)	
Asian	30 (14.2)	9 (40.9)	
Other	21 (10.0)	1 (4.5)	n/a
History of iron deficiency (n (%))	75 (35.5)	5 (22.7)	0.251
Has children (n (%))	29 (13.7)	0 (0)	n/a
Uses oral contraception (n (%))	74 (35.1)	5 (22.7)	0.178
Uses intra-uterine device (n (%))	9 (4.3)	0 (0.0)	n/a
SF-36 – Physical component score[5]	54.3 (50.4, 58.0)	55.1 (51.6, 59.3)	0.594
SF-36 – Mental component score	46.2 (38.1, 51.2)	43.6 (32.4, 49.9)	0.330
SF-36 – Physical functioning	54.9 (52.8, 57.0)	53.9 (48.6, 57.0)	0.041
SF-36 – Role physical	54.4 (47.1, 56.9)	50.7 (46.4, 56.9)	0.313
SF-36 – Bodily pain	51.1 (46.1, 55.4)	55.4 (51.1, 56.0)	0.101
SF-36 – General health	50.6 (43.4, 57.7)	52.9 (45.8, 55.3)	0.878
SF-36 – Vitality	49.0 (39.6, 52.1)	45.8 (38.8, 53.7)	0.979
SF-36 – Social functioning	51.4 (40.5, 56.8)	45.9 (35.0, 56.8)	0.469
SF-36 – Role emotional	48.1 (36.4, 55.9)	44.2 (32.6, 52.0)	0.202
SF-36 – Mental health	50.0 (41.6, 52.8)	47.2 (35.9, 53.5)	0.329
MFSI-SF – General	9.0 (5.0, 14.0)	6.0 (4.0, 10.0)	0.029
MFSI-SF – Physical	3.0 (1.0, 6.0)	1.0 (0.0, 3.0)	0.008
MFSI-SF – Emotional	5.0 (3.0, 8.0)	4.0 (3.0, 6.5)	0.401
MFSI-SF – Mental	5.0 (3.0, 9.0)	4.0 (3.0, 7.3)	0.498
MFSI-SF – Vigour	12.0 (9.0, 15.0)	13.0 (8.8, 16.0)	0.453
MFSI-SF – Total	11.0 (0.0, 25.0)	3.5 (-2.3, 14.3)	0.017

[1]Difference between groups (independent t-test, Mann-Whitney test or chi-square test); [2]SF≥20 µg/L and Hb≥120 g/L; [3]SF<20 µg/L and Hb ≥120g/L; [4]Hb reported as mean (95% confidence interval), all other results expressed as median (25, 75 percentile) or n (%); [5]SF-36 scores are norm based scores; n/a – expected count less than 5; SF-36 - SF-36v2 Health Survey; MFSI-SF - Multidimensional Fatigue Symptom Inventory – Short Form

In the multivariate model, a higher BMI (*P*=0.002) and being of Asian ethnicity (*p*=0.001) were the only significant predictors of poorer SF-36 physical functioning, explaining 4.2% and 3.4% of the variance respectively. There was a tendency for iron depletion to be negatively associated with SF-36 physical functioning, but this was not statistically significant (*P*=0.035) (Table 6.3).

Table 6.3. Multiple regression model to determine factors associated with SF-36 Physical Functioning

SF-36 Physical Functioning scale	***β*[1]**	**99% CI *β***	**Standardised *β*[2]**	***P*-value**
Ethnicity (Asian *vs.* European)	-3.14	-5.60, -0.67	-0.23	0.001
Ethnicity (Other *vs.* European)	-0.09	-3.13, 2.96	-0.01	0.940
Body Mass Index	-0.26	-0.48, -0.05	-0.22	0.002
History of suboptimal iron status	-1.75	-3.59, 0.09	-0.16	0.015
Currently iron depleted	-2.42	-5.35, 0.52	-0.14	0.035
Has a current medical condition	-0.98	-3.46, 1.49	-0.07	0.305
Oral contraceptive agent user	0.62	-1.31, 2.55	0.06	0.406
Intra-uterine device user	-0.46	-4.95, 4.04	-0.02	0.793
Regular user of iron or multivitamin supplement	0.61	-1.81, 3.03	0.04	0.514
Has children	-0.30	-3.98, 3.38	-0.02	0.832
Current smoker	-0.20	-3.35, 2.96	-0.01	0.871
Age	0.01	-0.17, 0.18	0.01	0.916
R^2=0.149 (Adjusted R^2=0.103); Regression *p* <0.001				

[1]Change in the SF-36 physical functioning scale for each unit change in the predictor variable; [2]Number of standard deviations (SD) the SF-36 physical functioning scale will change with a one standard deviation (SD) change in the predictor variable; Positive β and standardised β values are associated with higher SF-36 Physical Functioning; A P-value <0.01 is considered statistically significant; SF-36 - SF-36v2 Health Survey

4.3 Current iron status and fatigue

Women with iron depletion reported lower levels of physical fatigue on the MFSI-SF questionnaire (*P*=0.008) (Table 6.2). However, being iron depleted was not a significant predictor of MFSI-SF physical fatigue (*P*=0.037) in the multiple regression model depicted in Table 6.4a. Having a history of iron deficiency (*P*<0.001), being a smoker (*P*<0.001), and having a current medical condition (*P*=0.006) were significant predictors of MFSI-SF physical fatigue, explaining 8.5%, 5.8% and 5.2% of the variance in MFSI-SF physical fatigue respectively.

Two other fatigue-related outcomes met the screening criteria and so were investigated further to determine the extent to which they were associated with iron status: MFSI-SF general fatigue, and MFSI-SF total fatigue. The MFSI-SF general fatigue scale produced a non-significant model (*P*=0.015; R^2=0.105; data not presented here). The only significant predictor of MFSI-SF total fatigue was smoking (Table 6.4b), which explained 3.7% of the variance (*P*=0.004). Current iron status was not associated with MFSI-SF total fatigue (*P*=0.084).

Table 6.4a. Multiple regression model to determine factors associated with MFSI-SF physical fatigue

MFSI-SF physical fatigue scale	***β*[1]**	**99% CI *β***	**Standardised *β*[2]**	***P*-value**
Current smoker	3.01	0.89, 5.14	0.22	<0.001
History of suboptimal iron status	2.08	0.84, 3.32	0.28	<0.001
Has a current medical condition	1.79	0.13, 3.46	0.17	0.006
Ethnicity (Asian *vs.* European)	1.45	-0.21, 3.11	0.15	0.025
Ethnicity (Other *vs.* European)	0.22	-1.83, 2.27	0.02	0.782
Currently iron depleted	-1.61	-3.58, 0.37	-0.13	0.037
Body Mass Index	0.11	-0.04, 0.25	0.13	0.055
Oral contraceptive agent user	0.68	-0.62, 1.98	0.09	0.178
Intra-uterine device user	1.36	-1.67, 4.39	0.07	0.249
Age	-0.04	-0.16, 0.08	-0.08	0.358
Has children	0.39	-2.09, 2.87	0.04	0.686
Regular user of iron or multivitamin supplement	0.06	-1.57, 1.69	0.01	0.922
R^2=0.225 (Adjusted R^2=0.183), Regression *P*<0.001				

[1]Change in the MFSI-SF physical fatigue scale for each unit change in the predictor variable; [2]Number of standard deviations (SD) the MFSI-SF physical fatigue scale will change with a one standard deviation (SD) change in the predictor variable; Positive β and standardised β values are associated with higher MFSI-SF scores; higher scores on the MFSI-SF physical fatigue scale is indicative of more fatigue; A P-value <0.01 is considered statistically significant; MFSI-SF - Multidimensional Fatigue Symptom Inventory – Short Form

Table 6.4b. Multiple regression model to determine factors associated with MFSI-SF total fatigue

MFSI-SF total fatigue scale	***β*[1]**	**99% CI *β***	**Standardised *β*[2]**	***P*-value**
Current smoker	11.73	1.30, 22.15	0.19	0.004
History of suboptimal iron status	6.11	0.03, 12.20	0.18	0.010
Has a current medical condition	6.52	-1.65, 14.68	0.14	0.041
Currently iron depleted	-6.54	-16.24, 3.17	-0.12	0.084
Ethnicity (Asian *vs.* European)	1.67	-6.47, 9.81	0.04	0.201
Ethnicity (Other *vs.* European)	-5.00	-15.06, 5.06	-0.09	0.597
Intra-uterine device user	4.49	-10.36, 19.35	0.05	0.436
Oral contraceptive agent user	-0.28	-6.65, 6.10	-0.01	0.911
Age	-0.09	-0.67, 0.48	-0.04	0.674
Body Mass Index	0.11	-0.61, 0.83	0.03	0.691
Regular user of iron or multivitamin supplement	0.76	-7.24, 8.75	0.02	0.808
Has children	-1.09	-13.25, 11.07	-0.02	0.817
R^2=0.122 (Adjusted R^2=0.074), Regression *P*=0.004				

[1]Change in the MFSI-SF total fatigue scale for each unit change in the predictor variable; [2]Number of standard deviations (SD) the MFSI-SF total fatigue scale will change with a one standard deviation (SD) change in the predictor variable; Positive β and standardised β values are associated with higher MFSI-SF scores; higher scores on the MFSI-SF total fatigue scale is indicative of more fatigue; A P-value <0.01 is considered statistically significant; MFSI-SF - Multidimensional Fatigue Symptom Inventory – Short Form

5 Discussion

There were no significant differences in self-perceived health, well-being and fatigue between iron sufficient and iron depleted women participating in this study. This finding is in keeping with most studies using validated scales, in which women were unaware of their iron status, and in which the majority of participants did not have iron deficiency anaemia (Duport et al 2003, Fordy and Benton 1994, Grondin et al 2008, Rangan et al 1998). Using the General Health Questionnaire, no relationship was found between iron status and psychological distress in female students (Fordy and Benton 1994, Rangan et al 1998) unless they were anaemic (Rangan et al 1998). No association has been observed between iron status and quality of life determined using the Duke Profile in menstruating women (Duport et al 2003). Similarly, Grondin et al (2008) found no significant difference in the physical component score, mental component score and vitality scores from the SF-36 between iron deficient and iron sufficient French female students, although general health was significantly lower in iron deficient students. However, in this study, haemoglobin concentrations (anaemia) were investigated only in some participants, some of whom had iron deficiency anaemia (Grondin et al 2008). In student populations, self-reported fatigue has not been found to be associated with iron deficiency (Ballin et al 1992, Fordy and Benton 1994, Mansson et al 2005, Rangan et al 1998).

In contrast, Patterson et al (2001) found mental component and vitality scores measured using the SF-36 questionnaire were lower, and fatigue measured using the Piper Fatigue Scale (PFS) (Piper et al 1989) was higher for iron deficient women than iron sufficient women at baseline of an intervention study. However, the women were aware of their iron status prior to completing the PFS scales, and the PFS assumes the presence of fatigue, both of which may have influenced their responses. In addition, the iron deficient group included women with iron deficiency anaemia (Hb 90-120g/L). The SF-36 mental component and vitality scores, and fatigue (PFS) improved on treatment with diet or iron supplements. However, no blinding was used, a placebo group was not included, and similar improvements were seen in the two treatment groups for the mental component score and vitality scores on the SF-36 questionnaire, and for the PFS, in spite of larger increases in serum ferritin in the supplement group. Furthermore, the improvements seen in vitality in the supplement group and PFS scores in the diet and supplement group were no longer significant when women with anaemia were excluded. In a double blind randomised trial, Verdon

et al (2003) found fatigue in women with an Hb>117g/L improved following four weeks of iron supplementation (measured on a visual analogue scale). However, it is unclear whether haemoglobin concentration improved (i.e. some participants may have had anaemia) and participants may have known which group they were in due to the side effects of iron supplementation.

In the present study, a history of suboptimal iron status was associated with reporting significantly higher fatigue, explaining 8.5% of the variation in MFSI-SF physical fatigue. In fact, when a history of iron deficiency was controlled for, current iron status no longer predicted physical fatigue (*P*=0.037). This is in keeping with the findings of the Australian Longitudinal Study on Women's Health which found that women who reported (ever) having had low iron levels diagnosed by a doctor reported a greater prevalence of constant tiredness and significantly lower vitality score on the SF-36 questionnaire than women without a history of iron deficiency (Patterson et al 2000). A women's knowledge of whether she has had previous iron deficiency may impact on how fatigued she feels. Kallich et al (2006) found subject knowledge of haemoglobin concentrations had a modest association with aspects of health related quality of life. Alternatively, women who have had symptoms, such as fatigue may be more likely to have had their iron status checked, and are therefore more likely to have a previous diagnosis of iron deficiency.

Being a smoker and having a current medical condition were also associated with greater fatigue in our study suggesting that future studies investigating fatigue should control for previous diagnosis of suboptimal iron status, smoking, and having a medical condition.

The prevalence of iron depletion and iron deficiency (SF<20µg/L, Hb≥120 g/L) (8.7%) in this group of students was considerably lower than other New Zealand studies (15.6% (Fawcett et al 1998) and 23% (Heath et al 2001)) and studies in female university students overseas (30.7% (Grondin et al 2008), 34% (SF<15µg/L) (Houston et al 1997), and 19.8% (Rangan et al 1997)). The prevalence of iron deficiency anaemia (2.9%) was slightly higher than in other New Zealand studies (1-2.2%) (Fawcett et al 1998, Heath et al 2001, Russell et al 1999), but lower than in studies of female students living in other countries (4.5-6%) (Houston et al 1997, Rangan et al 1997).

Female university students in this study had better physical health but poorer mental health scores than the US general population (non-institutionalised males and females, 18-96 years) (Ware et al 2007). Their physical component score from the SF-36 questionnaire of 54.4 was similar to the reference population (females aged 18-44 years from the US general population (53.9-55.2)) (Ware et al 2007), and slightly higher than that of females aged 15-24 and 25-44 years in the 1996/1997 New Zealand Health Survey (52.2 and 52.3 respectively) (Ministry of Health 1999), and French female secondary and university students (52.5-53.0) (Grondin et al 2008). Their SF-36 questionnaire mental component score was lower (45.8) than the reference population (48.3-50.2) (Ware et al 2007), similar to females aged 15-24 and 25-44 years living in New Zealand (46.1 and 47.7 respectively), (Ministry of Health 1999), but higher than that of French female secondary and university students (35.8-40.5) (Grondin et al 2008). The SF-36 vitality score (46.8) was similar to the reference population (45.9-49.0) (Ware et al 2007).

Individual MFSI-SF scale scores showed greater levels of fatigue across all dimensions in this student population compared with healthy participants (Lim et al 2005, Stein et al 1998), and similar levels of fatigue to breast cancer patients undergoing or having recently completed treatment (Stein et al 1998). It appears that female students may be more tired compared with the general female population. However, self-reported fatigue may have been influenced by response bias. Women may have been drawn to the study by recruitment strategies linking iron status and fatigue. The motivation to participate may have been higher for women experiencing fatigue or for women who had experienced iron deficiency previously. Current iron status was not an important predictor of fatigue in this study.

This study had several strengths. The SF-36 questionnaire has been extensively validated, and the MFSI-SF measures multiple aspects of fatigue without assuming the presence of fatigue. The current study investigated both history of iron deficiency and biochemical analysis of current iron status, and included CRP to ensure that serum ferritin was not confounded by infection. Participants were unaware of their iron status when completing the SF-36 and MFSI-SF. A limitation in comparing iron sufficient women to iron depleted women was the small number of participants who were iron depleted (n=22), making it more difficult to detect a small difference in our outcomes. There is currently no agreement on what would constitute a clinically important difference in SF-36 scores, with differences of 2.1 (Patterson et al 2000) to approximately 10 (Ware et al 2007) having been suggested. These estimates would

require sample sizes of 5 to 408. Although there is a paucity of data on the magnitude of clinically important MFSI-SF scores, the component scores of the MFSI-SF are consistently in a direction suggesting less fatigue in iron depletion making it unlikely that a strong negative effect of iron depletion on fatigue would be found with a larger sample size. It is possible that there may have been a lag time between the onset of iron depletion and functional effects which this cross-sectional study did not detect. Due to possible recruitment bias (as discussed earlier) conclusions cannot be drawn regarding the prevalence of fatigue in this population. Additionally we relied on a convenience sample of students, so it is not clear how these results might apply to all female students or to the general female population.

In conclusion, having depleted iron stores did not appear to be associated with self-perceived health, well-being or fatigue, although a history of iron deficiency, smoking and having a medical condition explained almost one fifth of the variance in MFSI-SF physical fatigue. Future studies investigating the associations between fatigue and other conditions, such as iron status, should consider a women's perception of the link between her being iron deficient previously, and how fatigued she feels. Prospective studies in larger, representative samples of young women are needed to investigate the effects of iron status on self-perceived health and well-being, and fatigue. Participants should remain unaware of their iron status during testing.

6 References

Ballin A, Berar M, Rubinstein U, Kleter Y, Hershkovitz A, Meytes D (1992). Iron state in female adolescents. *Am J Dis Child* **146:** 803-805.

Bardwell WA, Burke SC, Thomas KS, Carter C, Weingart K, Dimsdale JE (2006). Fatigue varies by social class in African Americans but not Caucasian Americans. *Int J Behav Med* **13:** 252-258.

Contopoulos-Ioannidis DG, Karvouni A, Kouri I, Ioannidis JPA (2009). Reporting and interpretation of SF-36 outcomes in randomised trials: systematic review. *Br Med J* **338:** 1-6.

Dittner AJ, Wessely SC, Brown RG (2004). The assessment of fatigue - A practical guide for clinicians and researchers. *J Psychosom Res* **56:** 157-170.

Duport N, Preziosi P, Boutron-Ruault MC, Bertrais S, Galan P, Favier A *et al* (2003). Consequences of iron depletion on health in menstruating women. *Eur J Clin Nutr* **57:** 1169-1175.

Fawcett JP, Brooke M, Beresford CH (1998). Iron deficiency and anaemia in a longitudinal study of New Zealanders at ages 11 and 21 years. *N Z Med J* **111:** 400-402.

Field A (2005). *Discovering Statistics Using SPSS*, 2nd edn. SAGE Publications Ltd: London.

Fordy J, Benton D (1994). Does iron status influence psychological functioning? *J Hum Nutr Diet* **7:** 127-133.

Grondin M-A, Ruivard M, Perreve A, Derumeaux-Burel H, Perthus I, Roblin J *et al* (2008). Prevalence of iron deficiency and health-related quality of life among female students. *J Am Coll Nutr* **27:** 337-341.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Hendricks KM, Herbold N, Fung T (2004). Diet and other lifestyle behaviors in young college women. *Nutr Res* **24:** 981-991.

Horwath CC (1991). Dietary intake and nutritional status among university undergraduates. *Nutr Res* **11:** 395-404.

Houston MS, Summers SL, Soltesz KS (1997). Lifestyle and dietary practices influencing iron status in university women. *Nutr Res* **17:** 9-22.

Hulthen L, Lindstedt G, Lundberg P-A, Hallberg L (1998). Effect of mild infection on serum ferritin concentration - clinical and epidemiological implications. *Eur J Clin Nutr* **52:** 376-379.

Kallich J, McDermott A, Xu X, Fayers P, Cella D (2006). The relationship between patient knowledge of hemoglobin levels and health-related quality of life. *Qual Life Res* **15:** 57-68.

Lim WJ, Hong S, Nelesen R, Dimsdale JE (2005). The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. *Arch Intern Med* **165:** 910-915.

Mansson J, Johansson G, Wiklund M, Baigi A, Marklund B (2005). Symptom panorama in upper secondary school students and symptoms related to iron deficiency - Screening with laboratory tests, questionnaire and interventional treatment with iron. *Scand J Prim Health Care* **23:** 28-33.

Ministry of Health (1999). *Taking the Pulse - The 1996/97 New Zealand Health Survey*. Ministry of Health: Wellington.

Murray-Kolb LE, Beard JL (2007). Iron treatment normalizes cognitive functioning in young women. *Am J Clin Nutr* **85:** 778-787.

Patterson A, Brown W, Powers J, Roberts D (2000). Iron deficiency, general health and fatigue: Results from the Australian longitudinal study on women's health. *Qual Life Res* **9:** 491-497.

Patterson AJ, Brown WJ, Roberts DCK (2001). Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. *J Am Coll Nutr* **20:** 337-342.

Piper B, Lindsey A, Dodd M, Ferketich S, Paul S, Weller S (1989). Development of an instrument to measure the subjective dimension of fatigue. In: Funk S, Tournquist E, Champagne M, Copp L, Weise R (eds). *Key Aspects of Comfort: Management of Pain, Fatigue and Nausea*. Springer: New York. pp 199-208.

Rangan AM, Aitkin I, Blight GD, Binns CW (1997). Factors affecting iron status in 15-30 year old female students. *Asia Pac J Clin Nutr* **6:** 291-295.

Rangan AM, Blight GD, Winns CW (1998). Iron status and non-specific symptoms of female students. *J Am Coll Nutr* **17:** 351-355.

Ridsdale L, Evans A, Jerrett W, Mandalia S, Osler K, Vora H (1993). Patients with fatigue in general practice - a prospective study. *Br Med J* **307:** 103-106.

Russell D, Parnell WR, Wilson N, Faed J, Ferguson E, Herbison P *et al* (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*. Ministry of Health: Wellington.

SPSS Inc (2007). *SPSS for Windows, release 16.0*. SPSS Inc: Chicago.

Stein KD, Martin SC, Hann DM, Jacobsen PB (1998). A multidimensional measure of fatigue for use with cancer patients. *Cancer Pract* **6:** 143-152.

Stein KD, Jacobsen PB, Blanchard CM, Thors C (2004). Further validation of the multidimensional fatigue symptom inventory-short form. *J Pain Symptom Manage* **27:** 14-24.

Verdon F, Burnand B, Stubi CLF, Bonard C, Graff M, Michaud A *et al* (2003). Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *Br Med J* **326:** 1124-1127.

Ware JE, Kosinski M, Bjorner JB, Turner-Bowker DM, Gandek B, Maruish ME (2007). *User's Manual for the SF-36v2^{TM} Health Survey*, 2nd edn. QualityMetric Incorporated: Lincoln, Rhode Island.

Whitehead L (2009). The Measurement of Fatigue in Chronic Illness: A Systematic Review of Unidimensional and Multidimensional Fatigue Measures. *J Pain Symptom Manage* **37:** 107-128.

Zhu YI, Haas JD (1997). Iron depletion without anemia and physical performance in young women. *Am J Clin Nutr* **66:** 334-341.

CHAPTER 7

Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial

A food-based approach to improving iron deficiency offers a number of potential benefits. While it is well established that ascorbic acid enhances non-haem iron absorption, the effects of consuming ascorbic acid on iron status over the longer-term are less clear. This study investigated the effect of consuming an iron-fortified breakfast cereal with gold kiwifruit (high ascorbic acid, lutein, zeaxanthin-rich fruit) compared to a banana (low ascorbic acid, lutein, zeaxanthin) on iron status in women with low iron stores.

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1 Abstract

Background: Ascorbic acid, and more recently, the carotenoids lutein and zeaxanthin have been shown to enhance iron absorption. However, it is not clear whether iron status improves when foods high in ascorbic acid and carotenoids are consumed with iron-fortified meals.

Objective: This study aimed to investigate whether consuming high versus low ascorbic acid, lutein, zeaxanthin-rich fruit (gold kiwifruit versus banana) with iron-fortified breakfast cereal and milk improved iron status in women with low iron stores.

Methods: Healthy women 18-44 years (*n*=89) with low iron stores (serum ferritin ≤25µg/L, haemoglobin ≥115g/L) were randomly stratified to receive iron-fortified breakfast cereal (16 mg iron as ferrous sulphate), milk and either two gold kiwifruit or one banana (164 mg versus not detectable ascorbic acid; 526µg versus 22.90µg lutein and zeaxanthin, respectively) at breakfast every day for 16 weeks. Biomarkers of iron status and dietary intake were assessed at baseline and end in the final sample (*n*=69).

Results: Serum ferritin increased significantly in the kiwifruit group (*n*=33) compared to the banana group (*n*=36) with 10.0 (3.0, 17.5) versus 1.0 (-2.8, 6.5) µg/L (median [25^{th}, 75^{th} percentile]) (*P*<0.001). Median soluble transferrin receptor concentrations decreased significantly in the kiwifruit group compared to the banana group with -0.5 (-0.7, -0.1) versus 0.0 (-0.3, 0.4) mg/L (*P*=0.001).

Conclusions: Consumption of an iron-fortified breakfast cereal with kiwifruit compared to banana improved iron status. Addition of an ascorbic acid, lutein, zeaxanthin-rich fruit to a breakfast cereal fortified with ferrous sulphate is a feasible approach to improve iron status in women with low iron stores.

2 Introduction

Iron deficiency is the most common nutritional deficiency worldwide and premenopausal women are at particular risk (FAO/WHO 2002). Despite the ease of diagnosis and availability of supplements in countries such as New Zealand, iron deficiency remains prevalent (Heath et al 2001b).

Mild iron deficiency can be treated effectively through dietary intervention (Heath et al 2001a), including the use of iron-fortified foods (Hurrell et al 2004) or improving the bioavailability of iron within meals (Rossander-Hulthen and Hallberg 1996). It is well known that both synthetic and dietary ascorbic acid enhance iron absorption when added to meals (Cook and Monsen 1977, Diaz et al 2003). The effect of carotenoids and vitamin A on iron absorption is controversial. Carotenoids such as lutein and zeaxanthin (which lack provitamin A activity) have been shown to enhance iron absorption when added to a wheat-based breakfast (Garcia-Casal 2006), whereas the addition of vitamin A to corn bread did not improve iron absorption (Walczyk et al 2003). It is unclear whether enhanced absorption of iron by ascorbic acid or carotenoids observed in single meal studies leads to an improved iron status over time. Previous studies investigating the effect of consuming synthetic or dietary ascorbic acid with meals over a period of several weeks have shown little or no effect on iron stores due to various reasons. Ascorbic acid enhances iron absorption from a single meal containing iron, but it appears to be less effective at enhancing iron absorption from complete diets (Cook and Reddy 2001). Dietary enhancers or inhibitors have less pronounced effects when iron absorption is measured over several meals or days of intake, compared to measurement in single meals controlled by researchers (Reddy et al 2000).

Furthermore, studies investigating the effects of ascorbic acid on iron status had major limitations, including small participant numbers (Cook et al 1984, Hunt et al 1990, Hunt et al 1994, Kandiah 2002), short duration of intervention (Hunt et al 1990, Hunt et al 1994, Kandiah 2002), participants not clinically iron deficient (Cook et al 1984, Kandiah 2002), and lack of suitable control groups (Cook et al 1984). A well-designed study by Garcia et al (2003) found the addition of 25mg of ascorbic acid as lime juice to two meals per day for eight months did not improve iron status in eighteen iron deficient Mexican women compared to a control group consuming a lime-flavoured beverage with no ascorbic acid (Garcia et al 2003). This was despite the lime juice

having previously been shown by the same research group to increase iron absorption when added to meals (Diaz et al 2003). In addition, it seems likely that when participants began the studies with low haemoglobin levels, absorbed iron was preferentially used for haemoglobin synthesis before iron stores (as indicated by serum ferritin (SF) levels) were increased and many of the studies were not continued long enough for this to be observed (Hunt et al 1990, Hunt et al 1994, Kandiah 2002).

Few of these studies have reported on the iron content of the meals to which ascorbic acid was added (Cook et al 1984) or have only reported total daily iron intake (Garcia et al 2003, Hunt et al 1990, Hunt et al 1994). In one study that did report the iron content of meals, only 2.24mg of dietary iron was consumed at each meal (Kandiah 2002). Ascorbic acid may be more likely to improve iron status if consumed with meals containing substantial amounts of fortificant iron (Garcia et al 2003), especially as ferrous sulphate (Hurrell 2002).

It is hypothesised that iron absorption from a breakfast cereal fortified with a substantial amount of iron as ferrous sulphate will be enhanced if consumed with kiwifruit – a rich source of ascorbic acid, lutein and zeaxanthin – and may consequently improve iron status. This study aimed to investigate whether women's iron status could be improved by consuming an iron-fortified breakfast cereal and milk with gold kiwifruit compared to consuming the same breakfast with banana (low in ascorbic acid, lutein and zeaxanthin), each day for 16 weeks.

3 Methods

The study protocol is described in detail in a publication by Beck et al (2010) (Appendix 4). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Massey University Human Ethics Committee: (Southern A), Reference No. 08/20. Written informed consent was obtained from all participants. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12608000360314).

3.1 Participants and location

The research was conducted in Auckland, New Zealand. The sample size (42 participants per group) was calculated with the assumption of a possible clinically important difference in serum ferritin of 2.5µg/L at 80% power and 5% significance (two-sided test). Power calculations were based on a standard deviation (SD) for serum ferritin calculated from a group of iron deficient women measured in our laboratory (unpublished observation, Beck and Coad, 2007).

Six hundred and twenty three women were screened using a range of recruitment methods. Eighty nine non-anaemic women aged 18 to 44 years with low iron stores (defined as a SF ≤25µg/L and haemoglobin (Hb) ≥115g/L) and complying with the exclusion criteria, were invited to take part. The cut-off value for serum ferritin (≤25µg/L) is based on the inverse relationship between serum ferritin and iron absorption (Taylor et al 1988). When serum ferritin concentrations are greater than 25µg/L there are only small differences in iron absorption from foods of varying iron bioavailability (Fairweather-Tait 1995).

Exclusion criteria included pregnancy, breastfeeding or any known health problems likely to influence iron status including inflammatory bowel disease, coeliac disease, history of gastric ulcers, red blood cell disorders, menorrhagia, haemorrhoids, haematuria or malaria. Women who reported any allergies or intolerances to any components of the breakfast meal were excluded. Participants had to be prepared not to donate blood or consume iron, ascorbic acid or calcium supplements (or supplements containing these nutrients) for the duration of the study. Women who regularly consumed iron supplements within the three month period before commencement of the study were also excluded.

3.2 Study design and procedures

The study was a 16-week, randomised controlled intervention trial. At baseline, participants were randomised to either the kiwifruit group (*n*=44) or the banana group (*n*=45) using stratified randomisation based on serum ferritin concentration and age. Participants visited the human nutrition research unit at Massey University at baseline and after the 16-week intervention trial for blood samples, anthropometric

measurements, questionnaires (demographic, health, blood loss), and dietary assessment.

A fasted venipuncture blood sample was taken for determination of haemoglobin, serum ferritin, soluble transferrin receptor (sTfR), C-reactive protein (CRP), carotenoids and plasma ascorbic acid. Details of blood sample collection and processing procedures and analytical methods are described in Beck et al (2010). Samples collected at baseline and end of the study for each participant were analysed in the same assay run to eliminate inter-assay variability.

Height and weight were measured in duplicate at baseline and end by a trained researcher using the International Society for the Advancement of Kinanthropometry protocols (Marfell-Jones et al 2006). Quetelet's Body Mass Index (BMI) was calculated from height and weight.

Participants completed a face-to-face interview with a researcher at baseline including demographic questions (e.g. age, number of children); lifestyle questions (e.g. smoking); and a medical history (e.g. illness, medication, and supplement use in the past year) (Appendix 7).

Blood loss is known to affect iron status (Heath et al 2001b). Participants completed a previously validated blood loss questionnaire (Heath et al 1998) at baseline and end regarding menstruation, blood donation and nose bleeds. The questionnaire was updated to include details on sanitary items (brand and absorbency) and was completed online.

3.3 Dietary intervention and adherence

Both groups consumed standard portions of an iron-fortified breakfast cereal and milk every day as breakfast for 16 weeks. The kiwifruit and banana groups respectively consumed two ZESPRI® GOLD (*Actinidia chinensis* var Hort16A) kiwifruit or one banana with their breakfasts. Participants were asked to maintain their normal daily routine, including eating patterns (apart from the breakfast), physical activity, alcohol consumption and smoking habits, for the duration of the study.

Each breakfast consisted of pre-packaged individual portions of 64.4g breakfast cereal, 150mL milk (Pam's Long Life Slim low-fat 0.1% ultra heat treated) and either two ZESPRI® GOLD kiwifruit (171g) (ZESPRI® International Ltd, Mount Maunganui, New Zealand) or a banana (104g) (MG Marketing, Auckland, New Zealand). The wheat flake cereal with dried apricot pieces was developed by food technologists at Hubbard Foods Ltd., Mangere, Auckland, New Zealand. Ferrous sulphate AAS® (Zenica BioPlus Pty Ltd, Melbourne, Australia) was added to provide 16mg iron (1.92mg natural iron, 14.1mg ferrous sulphate) per serve. Fruit of a consistent size was delivered to participants each week for the bananas (in various stages of ripeness) and every fortnight for the kiwifruit (stored at 1ºC until delivery). Details of sampling and analytical methods for the breakfast meals are described in Beck et al (2010). The breakfast meals and intervention food items were analysed and their nutritional composition are reported in Table 7.1. The ascorbic acid content of the two gold kiwifruit decreased from 182mg (first six weeks of the study) to 144mg (final six weeks) during storage of the fruit.

Table 7.1. Analysis of nutrient content of the intervention meals and fruit

	Iron-fortified cereal (64.4g) & milk (150mL)	**Iron-fortified cereal, milk & kiwifruit (171g)**	**Iron-fortified cereal, milk & banana (104g)**	**Kiwifruit (171g)**	**Banana (104g)**
Gross energy (kJ)	1598.0	2068.0	2004.0	470.0	406.0
Protein (g)	16.2	17.7	17.3	1.5	1.1
Fat – total (g)	2.2	2.7	2.3	0.5	0.1
Carbohydrate (total) (g)	60.8	79.1	80.1	18.3	19.3
Ascorbic acid (mg)	1.4	164.0	1.4	163.0	ND
Iron (mg)	16.2	16.6	16.4	0.4	0.2
Dietary fibre (g)	4.3	6.5	5.8	2.1	1.5
Calcium (mg)	391.0	426.0	392.0	35.0	1.0
Phosphorus (mg)	437.0	488.0	462.0	51.0	25.0
Vitamin A (as retinol) (µg)	ND	ND	ND	ND	ND
Lutein and zeaxanthin (µg)	ND	194.9	22.9	194.9	22.9
Citric acid (mg)	279.0	2328.0	591.0	2049.0	312.0
Polyphenols (mg)	44.6	79.6	55.6	35.0	11.0
Phytic acid (mg)	238.0	272.0	238.0	34.0	ND

ND - not detectable

Participants were asked to eat the fruit with, immediately before or immediately after consuming the breakfast cereal and not to consume any other food or fluids (apart from plain tap water) with the breakfast meal or one hour before or after consuming the breakfast meal (Appendix 8).

All participants completed a daily compliance diary (Appendix 8), including whether they ate breakfast, the time of consumption, any food not consumed, or any other food eaten with or consumed within one hour either side of the breakfast. As part of this diary, participants reported in a weekly section on illnesses, medication use, symptoms experienced from the intervention, changes to their normal daily routine, supplement use, and any practical problems with eating the breakfast. Participants were e-mailed twice and telephoned at weeks 2, 4, 8 and 12 of the study to provide support, maintain participant motivation, and answer any questions regarding the intervention.

3.4 Dietary assessment

A 144-item FFQ was used for this study, grouping foods known to affect iron absorption according to their similarities, frequency of consumption and nutrient content per common standard measure (Appendix 5). Participants completed the FFQ at baseline and end. The FFQ was used to assess their food intake over the past month and to assess any changes in intake within and between groups over the course of the study. Responses were converted into serving sizes per week for each participant and then combined into the following food groupings: gold kiwifruit; bananas; breakfast cereal; fruit (medium and high ascorbic acid content); all other fruit; vegetables (medium and high ascorbic acid content); all other vegetables; grains, breads, cakes and biscuits; red meat and offal; white meat; fish and seafood; eggs; nuts and seeds; tofu and soy products; legumes; dairy products; coffee, black and herbal teas; fruit juice and alcohol.

3.5 Statistical analysis

Statistical analysis was performed using SPSS for Windows Version 16 (SPSS Inc 2007). Participants with a CRP concentration greater than 10mg/L at either baseline or end were excluded from the analysis, as a high CRP is associated with infection and elevated serum ferritin concentrations, which can lead to over estimation of body iron stores (Hulthen et al 1998). Descriptive statistics were used to describe the study

population using mean (SD), median (25, 75 percentile) or frequency summary statistics. Normality of distribution was evaluated using the Kolmogorov-Smirnov test. Non-normally distributed variables were transformed into approximately normal distributions by logarithmic transformations and again tested for normality.

Comparisons between groups at baseline were made using the independent t-test for parametric data, the Mann-Whitney test for non-parametric data, and the chi-square test for frequencies. Participants who did not complete the study or who were excluded from the analysis were compared with participants who completed the study using these same statistical tests. The main analysis was the comparison of the change in serum ferritin from baseline to 16 weeks between the kiwifruit and the banana groups. Changes in haemoglobin, soluble transferrin receptor, soluble transferrin receptor: serum ferritin ratio, plasma ascorbic acid, serum lutein/zeaxanthin and dietary intake between groups were also investigated. Independent t-tests were used for parametric data and the Mann-Whitney test for non-parametric data. Comparisons were made within the kiwifruit and banana groups between baseline and endpoint measures using the dependent t-test for parametric data and the Wilcoxon signed-rank test for non-parametric data. Serum ferritin, soluble transferrin receptor, soluble transferrin receptor: serum ferritin ratio and ascorbic acid were tested using one-sided tests. Two-sided tests were used for all other variables.

Since BMI levels differed between the two groups at baseline a two-way ANOVA was used to test for interaction or main effects between BMI and the intervention and its impact on change in serum ferritin. Significance was set at $P<0.05$. The percentage of days each participant consumed the study breakfast meal was calculated from compliance diaries (number of days the study breakfast was consumed/112 (total number of days on the study) x 100).

4 Results

4.1 Participants

Of the 89 women with low iron stores enrolled in the study, 69 (33 women in the kiwifruit group and 36 women in the banana group) were included in the final analysis. Eleven participants withdrew from the study (five from the kiwifruit group and six from the banana group), and a further nine participants were excluded from the final

analysis due to non-compliance, use of supplements, medical conditions or elevated CRP concentrations. Baseline serum ferritin concentrations were significantly higher (*P*=0.02) in women who withdrew or were excluded from the final analysis (median [25th, 75th percentile]) (21.0 [16.3, 31.8]µg/L) compared with women who completed the study (17.0 [10.0, 21.0]µg/L). Women who did not complete the study or who were excluded from final analysis did not differ with regard to other baseline characteristics.

4.2 Adherence

Participants completed 112 days on the study. Compliance diaries were available for all women (100%) in the kiwifruit group (*n*=33) and for 34 women (94.4%) in the banana group. The mean (SD) compliance rate in the kiwifruit group was 97 (5.12) % compared to 98 (3.68) % in the banana group. Ninety one percent of women in the kiwifruit group and 97% of women in the banana group reported a compliance rate of greater than 90%.

4.3 Baseline characteristics

The baseline characteristics of the women are shown in Tables 7.2 and 7.3. At baseline there were no significant differences between the groups for any of the variables or factors affecting iron status such as having children, menstrual blood loss or blood donation. BMI was significantly lower in the kiwifruit group compared to the banana group at baseline (*P*=0.02) although this difference was very small. These differences in BMI at the beginning of the study had no effect on the change in serum ferritin concentrations. BMI and menstrual blood loss did not change within or between groups over the course of the study.

Table 7.2. Baseline characteristics of study participants

	Kiwifruit group (*n*=33)	**Banana group (*n*=36)**	***P* value[1]**
Age (years)	31.0 (24.0, 39.5)	35.0 (22.5, 41.0)	0.41
Weight (kg)	62.4 (7.2)	66.1(13.8)	0.16
Height (cm)	166.3 (6.1)	163.7 (6.8)	0.10
BMI (kg/m^2)	22.4 (21.2, 23.7)	24.1 (21.8, 26.7)	0.02
Menstrual blood loss (BLU)	46.5 (22.8, 57.0)	47.5 (28.8, 66.0)	0.36
Blood donor in the past year (%)	24.2	19.4	0.77
Has children (%)	54.5	61.1	0.63

Results expressed as either median (25, 75 percentile) or mean (SD); BMI values are reported as geometric means (95% CI); [1]Difference between groups (independent t-test, Mann-Whitney test or chi-square test); BLU – blood loss units; BMI – Body Mass Index

4.4 Biochemical assessment

The changes in iron status, plasma ascorbic acid and serum lutein/zeaxanthin from baseline to end are presented in Table 7.3. Serum ferritin concentrations increased significantly in the kiwifruit group from baseline to end (*P*<0.001), but there was no effect in the banana group (*P*=0.09). The difference in the change between the two groups was also highly significant (*P*<0.001). Haemoglobin concentrations increased significantly in the kiwifruit group (*P*=0.005), but not within the banana group (*P*=0.30), and the difference in change between groups was not significantly different (*P*=0.14). Soluble transferrin receptor concentrations decreased significantly in the kiwifruit group (*P*<0.001), but not in the banana group (*P*=0.32), and the difference in change between the groups was statistically significant (*P*=0.001). The soluble transferrin receptor:serum ferritin ratio decreased significantly in the kiwifruit group (*P*<0.001), with no change in the banana group (*P*=0.20). The difference in change between the groups was statistically significant (*P*=0.008).

Plasma ascorbic acid concentrations increased significantly (*P*=0.002) in the kiwifruit group from baseline to end, but there was no change in the banana group (*P*=0.15). The difference in the changes between the two groups was almost significant (*P*=0.07). Serum lutein/zeaxanthin increased significantly within the kiwifruit group (*P*=0.002), but not in the banana group (*P*=0.33), and the difference in the change between groups was significant (*P*=0.03).

Table 7.3. Changes from baseline to end measures of iron status, plasma ascorbic acid and serum lutein/zeaxanthin within and between kiwifruit and banana groups

	Kiwifruit (*n*=33)	**Banana (*n*=36)**	***P*[1]**
Serum ferritin[2] (Normal reference range 20-160µg/L, Diagnostic MedLab, Auckland)			
Baseline	17.0 (10.5, 22.0)	16.5 (10.0, 20.8)	0.40
End	25.0 (20.0, 32.0)	17.5 (12.3, 22.8)	
Change[3]	10.0 (3.0, 17.5)	1.0 (-2.8, 6.5)	<0.001
P[4]	<0.001	0.09	
Haemoglobin[5] (Normal reference range 115-160g/L, Diagnostic MedLab, Auckland)			
Baseline	126.0 (8.8)	125.0 (9.0)	0.78
End	130.0 (7.6)	126.0 (9.0)	
Change[3]	3.8 (7.2)	1.2 (6.9)	0.14
P[4]	0.005	0.30	
Soluble transferrin receptor[2] (Normal reference range 2.2-4.5mg/L, LabPlus, Auckland)			
Baseline	3.1 (2.5, 3.8)	3.5 (2.7, 4.0)	0.13
End	2.7 (2.1, 3.0)	3.6 (2.6, 4.2)	
Change[3]	-0.5 (-0.7, -0.1)	0.0 (-0.3, 0.4)	0.001
P[4]	<0.001	0.32	
Soluble transferrin receptor: serum ferritin ratio[2] (Normal reference range < 100 (Skikne et al 1990))			
Baseline	188.0 (116.0, 288.0)	200.0 (157.0, 333.0)	0.32
End	116.0 (77.2, 150.0)	186.0 (132.0, 346.0)	
Change[3]	-89.5 (-183.0, -38.4)	-0.2 (-124.0, 65.9)	0.008
P[4]	<0.001	0.20	
Plasma ascorbic acid[2] (Normal reference range 20-80µmol/L, LabPlus, Auckland)			
Baseline	70.0 (52.0, 79.5)	65.0 (56.3, 72.8)	0.20
End	76.0 (69.0, 83.0)	66.0 (56.3, 72.0)	
Change[3]	7.0 (-3.5, 19.0)	3.0 (-4.8, 10.8)	0.07
P[4]	0.002	0.15	
Serum Lutein/Zeaxanthin[2]			
Baseline	413.0 (311.0, 540.0)	378.0 (269.0, 506.0)	0.27
End	497.0 (356.0, 658.0)	438.0 (288.0, 496.0)	
Change[3]	64.0 (11.5, 132.0)	12.0 (-59.0, 95.0)	0.03
P[4]	0.002	0.33	

[1]Difference between groups (independent t-test or Mann-Whitney test); [2]Values are medians; 25th, 75th percentiles in parentheses; [3]Change: End value – Baseline value; [4]Difference between baseline and end (dependent t-test or Wilcoxon signed-rank test); [5]Change are means; SD in parentheses

4.5 Dietary assessment

There were no significant differences in dietary intake between the kiwifruit and banana groups at baseline. As expected, participants in the kiwifruit and banana groups significantly increased their intake of gold kiwifruit (*P*<0.001) and bananas (*P*=0.02) respectively from baseline to end. The intake of medium to high ascorbic acid containing fruits decreased significantly over the course of the study in the kiwifruit group (-2.9 [-5, 0.1] servings/week) compared to the banana group (-0.8 [-2.5, 0.7] servings/week) (*P*=0.02). Red meat and offal intake decreased significantly in both the kiwifruit (*P*=0.02) and banana (*P*=0.01) groups although the decreases were small (-0.1 [-2.0, 0.2] and -0.4 [-2.1, 0.0] servings per week respectively). The difference in change between groups was not significant (*P*=0.63). Similarly, the intake of grains, breads, cakes and biscuits decreased significantly in both the kiwifruit (*P*<0.001) and banana (*P*=0.05) groups, although the difference in change between the groups was not significant (*P*=0.61). In this instance however, the servings consumed decreased in both groups by about four to six servings showing the possible replacement due to the consistent daily intake of breakfast cereal.

4.6 Side effects

The majority (n=40, 58%) of the women who completed the study (kiwifruit group, n=19; banana group, n=21) reported no side effects of eating the breakfast meal. Positive effects such as regular bowel movements, better skin, not feeling as hungry and a feeling of more energy were reported by 15 (21.7%) of the women (kiwifruit group n=7, banana group n=8). In total 26.1% (n=18) of the women reported a negative side effect associated with the breakfast (kiwifruit group n=7, banana group n=11). Most of the reported negative effects related to initial nausea, constipation or diarrhoea which subsided during the course of the study.

5 Discussion

As far as we are aware, this is the first randomised controlled intervention trial to investigate the effect of an iron-fortified breakfast cereal plus an ascorbic acid, lutein and zeaxanthin-rich kiwifruit compared with banana (low ascorbic acid, lutein and zeaxanthin) on iron status in healthy women with low iron stores. The results showed

an improvement in iron status (increased serum ferritin; decreased soluble transferrin receptor and soluble transferrin receptor: serum ferritin ratio) following the 16-week intervention.

Ascorbic acid is the most widely used enhancer of fortified iron (Hurrell 2002) and increases iron absorption in a dose dependent manner (Cook and Monsen 1977). Ascorbic acid enhances iron absorption by reducing ferric iron to ferrous iron, for transport by divalent metal transport protein 1 into the intestinal mucosal cell. Ascorbic acid also forms a soluble chelate with iron, preventing it being precipitated as insoluble compounds, such as ferric hydroxide or ferric phosphate, or binding to inhibitory ligands such as phytate (Allen and Ahluwalia 1997). An ascorbic acid to iron molar ratio of 4:1 is needed to increase iron absorption from fortified foods high in phytic acid (Hurrell 2002). The ascorbic acid to iron molar ratio of the kiwifruit breakfast meal provided in this study was 3.7:1. Ascorbic acid may be unstable during food processing, storage and cooking, therefore consuming natural sources of ascorbic acid such as fruits and vegetables with iron-fortified foods is recommended (Teucher et al 2003). Hallberg et al (1986) suggested that 50mg of ascorbic acid is needed to enhance iron absorption in a meal, although this is likely to depend on the components of the meal and other studies have shown smaller amounts of ascorbic acid to be successful in enhancing iron absorption (Diaz et al 2003). The kiwifruit supplied in this study provided 163mg of ascorbic acid.

Although it is well established that ascorbic acid increases iron absorption, previous studies have not shown an improvement in iron status over time with the consumption of ascorbic acid. These studies had several limitations (sample size, short duration, iron replete participants, no control groups, ascorbic acid not specifically used with absorbable iron) (Cook et al 1984, Hunt et al 1990, Hunt et al 1994, Kandiah 2002). These limitations were all addressed within the current study as follows: a sufficient sample size to detect the expected small changes in biomarkers of iron status; a 16-week intervention allowing sufficient time for red blood cell turnover and improvement of iron stores; a control group was used; only participants with low iron stores were included; the ascorbic acid-rich fruit was added to a meal containing a significant amount of iron (16 mg iron as ferrous sulphate).

Serum ferritin is routinely used and is a well standardised and sensitive measure of iron stores (Cook 2005). Soluble transferrin receptor is a stable and highly sensitive indicator of functional or tissue iron, not subject to biological variation or affected by

inflammation (Cook 2005, Gibson 2005). Serum ferritin is reduced during the depletion of iron stores, while soluble transferrin receptor is not affected. During functional iron deficiency however, soluble transferrin receptor increases, while serum ferritin remains unchanged. Therefore, the decrease in the ratio of soluble transferrin saturation and serum ferritin is a more specific and sensitive indicator of improved iron status than either measure alone (Baynes 1996). This ratio decreased significantly in the kiwifruit group compared to the banana group. The women who consumed kiwifruit had improved functional iron and stored iron at the end of the study, whereas women who consumed bananas, despite receiving an equivalent amount of iron, did not improve their iron status.

The plasma ascorbic acid status of the women in the kiwifruit group showed a significant increase, from 70 to 76µmol/L, over the course of the intervention compared with no change in the banana group. Plasma ascorbic acid concentrations have been shown to increase linearly with dietary intake (up to 60 to 70mg/day), but only up to a plateau of 75µmol/L (Gibson 2005). Therefore, further increases in plasma ascorbic acid were not expected to be seen with the large quantities of ascorbic acid provided by the kiwifruit. Although the intake of fruits with medium and high ascorbic acid content decreased in the kiwifruit group compared to the banana group, the iron status in the kiwifruit group still improved, probably indicating that it is more important to consider whether ascorbic acid is added to a meal containing iron than to focus on the total daily intake of ascorbic acid.

Lutein and zeaxanthin have been shown to improve iron absorption in humans (Garcia-Casal 2006), however, no studies have investigated their effects on iron status when consumed with meals for an extended period of time. Serum lutein/zeaxanthin concentrations increased significantly in the kiwifruit group compared to the banana group. The improvement seen in iron status in the kiwifruit group could have been due to the ascorbic acid, carotenoids or a combination of nutritional factors in kiwifruit. The kiwifruit contained over 2000mg of citric acid (more than six times the citric acid content of the banana) which may also have contributed to the increased iron status seen in the kiwifruit group. The effect of citric acid has been shown to be additive to the effect of ascorbic acid on iron absorption (Ballot et al 1987).

The level of iron fortification within this study was set at 16mg per serve, providing more than 80% of the current Recommended Daily Intake (18mg) for iron (Commonwealth Department of Health and Ageing Australia et al 2006). Zimmermann

et al (2005) found that adding 12mg of iron per day as snack foods increased iron status in women with low iron stores (Zimmerman et al 2005). Despite this, the banana group did not show an improvement in iron status. Iron fortification at 16mg iron as ferrous sulphate per serve alone may not have been adequate to overcome the inhibitory effects on iron absorption of the calcium or phytic acid contained in the breakfast meals (Cook et al 1997, Hallberg et al 1991). While calcium inhibits iron absorption (Hallberg et al 1991), supplemental calcium consumed with meals (Minehane and Fairweather-Tait 1998) or calcium supplementation as high has 1200mg per day does not appear to affect iron status in iron replete women over time (Bendich 2001). However, further research is needed to see whether the iron stores of iron depleted women (such as those in the current study) are affected by high calcium intakes (Bendich 2001). Phytic acid has a strong inhibitory effect on iron absorption (Hallberg et al 1989). Sodium phytate decreased iron absorption in a dose-dependent manner when added to a wheat roll, while the addition of 50 and 100mg ascorbic acid significantly counteracted the effect of sodium phytate (Hallberg et al 1989). Hurrell et al (1992) suggested that decreasing the phytic acid content of a meal from 220 to 110mg would have little effect on improving iron absorption, but by decreasing the phytic acid content to <10mg per meal, iron absorption would increase substantially. The phytic acid content of the banana meal was 238mg and could have therefore had a negative effect on iron absorption if not counteracted by ascorbic acid. Very few studies have investigated the long-term effect of phytic acid on iron status. Kristensen et al (2005) found that serum ferritin concentrations decreased significantly in iron replete women given fibre-rich bread containing phytate over a four month period. In another study, soy protein containing native phytate significantly reduced serum ferritin concentrations in participants after six weeks (Hanson et al 2006). It is likely that the phytic acid contained in the breakfast cereal may have inhibited iron absorption, which the kiwifruit but not the banana was able to overcome. It is unlikely that the banana itself inhibited iron absorption. The banana contained similar or lower levels of dietary fibre, calcium, polyphenols and phytic acid (substances all known to inhibit iron absorption) than the kiwifruit. Thus, adding 16mg of iron as ferrous sulphate to a breakfast cereal eaten with milk was ineffective at improving iron status unless eaten with an ascorbic acid, lutein and zeaxanthin-rich fruit.

Breakfast cereal was chosen as a suitable vehicle to fortify with iron (Hurrell 1997), and as a food item that is acceptable to consume with fruit. A food-based approach represents a desirable and potentially sustainable method of addressing the issue of iron deficiency (World Health Organization 2001), which may also confer other

nutritional benefits compared to simple nutrient supplementation. Self-reported compliance to the breakfast protocol was high, probably because the breakfast was feasible for women to incorporate into their daily lives and due to the high levels of support received from the researchers during the study. Women simply replaced one breakfast item for another, which meant little interference with the rest of the family. Few women reported any side-effects due to the breakfast meal. Negative side-effects included initial nausea which could be related to the size of the breakfast being consumed, constipation or diarrhoea possibly due to the fortificant which subsided during the course of the study as tolerance increased. Several positive effects were also noted including regular bowel movements due to the cereal and fruit combination, healthier skin, reduced hunger and higher energy levels which may be related to healthier eating habits. An alternative to dietary intervention is iron supplementation, but this may not be feasible due to poor compliance, often attributed to the side-effects experienced (Galloway and McGuire 1994). Increasing the intake of meat, fish and poultry is often recommended to improve iron status. However, a study in New Zealand found that women were only able to increase their intake of flesh food by 31g per day (equivalent to one third of a meat serving per day), despite receiving intensive dietary counselling to improve the iron bioavailability in their diet. However, these women increased their intake of ascorbic acid by 136mg per day (Heath et al 2001a). Therefore, the inclusion of a combination of fruit high in ascorbic acid, lutein and zeaxanthin and an iron-fortified breakfast cereal may be far more achievable for these women to improve their iron status compared to other approaches. The optimal level of iron fortification in various foods, and the impact of other fruit or foods high in ascorbic acid, lutein and zeaxanthin consumed with iron-fortified foods on iron status should be investigated, also taking into account consumer acceptability and accessibility.

In conclusion, the consumption of an ascorbic acid, lutein and zeaxanthin-rich fruit (kiwifruit) compared to a banana (low ascorbic acid, lutein and zeaxanthin) with an iron-fortified breakfast cereal meal improved iron status in women with low iron stores. The addition of an ascorbic acid, lutein and zeaxanthin-rich fruit to a breakfast cereal fortified with ferrous sulphate may be a more feasible approach of addressing iron deficiency in young women compared to other approaches.

6 References

Allen LH, Ahluwalia N (1997). *Improving iron status through diet: The application of knowledge concerning dietary iron bioavailability in human populations*. MOST: The USAID Micronutrient Program http://www.mostproject.org/Archives.htm#IronArchives

Ballot D, Baynes RD, Bothwell TH, Gillooly M, MacFarlane BJ, MacPhail AP *et al* (1987). The effects of fruit juices and fruits on the absorption of iron from a rice meal. *Br J Nutr* **57:** 331-343.

Baynes RD (1996). Assessment of iron status. *Clin Biochem* **29:** 209-215.

Beck K, Conlon C, Kruger R, Coad J, Stonehouse W (2010). The effect of gold kiwifruit consumed with an iron fortified breakfast cereal meal on iron status in women with low iron stores: A 16 week randomised controlled intervention study. *BMC Public Health* **10:** 36.

Bendich A (2001). Calcium supplementation and iron status of females. *Nutrition* **17:** 46-51.

Commonwealth Department of Health and Ageing Australia, Ministry Of Health New Zealand, National Health and Medical Research Council (2006). *Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes*. Commonwealth of Australia: Canberra.

Cook JD, Monsen ER (1977). Vitamin C, the common cold and iron absorption. *Am J Clin Nutr* **30:** 235-241.

Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS (1984). The effect of high ascorbic acid supplementation on body iron stores. *Blood* **64:** 721-726.

Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF (1997). The influence of different cereal grains on iron absorption from infant cereal foods. *Am J Clin Nutr* **65:** 964-969.

Cook JD, Reddy MB (2001). Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *Am J Clin Nutr* **73:** 93-98.

Cook JD (2005). Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol* **18:** 319-332.

Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP (2003). The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78:** 436-440.

Fairweather-Tait SJ (1995). Iron Absorption. In: British Nutrition Foundation (ed). *Iron: Nutritional and physiological significance*. Chapman and Hall: London. pp 3-12.

FAO/WHO (2002). Iron. *Human vitamin and mineral requirements: Report of a joint FAO/WHO expert consultation, Bangkok, Thailand*. FAO: Rome. pp 195-221.

Galloway R, McGuire J (1994). Determinants of compliance with iron supplementation - supplies, side-effects, or psychology. *Soc Sci Med* **39:** 381-390.

Garcia-Casal MN (2006). Carotenoids increase iron absorption from cereal-based food in the human. *Nutr Res* **26:** 340-344.

Garcia OP, Diaz M, Rosado JL, Allen LH (2003). Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. *Am J Clin Nutr* **78:** 267-273.

Gibson RS (2005). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York.

Hallberg L, Brune M, Rossander L (1986). Effect of ascorbic acid on iron absorption from different types of meals: Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum Nutr: Clin Nutr* **40A:** 97-113.

Hallberg L, Brune M, Rossander L (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am J Clin Nutr* **49:** 140-144.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Hanson LN, Engelman HM, Lee Alekel D, Schalinske KL, Kohut ML, Reddy MB (2006). Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. *Am J Clin Nutr* **84:** 774-780.

Heath AL, Skeaff CM, Gibson RS (1998). Validation of a questionnaire method for estimating extent of menstrual blood loss in young adult women. *J Trace Elem Med Biol* **12:** 231-235.

Heath ALM, Skeaff CM, O'Brien SM, Williams SM, Gibson RS (2001a). Can dietary treatment of non-anemic iron deficiency improve iron status? *J Am Coll Nutr* **20:** 477-484.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001b). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Hulthen L, Lindstedt G, Lundberg P-A, Hallberg L (1998). Effect of mild infection on serum ferritin concentration - clinical and epidemiological implications. *Eur J Clin Nutr* **52:** 376-379.

Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH (1990). Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *Am J Clin Nutr* **51:** 649-655.

Hunt JR, Gallagher SK, Johnson LK (1994). Effect of ascorbic acid on apparent iron absorption by women with low iron stores. *Am J Clin Nutr* **59:** 1381-1385.

Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD (1992). Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr* **56:** 573-578.

Hurrell RF (1997). Preventing iron deficiency through food fortification. *Nutr Rev* **55:** 210-222.

Hurrell RF (2002). Fortification: Overcoming technical and practical barriers. *J Nutr* **132:** 806S-812S.

Hurrell RF, Lynch S, Bothwell T, Cori H, Glahn RP, Hertrampf E *et al* (2004). Enhancing the absorption of fortification iron: A SUSTAIN task force report. *Int J Vitam Nutr Res* **74:** 387-401.

Kandiah J (2002). Impact of tofu or tofu + orange juice on hematological indices of lacto-ovo vegetarian females. *Plant Foods Hum Nutr* **57:** 197-204.

Kristensen MB, Tetens I, Jorgensen ABA, Dal Thomsen A, Milman N, Hels O *et al* (2005). A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread. *Eur J Nutr* **44:** 334-340.

Marfell-Jones M, Olds T, Stewart A, Carter L (2006). *International standards for anthropometric assessment*. ISAK: Potchefstroom.

Minehane A, Fairweather-Tait S (1998). Effect of calcium supplementation on daily nonheme iron absorption and long term iron status. *Am J Clin Nutr* **68:** 96-102.

Reddy MB, Hurrell RF, Cook JD (2000). Estimation of nonheme-iron bioavailability from meal composition. *Am J Clin Nutr* **71:** 937-943.

Rossander-Hulthen L, Hallberg L (1996). Dietary factors influencing iron absorption - an overview. In: Hallberg L, Asp N (eds). *Iron Nutrition in Health and Disease*. John Libbey and Company Ltd: London. pp 105-115.

Skikne BS, Flowers CH, Cook JD (1990). Serum transferrin receptor - a quantitative measure of tissue iron deficiency *Blood* **75:** 1870-1876.

SPSS Inc (2007). *SPSS for Windows, release 16.0*. SPSS Inc: Chicago.

Taylor P, Martinez-Torres C, Leets I, Ramirez J, Nieves-Garcia-Casal M, Layrisse M (1988). Relationships among iron absorption, percent saturation, of plasma transferrin and serum ferritin concentration in humans. *J Nutr* **118:** 1110-1115.

Teucher B, Olivares M, Cori H (2003). Enhancers of iron absorption: Ascorbic acid and other organic acids. *Int J Vitam Nutr Res* **74:** 403-419.

Walczyk T, Davidsson L, Rossander-Hulthen L, Halberg L, Hurrell RF (2003). No enhancing effect of Vitamin A on iron absorption in humans. *Am J Clin Nutr* **77:** 144-149.

World Health Organization (2001). *Iron Deficiency Anaemia: Assessment, Control and Prevention. A guide for programme managers*. World Health Organization: Geneva.

Zimmerman MB, Winichagoon P, Gowachirapant S, Hess SY, Harrington M, Chavasit V *et al* (2005). Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. *Am J Clin Nutr* **82:** 1276-1282.

CHAPTER 8

Discussion and conclusions including recommendations for future research

1 Introduction

This thesis investigated the causes and some of the consequences of iron deficiency in young women, and explored a possible solution to iron deficiency. The discussion will bring together the different studies to focus on the main results observed, methodological issues, the significance of the results and future research recommendations. Final conclusions will also be drawn.

2 Summary of findings

The use of dietary patterns provides a novel approach to investigating associations between diet and iron status. This approach considers the whole diet, which is particularly relevant to iron nutrition where a number of food components enhance and inhibit iron absorption. In this research, a computerised iron food frequency questionnaire (FeFFQ) was developed which contained an extensive list of foods grouped according to their potential impact on iron intake and bioavailability. Using a robust and rigorous study design, the FeFFQ was found to be a reproducible and reasonably valid tool for determining the frequency of intake of food groupings and identifying iron-related dietary patterns in premenopausal women **(hypothesis 1 accepted)**. As far as we are aware, this is the first study to determine the validity and reproducibility of a food frequency questionnaire (FFQ) designed specifically to identify iron-related dietary patterns ('validation' study; chapter three).

Once validated, the FeFFQ was used to determine the association between iron status and dietary patterns in 375 premenopausal women. It was found that following a 'meat & vegetable' dietary pattern reduced the risk of suboptimal iron status (serum ferritin (SF) <20µg/L, haemoglobin (Hb) < or ≥120g/L) by 0.6 times and following a 'milk & yoghurt' pattern increased the risk of suboptimal iron status by 1.5 times **(hypothesis 2 accepted)**. These findings reinforce our knowledge of meat's beneficial effect on iron status, and provide some evidence for calcium (or another component in milk and yoghurt) having a negative effect on iron status ('dietary patterns and iron status' study, chapter four). These dietary patterns remained important predictors of suboptimal iron status when non-dietary factors that might impact on iron status were also considered (the

'meat and vegetable' dietary pattern continued to reduce the risk of suboptimal iron status by 0.6 times, and the 'milk and yoghurt' dietary pattern increased the risk of suboptimal iron status by 1.4 times (instead of 1.5 times when only dietary factors were considered). Blood donation in the past year and being of Asian ethnicity were the strongest predictors of suboptimal iron status, increasing the risk by 6.7 and 5.2 times respectively. Other predictors for suboptimal iron status included having children (increasing the risk of suboptimal iron status 2.7 times), previous iron deficiency (2.1 times), and having a longer menstrual period (1.3 times) **(hypothesis 3 accepted)** ('determinants of iron status' study, chapter five).

The effect of iron status on self-perceived health, well-being and fatigue was investigated in female university students. Participants remained unaware of their iron status and no significant differences were observed in self-perceived health, well-being and fatigue between iron sufficient (SF≥20µg/L, Hb≥120g/L) and iron depleted (SF<20µg/L, Hb≥120g/L) students as determined using the SF-36v2 Health Survey (SF-36 questionnaire) and the Multidimensional Fatigue Symptom Inventory – Short Form (MFSI-SF questionnaire) **(hypothesis 4 rejected)**. Having a history of iron deficiency, smoking and having a medical condition explained almost one fifth of the variance in MFSI-SF physical fatigue. The prevalence of iron depletion or iron deficiency (SF<20µg/L, Hb≥120g/L) (8.7%) and iron deficiency anaemia (SF<20µg/L, Hb<120g/L) (2.9%) in female university students was unexpectedly low compared to that observed in other studies of young New Zealand women (Fawcett et al 1998, Heath et al 2001b, Horwath 1991) ('well-being and fatigue' study, chapter six).

The literature review highlighted a lack of well-designed dietary interventions for treating iron deficiency. The final study was a randomised controlled trial to investigate the effect of an iron-fortified breakfast cereal plus an ascorbic acid, lutein and zeaxanthin-rich kiwifruit compared with banana (low ascorbic acid, lutein and zeaxanthin) on iron status in 89 healthy women with low iron stores (SF≤25µg/L, Hb ≥115g/L). The results showed an improvement in iron status (increased serum ferritin, decreased soluble transferrin receptor and soluble transferrin receptor: serum ferritin ratio) following the 16-week intervention **(hypothesis 5 accepted)**. The use of an iron-fortified breakfast cereal and kiwifruit may be a feasible approach to addressing iron deficiency in young women ('breakfast cereal and fruit intervention' study, chapter seven).

3 Methodological considerations

A robust series of studies was designed to investigate the causes, consequences and a possible solution to iron deficiency in premenopausal women. When examining the internal and external validity of these studies, a number of methodological strengths and limitations need to be taken into consideration. These include the study population, the study design, measurement of iron status, dietary assessment issues, the use of empirical methods for identifying and validating dietary patterns, and the tools used to measure well-being and fatigue. Each of these issues will be discussed below.

3.1 Study population

Recruitment strategies that focussed on iron status, diet, health and fatigue may have attracted volunteers who were health conscious, or volunteers who have previously experienced iron deficiency or fatigue. The high compliance rate observed in both the 'validation' (100% compliance) and 'breakfast cereal and fruit' studies may have been due to the women's interest in their health (and subsequently, motivation to change their diet and improve their iron status in the 'breakfast cereal and fruit' study), or alternatively, due to the high levels of support and follow up provided in both studies. However, one disadvantage of using a convenience sample of volunteer subjects, meant participants were not necessarily representative of women aged 18 to 44 years living in New Zealand.

We used a separate study population for the 'validation' study and the 'dietary patterns'/'determinants of iron status' studies. Due to the different women included in each of the studies, different dietary patterns were obtained. While both were convenience samples of women aged 18 to 44 years, women in the 'validation' study were older (median age 33 versus 25.5 years) and of mainly European ethnicity (85.2 versus 75.4%). More Asian women were included in the 'dietary patterns'/'determinants of iron status' studies (15.0 compared with 7.0% in the 'validation' study). It was not realistic to carry out the 'validation' study using the same women (including numbers of women) that were in the 'dietary patterns'/'determinants of iron status' studies due to the high levels of participant burden. However, the 'validation' study enabled us to explore dietary patterns

further in a larger, more ethnically diverse group in which a greater number of dietary patterns were identified e.g. an Asian dietary pattern.

In planning the research, our studies were adequately powered to observe clinically significant results. However, the 'well-being and fatigue' study was limited by the surprisingly small percentage of women with depleted iron stores. Power calculations were based on a prevalence of depleted iron stores of 20%, but only 9.4% of women in this study had depleted iron stores. Due to the unexpectedly low numbers of women with depleted iron stores, it would be desirable to expand the 'well-being and fatigue' study to include a larger number of participants.

3.2 Study design

Randomised controlled trials are the 'gold standard' of study design to determine cause and effect relationships (Boushey et al 2006). The 'breakfast cereal and fruit' study was a randomised controlled trial. The methodology used in the 'breakfast cereal and fruit' study was robust compared to other intervention trials investigating the effect of ascorbic acid on iron status over time. The sample size was sufficient to detect clinically important changes in serum ferritin concentration, the 16-week intervention provided adequate time for red blood cell turnover and improvement of iron stores, only participants with low iron stores were included (therefore absorbing more iron) and a control group was used. Additionally, the gold kiwifruit were added to a meal containing a significant amount of iron, meaning the ascorbic acid could exert its effect. Since completing this study further research has shown green and gold kiwifruit to be a highly bioavailable source of ascorbic acid (Vissers et al 2011). In a mouse model, consumption of green and gold kiwifruit increased the delivery of ascorbic acid up to five times compared with ascorbic acid given with water (Vissers et al 2011). This may further help to explain the beneficial effects observed of kiwifruit on iron status.

Ideally randomised controlled trials should be double-blinded where both the participants and researcher are unaware of the treatment (Sibbald and Roland 1998). Due to the nature of the treatment provided (kiwifruit versus banana) it was not possible for the participants to remain blinded to the treatment they were receiving. With increased resources it may have been possible to blind the main researcher to the treatment the

participants received. Alternatively, a food product (e.g. a freeze-dried powder) could have been developed so that the kiwifruit and banana were indistinguishable. However, the aim of the study was to determine the effect of an iron-fortified breakfast cereal meal and gold kiwifruit in its natural form (i.e. test a real-life situation) on iron status.

With the 'breakfast cereal and fruit' study, iron status improved in the kiwifruit but not the banana group. The use of a banana as the control group may have had several limitations, due to its own unique properties. It is possible that in the banana group, the level of iron fortification was inadequate to overcome the inhibitory effects of calcium (Hallberg et al 1991) and phytic acid (Hurrell et al 2003) contained in the breakfast meals. It is unlikely the banana inhibited iron absorption, as it contained similar or lower levels of dietary factors known to inhibit iron absorption (fibre, phytic acid, calcium and polyphenols) compared to kiwifruit. However, there is also a possibility that bananas contain an as yet unidentified factor which inhibits iron absorption. Ideally a third (control) group could have been included who consumed a breakfast cereal without the addition of fruit. However, to ensure the study had adequate power, significantly more women would need to have been screened to identify sufficient numbers of women with low iron stores. As it was, 623 women were screened to find 89 women with low iron stores. Another option would have been to include a 'breakfast cereal and kiwifruit' group, and a 'breakfast cereal with no fruit' group. However, it was decided to include a 'breakfast cereal and banana' group to keep the energy content of the two breakfast meals as similar as possible. The banana was chosen as an attractive fruit option to consume with the breakfast cereal, and also for its nutritional properties (relatively similar energy content to two gold kiwifruit, and low levels of ascorbic acid, lutein and zeaxanthin, all factors which enhance iron absorption.

The 'dietary patterns and iron status', 'determinants of iron status' and 'well-being and fatigue' studies were all cross-sectional studies. Cross-sectional studies are useful for generating hypotheses and informing future research. A major limitation of cross-sectional studies is that causality cannot be determined. For example, it is not known whether particular dietary patterns cause suboptimal iron status, or whether women with suboptimal iron status are more likely to consume these particular dietary patterns. Future studies could be improved by using a longitudinal study design where subjects are followed up over time or a randomised controlled trial where for example, well-being and

fatigue are measured in iron deficient participants prior to and after iron supplementation. However, both these types of studies require highly motivated subjects and are more expensive due to the increased resources required (Truswell and Mann 2007).

3.3 Measurement of iron status

It is recommended that at least two biochemical measures be undertaken when determining an individual's iron status (Gibson 2005a). Using two measurements minimises the misclassification that can occur due to overlapping normal and abnormal values when a single measure is used, as well as enabling the severity of iron deficiency to be known (Gibson 2005a). In all studies where a measure of iron status was required we measured both serum ferritin (to determine the extent of iron deficiency) and haemoglobin (to identify anaemia) concentrations.

Serum ferritin is regarded as one of the best measures of iron status in developed countries such as New Zealand due to its high sensitivity and relationship to body iron stores (Gibson 2005a). One of the disadvantages of using serum ferritin is that it is elevated during infection and inflammation (Hulthen et al 1998). For this reason, we excluded all women from the data analysis who had a C reactive protein (CRP) >10mg/L (Zimmermann and Hurrell 2007). Serum ferritin also has a large day-to-day variability. One study found serum ferritin had a total intra-individual variation in women of 27.4%, and four venous serum samples were needed to estimate serum ferritin within 20% of its true value, 95% of the time (Cooper and Zlotkin 1996). We may therefore have not always obtained an accurate measure of an individual's serum ferritin concentration.

Within the literature, a range of serum ferritin concentrations (10-22µg/L) have been used to determine the cut-off point for depleted iron stores and iron deficiency (Pynaert 2007). This is further complicated by the different names used to label iron depletion. Even within this research we have used various names and categories to identify women with high versus low iron stores. This was due primarily to the differing objectives of each of the studies. In all studies, women who had normal iron stores (SF≥20µg/L, Hb≥120g/L) were referred to as **iron sufficient**. In the 'well-being and fatigue' study, we specifically wanted to investigate women with non-anaemic iron deficiency (SF<20µg/L, Hb≥120g/L). These women were defined as **iron depleted**. In the 'dietary patterns'/'determinants of

iron status' studies, we compared iron sufficient women with women who had non-anaemic iron deficiency (SF<20µg/L, Hb≥120g/L) or iron deficiency anaemia (SF<20µg/L, Hb<120g/L). They were collectively referred to as women with **suboptimal iron status**. In the 'breakfast cereal and fruit' study, women with **low iron stores** (SF≤25µg/L, Hb≥115g/L) were included. We chose a higher cut-off for serum ferritin (≤25 µg/L) as this is a clinically relevant cut-off to demonstrate increased iron absorption with dietary factors (Fairweather-Tait 1995). Diagnostic MedLab's cut-off was used to define adequate haemoglobin concentrations (Hb≥115g/L for women). These wider values also enabled more women to be included in a study which was difficult to recruit for due to an expected prevalence rate of approximately 20% for women with low iron stores (Heath et al 2001b).

Haemoglobin provides a useful measure of iron deficiency anaemia. However, the physiological range of normal haemoglobin is rather broad (115-160g/L (Diagnostic MedLab, Auckland)) and haemoglobin is an insensitive measure of iron deficiency (Gibson 2005a). For haemoglobin and soluble transferrin receptor the total coefficients of variation in women were 4.4 (Borel et al 1991) and 12.9% (Cooper and Zlotkin 1996) respectively, both requiring just one measurement to accurately determine their true value.

In the 'breakfast cereal and fruit' study, soluble transferrin receptor was also measured. Soluble transferrin receptor is a highly sensitive indicator of functional or tissue iron deficiency, and is not affected by inflammation (Gibson 2005a). A reduced ratio of soluble transferrin receptor to serum ferritin provides a more specific and sensitive indicator of improved iron status than either measure alone (Baynes 1996, Cook et al 2003, Lynch 2011a, Skikne et al 1990). Using both serum ferritin and soluble transferrin receptor measurements to assess changes in iron status increased the robustness of the dietary intervention study.

3.4 Dietary assessment

An iron FFQ was developed for this research and was used in all of the studies where dietary intake was measured. A unique feature of the FeFFQ was the grouping of foods according to their iron content and potential impact on iron bioavailability, in order to identify iron-related dietary patterns. Foods were grouped according to similarities,

frequency of consumption, and nutrient content of iron, ascorbic acid, calcium, fibre and vitamin A. A full description of the development of the FeFFQ is provided in chapter three. Previous studies investigating associations between dietary patterns and iron status (Broderstad et al 2011, Shi et al 2006) have used generic FFQs to identify dietary patterns. These FFQs do not appear to have been validated for nutrients, food groups or dietary patterns. In fact, very few studies have investigated the validity and reproducibility of FFQs designed to identify dietary patterns. This is the first study to determine the validity and reproducibility of an FFQ designed specifically to identify iron-related food groups and dietary patterns.

The advantages and disadvantages of FFQs have been described in detail in chapter two. In brief, they tend to be quick to complete, with a low respondent burden and therefore a high response rate. As they cover a longer period of time (e.g. one month) they are useful for describing the dietary habits, and therefore dietary patterns of a group. The FFQ is however dependent on participant memory and ability to recall their diet accurately, with the accuracy of the FFQ often being lower than other dietary assessment methods (Gibson 2005b).

The online nature of the FeFFQ assisted in complete data capturing. Additional safeguards were put in place to ensure all questions were completed before the FeFFQ results were submitted to the database. Researchers were available when participants completed the FeFFQ to answer any questions that arose. A number of statistical methods were used to assess the validity and reproducibility of the FeFFQ, ensuring the robustness of the FeFFQ and 'validation' study.

The FeFFQ was validated for food groupings and iron-related dietary patterns against a weighed food record. This assumes the weighed food record is the gold standard for measuring dietary intake (Crozier et al 2008). However, errors may occur in recording dietary intake or if participants change their dietary intake to impress the researcher or ease the recording process (Gibson 2005c). We aimed to minimise these errors by providing participants with clear written and verbal instructions from a registered dietitian on how to complete the food record. Participants were provided with electronic food scales, household measuring cups and spoons and a photographic portion guide to aid them in completing the food record.

Under-reporting is an issue with all dietary assessment methods. In the 'validation' study, food records were reviewed with all participants to check for missing or incomplete reporting of foods. Within the literature, agreement is lacking as to whether under-reporters should be excluded from the analyses (Heath et al 2000). Using the Goldberg method (Goldberg et al 1991), it was estimated that 14 (12.2%) of participants under-reported their energy intake when completing the food record. However, the Goldberg method assumes all participants were in energy balance. We were unable to determine whether participants were trying to lose weight when they completed FeFFQ1. However six of these 14 women (42.9%) lost more than 1kg in the month following the completion of FeFFQ1, compared with only eight (7.9%) of the remaining participants who were not categorised as under-reporters. It is quite likely that these women were not under-reporting, but instead following a low energy diet. As the FeFFQ was not designed to measure energy intake, we were unable to determine whether the same women under-reported when completing the FeFFQ. For these reasons, under-reporters were not excluded from our analyses in the 'validation' study.

The FeFFQ proved to be a reproducible and reasonably valid method of assessing frequency of food grouping intakes and iron-related dietary patterns. This enabled the FeFFQ to be used in the 'dietary patterns and iron status' study. The FeFFQ was also used in the 'breakfast cereal and fruit' study, where food groupings were further combined to ensure dietary intake did not change prior to and at the end of the dietary intervention.

The dietary practices questionnaire was not validated, meaning we cannot be sure it measured what it was intended to measure. It may also not have been sensitive enough to account for the effects of all possible combinations of foods eaten on iron status.

3.5 Use of empirical methods for identifying and validating dietary patterns

Two empirical methods are commonly used to determine dietary patterns: factor analysis and cluster analysis. Factor analysis reduces data into dietary patterns based on correlations between food items. Factor scores for each participant on each dietary pattern are produced as continuous variables which is seen as advantageous compared

to cluster analysis (Crozier et al 2006), which aggregates individuals into groups (or clusters) based on similarities in dietary intake (Crozier et al 2008). For this reason, we chose to use factor analysis to identify dietary patterns.

As discussed in chapter's two to four, factor analysis involves the researcher making a number of subjective decisions based on the literature and available evidence. These include the number of food groupings to enter into the factor analysis, whether to adjust input variables (e.g. for energy intake), cut-off points for eigenvalues, methods of rotation, the number of factors (dietary patterns) to extract, and the naming and interpretation of dietary patterns (Newby and Tucker 2004). While these decisions were carefully considered (and documented in chapters three and four) by three members of the research group, the factors/dietary patterns identified may have been different, had alternative decisions been made.

Unlike other studies which have collapsed food groupings into smaller groups, we entered only the top 30 most frequently consumed foods into the factor analysis. The reasons for this were the FeFFQ was specifically developed to group foods based on their content of nutrients likely to affect iron intake and bioavailability. By collapsing these food groups further we would have overridden the original purpose of the questionnaire. Food groupings consumed less than twice per week were thought to have minimal effect on iron status, and for most people consuming 15-20 different foods per week is usual, with 30 or more different foods recommended per week for optimal health (Hodgson et al 1994). This aligns with the approach used. The grouping of foods usually depends on the study question (e.g. one of the criteria for grouping meats was their iron content). Differences between studies in the grouping of food item variables or number of variables entered into the factor analysis may have affected the dietary patterns derived (Newby and Tucker 2004).

3.6 Measurement of well-being and fatigue

The 'well-being and fatigue' study investigated both history of iron deficiency and current iron status. Participants remained unaware of their iron status when completing questionnaires on quality of life, so that knowledge of current iron status was unlikely to affect participant responses. Knowledge of previous iron deficiency may have however,

had an impact on participant responses. Two tools were used to measure self-perceived health, well-being and fatigue. The SF-36 questionnaire has been used previously to investigate the relationship between iron status and self-perceived health, well-being and fatigue (Grondin et al 2008, Patterson et al 2000, Patterson et al 2001). It is one of the most extensively validated tools for measuring quality of life, with the vitality subscale of the SF-36 recognised as an established measure of fatigue (Contopoulos-Ioannidis et al 2009). The MFSI-SF was also used to investigate the relationship between iron status and fatigue. We chose to use this tool as it does not refer to any medical diagnosis or disease (Dittner et al 2004) or assume the presence of fatigue (Stein et al 2004), and it had good psychometric properties (Stein et al 2004, Whitehead 2009). However, the MFSI-SF has been validated in cancer patients only, and therefore may not have been the most appropriate tool to measure the relationship between iron status and fatigue. In future studies investigating the association between iron status and quality of life, it is recommended that the SF-36 questionnaire be used due to its extensive validation and so that any results can be compared with previous studies.

4 Discussion of main results

4.1 Causes of iron deficiency in young women

A major objective of this research was to determine the causes of suboptimal iron status in premenopausal women. The determination of factors that affect iron status enables preventative strategies and solutions for iron deficiency to be put in place. Most studies investigating the causes of iron deficiency have considered the effects of individual nutrients and foods, which has several limitations (Hu 2002, Newby and Tucker 2004). As iron bioavailability is affected by a number of components in foods (Benkhedda et al 2010, Diaz et al 2003, Hurrell et al 1999, Hurrell et al 2003, Hurrell et al 2006), the consideration of how foods are consumed in combination (e.g. dietary pattern analysis) may be a more powerful approach to investigate determinants of iron status.

Unlike other studies investigating the impact of dietary patterns on iron status (Broderstad et al 2011, Shi et al 2006), we focussed exclusively on premenopausal women, used two measures of iron status (serum ferritin and haemoglobin), and considered a wide range of

non-dietary factors likely to impact on iron status. Blood donation in the past year and being of Asian ethnicity were the strongest predictors of suboptimal iron status followed by having children, previous iron deficiency, following a 'milk & yoghurt' dietary pattern, and having a longer menstrual period. Following a 'meat & vegetable' dietary pattern reduced the risk of suboptimal iron status.

All of these factors should be considered in the treatment and prevention of iron deficiency. Modifiable risk factors include blood donation, dietary patterns and duration of menstrual period. Blood donation was found to increase the risk of suboptimal iron status considerably. Donating blood means an additional loss equivalent to 1-1.35mg of iron per day, assuming six months between donations (Coad and Conlon 2011). In New Zealand, women are able to donate blood every four months if they are not anaemic (New Zealand Blood Service 2010), potentially making it extremely difficult to compensate for this extra iron loss. Preventing blood donation in women at risk of iron deficiency or reducing the number of times blood is donated (i.e. lengthening the time period between calling women back for blood donation) may help to reduce the risk of suboptimal iron status in some women. However, this may not be an acceptable approach for women who feel a moral responsibility to donate blood.

Another approach is to focus on diet, particularly for those women at risk of suboptimal iron status (e.g. blood donors). The time of blood donation may be an opportune time to reinforce dietary messages regarding the improvement of iron status. A 'meat & vegetable' dietary pattern was associated with a reduced risk of suboptimal iron status. While it seems obvious to encourage women to increase their intake of meat, other factors must also be taken into consideration. For example, many women choose to avoid meat for a variety of reasons (Craig et al 2009) and a high meat intake has been associated with increased risk of some cancers (Gonzalez and Riboli 2010). While milk and yoghurt were shown to increase the risk of suboptimal iron status, they provide essential nutrients and there is evidence to support their role in the prevention of osteoporosis (Alvarez-Leon et al 2006). More research is needed regarding the effect of dairy product consumption (e.g. timing of intake) on iron status.

Women at risk of suboptimal iron status and who have a longer duration of menstrual period could consider the use of the oral contraceptive pill to reduce menstrual bleeding.

However, this type of intervention would need to be considered on an individual basis and under the supervision of a medical doctor.

Non-modifiable risk factors for suboptimal iron status including ethnicity, having children, and previous iron deficiency should all be considered when identifying women at risk of iron deficiency. Being of Asian ethnicity increased the risk of suboptimal iron status by more than five times. Another New Zealand study found Asian female high school students to also have an increased risk of iron deficiency and iron deficiency anaemia (Schaaf et al 2000). This may be due to genetic (Whitfield et al 2003) or lifestyle factors. However in the 'dietary patterns' study, the 'Asian' dietary pattern was not associated with iron status, despite significantly more Asian women being in quintile five of this dietary pattern compared with quintile one. There has not been a large amount of research undertaken regarding the effects of having children and previous iron deficiency on risk of suboptimal iron status. However, these would be easy questions to include when screening for women at risk of iron deficiency.

In total these factors explained more than 20% of the variance in suboptimal iron status, meaning a large percentage of the variation in suboptimal iron status remained unaccounted for. As the nature and location of the genes affecting iron status are identified, future research should consider the extent to which genetic variability impacts on iron status (Zimmermann et al 2010).

It is difficult to compare the results of this study with those of other studies due to the different populations used, the varying cut-offs used to determine iron deficiency, and the range of variables investigated in different studies. For example, many cross-sectional studies in young women have not investigated the influence of ethnicity on iron status (Heath et al 2001b, Pynaert et al 2009), possibly due to lack of access to ethnically diverse populations.

While dietary pattern analysis provides excellent insight into the effect of combinations of food on iron status, it is not clear how dietary patterns can be used effectively in both clinical practice and in public health nutrition. To further establish the role of dietary patterns more research is needed which focuses on how dietary patterns can be used in the assessment, treatment and education of individuals, as well as how dietary patterns

can be used to make food-based dietary guidelines for the general population. A review paper suggested more research is needed regarding the methodological issues in dietary pattern analysis such as the validity and reproducibility of dietary patterns, energy adjustment and associations with biochemical markers and disease (Newby and Tucker 2004). Our study on the validity and reproducibility of the FeFFQ helped in part to address this.

4.2 Consequences of iron deficiency in young women

There is a lack of clear consensus regarding the effect of non-anaemic iron deficiency on self-perceived health, well-being and fatigue. This is due in part to variations in the methodologies used (e.g. participant inclusion criteria, assessment tools used and participants awareness of their iron status). In this study, we observed no significant differences between iron sufficient and iron depleted women (without anaemia) in self-perceived health, well-being and fatigue. Smoking, a history of suboptimal iron status, and having a current medical condition were significant negative predictors of MFSI-SF physical fatigue, and should therefore be controlled for in any study investigating causes of fatigue. This study was limited by the unexpectedly low number of participants with depleted iron stores. For more conclusive results, future studies should be carried out in a larger population group. Both longitudinal studies and randomised controlled trials should be considered. Validated assessment tools should be used and participants should remain unaware of their iron status during testing.

4.3 Solutions for iron deficiency in young women

Iron deficiency is common in developing and developed countries such as New Zealand (McLean et al 2008, University of Otago and Ministry of Health 2011). It is therefore important that appropriate solutions to iron deficiency are found for the target population involved. Solutions may include iron supplementation, iron fortification of foods or education to improve dietary practices which enhance the iron bioavailability of the diet (Food and Agricultural Organization of the United Nations/World Health Organization 2004). This study presents one possible solution, with results showing an iron-fortified

breakfast cereal consumed with kiwifruit compared to a banana improved iron status in healthy women with low iron stores.

There are both advantages and disadvantages to this approach. The positive results observed, minimal side effects experienced and high compliance of the women involved in the study suggests that consuming an iron-fortified breakfast cereal with kiwifruit is a feasible method of correcting iron deficiency in young women. This approach provides additional nutritional benefits including the consumption of a breakfast meal and fruit, and the increased consumption of other nutrients (e.g. vitamin E). Eating a breakfast cereal and fruit meal appeared to be easy to incorporate into these women's everyday life, with minimal impact on other family members. Breakfast is also a meal where people are more likely to eat exactly the same type of food each day, and if implemented in the 'real world', it would be a cost-effective change for women to make to improve their iron status. While meat consumption is often recommended to improve iron status, this may be more difficult to achieve. In a study where participants received intensive counselling to increase the iron intake and bioavailability of the diet, meat intake only increased by 31g per day (Heath et al 2001a).

However, the 'breakfast cereal and fruit' approach to correcting iron deficiency also has several limitations which need to be considered. Participants received high levels of support and encouragement throughout the study (e.g. telephone calls, emails) to assist them in eating the breakfast cereal and fruit each day. The level of support provided would not be practical in a real-life situation and therefore compliance may not be so high.

The iron added to the breakfast cereal was encapsulated ferrous sulphate. Ferrous sulphate is highly bioavailable (Hurrell 2002), and encapsulation protects against the sensory changes, and fat oxidation associated with the addition of ferrous sulphate to foods. However, encapsulated ferrous sulphate is more expensive than the less bioavailable elemental iron powders typically added to breakfast cereals (Hurrell 2002). Food manufacturers are unlikely to be willing to pay for this added expense, unless it is seen as a profitable venture. Furthermore, a significant dose of iron (16mg per serve) was added to each serving (64g) of breakfast cereal. In New Zealand, the maximum claim that can be made for iron added to breakfast cereals is 3mg per reference quantity (Food Standards Australia New Zealand 2011). Most iron-fortified breakfast cereals in

New Zealand contain 6-7mg iron per 100g. More research is needed to determine if smaller doses of less bioavailable iron would result in similar findings.

When considering the suitability of potential solutions to iron deficiency the target group must be considered. This research was undertaken in New Zealand, a developed country. Market-driven fortification is common in developed countries and includes initiatives led by the manufacturer to enhance profits including the addition of micronutrients to breakfast cereals (Lynch 2011b). The 'breakfast cereal and fruit' approach may be most suitable in developed countries where women are able to afford products with additional micronutrients. This approach may be less suitable for addressing iron deficiency in developing countries, where women are at increased risk of iron deficiency (McLean et al 2008). Women may not consume breakfast cereal on a regular basis, and for many women, breakfast cereal may be seen as a luxury food item which few can afford. For mass fortification to occur, breakfast cereal would need to be a food commonly consumed by the general population (Lynch 2011b). However, the methodology used in this study could be replicated in food staples consumed in developing countries, to assess their feasibility and impact on improving iron status.

There are also limitations associated with the use of kiwifruit to address iron deficiency. Availability and logistical issues such as transport and storage need to be considered when using fresh fruit in dietary interventions. Kiwifruit are a seasonal fruit, meaning other foods and drinks high in ascorbic acid would need to be consumed when kiwifruit are unavailable. It is assumed these foods or drinks would have similar effects on iron status, but this needs to be confirmed. Furthermore, both green and gold kiwifruit are allergenic (Bublin et al 2004, Lucas et al 2003, Lucas et al 2005, Lucas et al 2007), meaning they are not suitable for all people to consume. Crops such as kiwifruit are also vulnerable to environmental conditions and disease. In November 2010, the presence of *Psuedomona's Syringae* pv. *Actinidiae* (Psa-V) (bacteria which causes the death of kiwifruit vines) was identified in some kiwifruit orchards in New Zealand. Hort 16A gold kiwifruit vines were particularly affected, with reports of gold kiwifruit exports being reduced by two thirds (Ministry of Agriculture and Forestry 2011).

The 'breakfast cereal and fruit' study overcame many of the limitations of previous studies investigating the effect of ascorbic acid on iron status over extended periods. However,

further research is needed to determine the optimal level of iron and ascorbic acid needed to produce clinically significant results. For example, would the same results be obtained with a lower dose of iron or a different form of iron, such as the elemental iron typically found in breakfast cereals. Other food vehicles should also be investigated as alternatives to breakfast cereal, which has a high phytic acid content and therefore, an inhibitory effect on iron absorption. There may also be the potential to develop a targeted food product (containing iron and ascorbic acid) for young women at risk of iron deficiency. When considering possible solutions to iron deficiency consumer acceptability and accessibility to any food product must be considered, as well as the cost and purpose for which iron fortification was intended. Finally, while a dietary intervention such as this one may improve iron status, it would be useful to investigate the longer term effects of such an intervention. For example, the dietary practices and iron status of participants several months after the dietary intervention is completed.

5 Final conclusions

An iron food frequency questionnaire was developed which showed reasonable validity and high reproducibility for determining the frequency of intake of food groupings and identifying iron-related dietary patterns. Using this food frequency questionnaire, it was determined that premenopausal women who followed a 'meat & vegetable' dietary pattern had a reduced risk of suboptimal iron status, while those who followed a 'milk & yoghurt' dietary pattern had an increased risk of suboptimal iron status. Blood donation in the past year, being of Asian ethnicity, having children and previous iron deficiency were stronger predictors of suboptimal iron status than both dietary patterns. However, both dietary patterns were stronger predictors of suboptimal iron status than the only significant measure of menstrual blood loss (longer duration of menstrual period). No significant differences were observed in self-perceived health, well-being and fatigue between iron sufficient and iron depleted female university students. Consumption of an iron-fortified breakfast cereal with kiwifruit (ascorbic acid, lutein, zeaxanthin-rich) compared to banana (low ascorbic acid, lutein, zeaxanthin) improved iron status in healthy women with depleted iron stores over a 16 week period.

6 Research recommendations

1. Validate the dietary practices questionnaire against a food record. If it shows good validity, use in a larger population group to examine the effect of dietary practices (e.g. how and when dairy products are consumed) on iron status.

2. Investigate how dietary patterns can be used effectively in the clinical setting. For example, when a dietitian/nutritionist is consulted by clients with iron deficiency, dietary patterns could be used as an assessment, treatment or education tool.

3. Explore how dietary patterns can be used to develop food-based dietary guidelines for the general population.

4. Future research should consider the extent to which genetic variability impacts on iron absorption and status. For example, genetic variation in the expression of divalent metal transporter-1, and how this may affect iron absorption and therefore response to treatment.

5. Investigate self-perceived health, well-being and fatigue in a larger group of women using the SF-36 questionnaire. Consider the use of a longitudinal study or a randomised controlled trial in women who have non-anaemic iron deficiency.

6. Determine the optimal level or source of iron-fortificant and ascorbic acid (or ascorbic acid-rich fruit) needed to produce clinically significant results when added to a variety of food products, including consumer acceptability and the public health implications of using these food products.

7. Examine the longer term impact of dietary interventions on iron status and dietary practices by following up participants several months after the dietary intervention finishes.

7 References

Alvarez-Leon EE, Roman-Vinas B, Serra-Majem L (2006). Dairy products and health: a review of the epidemiological evidence. *Br J Nutr* **96:** S94-S99.

Baynes RD (1996). Assessment of iron status. *Clin Biochem* **29:** 209-215.

Benkhedda K, L'Abbe MR, Cockell KA (2010). Effect of calcium on iron absorption in women with marginal iron status. *Br J Nutr* **103:** 742-748.

Borel MJ, Smith SM, Derr J, Beard L (1991). Day-to-day variation in iron-status indices in healthy men and women. *Am J Clin Nutr* **54:** 729-735.

Boushey C, Harris J, Bruemmer B, Archer SL, Van Horn L (2006). Publishing nutrition research: A review of study design, statistical analyses, and other key elements of manuscript preparation, part 1. *J Am Diet Assoc* **106:** 89-96.

Broderstad AR, Melhus M, Brustad M, Lund E (2011). Iron stores in relation to dietary patterns in a multiethnic population: the SAMINOR study. *Public Health Nutr* **14:** 1-8.

Bublin M, Mari A, Ebner C, Knulst A, Scheiner O, Hoffmann-Sommergruber K *et al* (2004). IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *J Allergy Clin Immunol* **114:** 1169-1175.

Coad J, Conlon C (2011). Iron deficiency in women: assessment, causes and consequences *Curr Opin Clin Nutr Metab Care* **14:** 625-634.

Contopoulos-Ioannidis DG, Karvouni A, Kouri I, Ioannidis JPA (2009). Reporting and interpretation of SF-36 outcomes in randomised trials: systematic review. *Br Med J* **338:** 1-6.

Cook JD, Flowers CH, Skikne BS (2003). The quantitative assessment of body iron. *Blood* **101:** 3359-3364.

Cooper MJ, Zlotkin SH (1996). Day-to-day variation of transferrin receptor and ferritin in healthy men and women. *Am J Clin Nutr* **64:** 738-742.

Craig WJ, Mangels AR, Ada (2009). Position of the American Dietetic Association: Vegetarian Diets. *J Am Diet Assoc* **109:** 1266-1282.

Crozier SR, Robinson SM, Borland SE, Inskip H, SWS Study Group (2006). Dietary patterns in the Southampton Women's Survey. *Eur J Clin Nutr* **60:** 1391-1399.

Crozier SR, Inskip HM, Godfrey KM, Robinson SM (2008). Dietary patterns in pregnant women: a comparison of food-frequency questionnaires and 4d prospective diaries. *Br J Nutr* **99:** 869-875.

Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP (2003). The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78:** 436-440.

Dittner AJ, Wessely SC, Brown RG (2004). The assessment of fatigue - A practical guide for clinicians and researchers. *J Psychosom Res* **56:** 157-170.

Fairweather-Tait SJ (1995). Iron Absorption. In: British Nutrition Foundation (ed). *Iron: Nutritional and physiological significance*. Chapman and Hall: London. pp 3-12.

Fawcett JP, Brooke M, Beresford CH (1998). Iron deficiency and anaemia in a longitudinal study of New Zealanders at ages 11 and 21 years. *N Z Med J* **111:** 400-402.

Food and Agricultural Organization of the United Nations/World Health Organization (2004). Iron. *Vitamin and Mineral Requirements in Human Nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand*, 2nd edn. World Health Organization: Geneva. pp 246-278.

Food Standards Australia New Zealand (2011). Chapter 1 - General Food Standards - Standard 1.3.2 - Vitamins and Minerals. *Australia New Zealand Food Standards Code*. Food Standards Australia New Zealand: Canberra.

Gibson RS (2005a). Assessment of iron status. In: Gibson RS (ed). *Principles of Nutrition Assessment*, 2nd edn. Oxford University Press: New York. pp 446-469.

Gibson RS (2005b). Measuring food consumption of individuals. In: Gibson RS (ed). *Principles of Nutritional Assessment*, 2nd edn. Oxford University Press: New York. pp 41-64.

Gibson RS (2005c). Measurement errors in dietary assessment. In: Gibson RS (ed). *Principles of Nutrition Assessment*, 2nd edn. Oxford University Press: New York. pp 105-128.

Goldberg G, Black A, Jebb S, Cole T, Murgatroyed P, Coward W *et al* (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45:** 569-581.

Gonzalez CA, Riboli E (2010). Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur J Cancer* **46:** 2555-2562.

Grondin M-A, Ruivard M, Perreve A, Derumeaux-Burel H, Perthus I, Roblin J *et al* (2008). Prevalence of iron deficiency and health-related quality of life among female students. *J Am Coll Nutr* **27:** 337-341.

Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991). Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* **53:** 112-119.

Heath ALM, Skeaff CM, Gibson RS (2000). The relative validity of a computerized food frequency questionnaire for estimating intake of dietary iron and its absorption modifiers. *Eur J Clin Nutr* **54:** 592-599.

Heath ALM, Skeaff CM, O'Brien SM, Williams SM, Gibson RS (2001a). Can dietary treatment of non-anemic iron deficiency improve iron status? *J Am Coll Nutr* **20:** 477-484.

Heath ALM, Skeaff CM, Williams S, Gibson RS (2001b). The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. *Public Health Nutr* **4:** 197-206.

Hodgson JM, Hsu-Hage B, Wahlqvist M (1994). Food variety as a quantitative descriptor of food intake. *Ecol Food Nutr* **32:** 137-148.

Horwath CC (1991). Dietary intake and nutritional status among university undergraduates. *Nutr Res* **11:** 395-404.

Hu FB (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13:** 3-9.

Hulthen L, Lindstedt G, Lundberg P-A, Hallberg L (1998). Effect of mild infection on serum ferritin concentration - clinical and epidemiological implications. *Eur J Clin Nutr* **52:** 376-379.

Hurrell RF, Reddy M, Cook JD (1999). Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br J Nutr* **81:** 289-295.

Hurrell RF (2002). Fortification: Overcoming technical and practical barriers. *J Nutr* **132:** 806S-812S.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am J Clin Nutr* **77:** 1213-1219.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2006). Meat protein fractions enhance nonheme iron absorption in humans. *J Nutr* **136:** 2808-2812.

Lucas JSA, Lewis SA, Hourihane JO (2003). Kiwi fruit allergy: A review. *Pediatr Allergy Immunol* **14:** 420-428.

Lucas JSA, Lewis SA, Trewin JB, Grimshaw KEC, Warner JO, Hourihane JO (2005). Comparison of the allergenicity of Actinidia deliciosa (kiwi fruit) and Actinidia chinensis (gold kiwi). *Pediatr Allergy Immunol* **16:** 647-654.

Lucas JSA, Nieuwenhuizen NJ, Atkinson RG, MacRae EA, Cochrane SA, Warner JO *et al* (2007). Kiwifruit allergy: actinidin is not a major allergen in the United Kingdom. *Clin Exp Allergy* **37:** 1340-1348.

Lynch S (2011a). Improving the assessment of iron status. *Am J Clin Nutr* **93:** 1188-1189.

Lynch SR (2011b). Why Nutritional Iron Deficiency Persists as a Worldwide Problem. *J Nutr* **141:** 763S-768S.

McLean E, Cogswell M, Egli I, Woidyla D, de Benoist B (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr* **12:** 444-454.

Ministry of Agriculture and Forestry (2011). *Situation and outlook for New Zealand agriculture and forestry June 2011*. Ministry of Agriculture and Forestry: Wellington.

New Zealand Blood Service (2010). *All about donating blood*. New Zealand Blood Service: Auckland.

Newby PK, Tucker KL (2004). Empirically derived eating patterns using factor or cluster analysis: A review. *Nutr Rev* **62:** 177-203.

Patterson A, Brown W, Powers J, Roberts D (2000). Iron deficiency, general health and fatigue: Results from the Australian longitudinal study on women's health. *Qual Life Res* **9:** 491-497.

Patterson AJ, Brown WJ, Roberts DCK (2001). Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. *J Am Coll Nutr* **20:** 337-342.

Pynaert I (2007). *Public health aspects of iron intake and iron status*. Ghent University: Ghent.

Pynaert I, De Bacquer D, Matthys C, Delanphe J, Temmerman M, De Backer G *et al* (2009). Determinants of ferritin and soluble transferrin receptors as iron status parameters in young adult women. *Public Health Nutr* **12:** 1775-1782.

Schaaf D, Scragg R, Metcalf P, Grant C, Buchanan J (2000). Prevalence of iron deficiency in Auckland high school students. *N Z Med J* **113:** 347-350.

Shi ZM, Hu XS, Yuan BJ, Pan XQ, Dai Y, Holmboe-Ottesen G (2006). Association between dietary patterns and anaemia in adults from Jiangsu Province in Eastern China. *Br J Nutr* **96:** 906-912.

Sibbald B, Roland M (1998). Understanding controlled trials - Why are randomised controlled trials important? *Br Med J* **316:** 201-201.

Skikne BS, Flowers CH, Cook JD (1990). Serum transferrin receptor - a quantitative measure of tissue iron-deficiency. *Blood* **75:** 1870-1876.

Stein KD, Jacobsen PB, Blanchard CM, Thors C (2004). Further validation of the multidimensional fatigue symptom inventory-short form. *J Pain Symptom Manage* **27:** 14-24.

Truswell AS, Mann J (2007). Introduction. In: Mann J, Truswell AS (eds). *Essentials of human nutrition*, 3rd edn. Oxford University Press: Oxford. pp 1-7.

University of Otago and Ministry of Health (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Ministry of Health: Wellington.

Vissers MCM, Bozonet SM, Pearson JF, Braithwaite LJ (2011). Dietary ascorbate intake affects steady state tissue concentrations in vitamin C-deficient mice: tissue deficiency after suboptimal intake and superior bioavailability from a food source (kiwifruit). *Am J Clin Nutr* **93:** 292-301.

Whitehead L (2009). The Measurement of Fatigue in Chronic Illness: A Systematic Review of Unidimensional and Multidimensional Fatigue Measures. *J Pain Symptom Manage* **37:** 107-128.

Whitfield JB, Treloar S, Zhu G, Powell LW, Martin NG (2003). Relative importance of female specific and non-female specific effects on variation in iron stores between women. *Br J Haematol* **120:** 860-866.

Zimmermann MB, Hurrell RF (2007). Nutritional iron deficiency. *Lancet* **370:** 511-520.

Zimmermann MB, Harrington M, Villalpando S, Hurrell RF (2010). Nonheme-iron absorption in first-degree relatives is highly correlated: a stable-isotope study in mother-child pairs. *Am J Clin Nutr* **91:** 802-807.

APPENDICES

APPENDIX 1

Published papers

Published papers

Beck KL, Kruger R, Conlon C, Heath ALM, Matthys C, Coad J, Stonehouse W (2013). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. *European Journal of Nutrition* 52:2, pages 467-476.

Beck KL, Kruger R, Conlon C, Heath ALM, Coad J, Matthys C, Stonehouse W (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women in New Zealand. *Journal of the Academy of Nutrition and Dietetics* 112: 8, pages 1177-1187.

Beck KL, Conlon C, Kruger R, Heath ALM, Matthys C, Coad J, Stonehouse W (2012). Iron status and self-perceived health, well-being and fatigue in female university students living in New Zealand. *Journal of the American College of Nutrition* 31:1, pages 45-53.

Beck KL, Conlon C, Kruger R, Coad J, Stonehouse W (2011). Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. *British Journal of Nutrition* 105, pages 101–109.

Beck KL, Conlon C, Kruger R, Coad J, Stonehouse W (2010). Study protocol: The effect of gold kiwifruit consumed with an iron fortified breakfast cereal meal on iron status in women with low iron stores: A 16 week randomised controlled intervention study. *BMC Public Health* **10:**36.

Beck KL, Conlon C, Kruger R, Matthys C, Coad J, Heath ALM, Stonehouse W (2008). Iron status of female university students living in New Zealand. *Proceedings of the 43rd Annual Conference of the Nutrition Society of New Zealand* 33, pages 46-50.

APPENDIX 2

Conference presentations and abstracts

Conference presentations and abstracts

2012

Beck KL, Conlon CA, Kruger R, Matthys C, Coad J, Heath ALM, Stonehouse W (2012). Dietary patterns and other potential determinants of iron status in premenopausal women living in New Zealand. In *Nutrition & Dietetics* Vol. 69 (supplement 1), p58.
Oral presentation at the *16th International Congress of Dietetics*, Sydney, Australia.

2011

Beck KL, Kruger R, Conlon C, Matthys C, Coad J, Heath ALM, Stonehouse W (2011). Suboptimal iron status and associated dietary patterns in pre-menopausal women living in Auckland, New Zealand. In *Proceedings of the Nutrition Society of Australia Vol. 35, p9.*
Oral presentation at the *35th Annual Scientific Meeting of the Nutrition Society of Australia in conjunction with the Nutrition Society of New Zealand,* Queenstown, New Zealand.

Beck KL, Kruger R, Conlon C, Matthys C, Coad J, Heath ALM, Stonehouse W (2011). Dietary patterns and iron status in young women. In *Nutrition & Dietetics* Vol. 68, p4.
Oral presentation at the *Dietitians New Zealand conference,* Nelson, New Zealand.
Winner of the FSANZ award for postgraduate research.

Beck KL, Stonehouse W, Kruger R, & Conlon C (2011). Changes in iron status and dietary habits in young women nine months after completing a dietary intervention aimed at improving iron status. In *Nutrition & Dietetics* Vol. 68, p11.
Poster presentation at *Dietitians New Zealand conference*, Nelson, New Zealand.

Beck K (2011). Determinants of iron status in young women.
Oral presentation at the *Auckland Nutrition Research Network student presentations*.

2010

Beck K, Conlon C, Kruger R, Coad J, & Stonehouse W. (2010). Kiwifruit and fortified breakfast cereal – a winning combination for iron status.
Oral presentation at the *New Zealand Institute of Food Science and Technology Conference,* Auckland, New Zealand.

Beck K (2010). Dietary patterns and iron status in young women.
Oral presentation at the *Auckland Nutrition Research Network student presentations*.
Winner of best presentation.

2009

Beck KL, Conlon CA, Kruger R, Coad J, & Stonehouse W. (2009). Consuming kiwifruit with an iron fortified breakfast cereal meal improves iron status in women with low iron stores. In *Annals of Nutrition and Metabolism* Vol. 55, p295.
Poster presentation at the *International Congress of Nutrition*, Bangkok, Thailand.

Beck K, Conlon C, Kruger R, Coad J, & Stonehouse W. (2009). Consuming kiwifruit with an iron fortified breakfast cereal improves iron status in women with low iron stores. In *Nutrition & Dietetics* Vol. 66, p60.
Oral presentation at the *Dietitians New Zealand Conference,* Napier, New Zealand.

Beck K, Conlon C, Kruger R, Coad J, & Stonehouse W. (2009). Consuming kiwifruit with an iron fortified breakfast cereal meal improves iron status in women with low iron stores. In *Proceedings of the Nutrition Society of Australia*, Vol 33. p96.
Poster presentation at the *33rd Annual Scientific Meeting of the Nutrition Society of Australia in conjunction with the Nutrition Society of New Zealand,* Newcastle, Australia.

Beck K, Kruger R, Conlon C, Matthys C, Coad J, & Stonehouse W. (2009). The development of a computerised iron habits assessment tool to determine dietary patterns and practices affecting iron status.
Poster presentation at the meeting of *7th International Conference on Diet and Activity Methods.* Washington, DC, USA.

Beck KL (2009). Consuming kiwifruit with an iron fortified breakfast cereal improves iron status in women with low iron stores: a randomised controlled trial.
Oral presentation at the *Auckland Nutrition Research Network*.

2008

K Beck, C Conlon, R Kruger, C Matthys, J Coad, ALM Heath & Stonehouse W (2008). Iron status of female university students living in New Zealand.
Oral presentation at the *Annual Nutrition Society of New Zealand Conference.* Christchurch, New Zealand.

APPENDIX 3

Contribution of Authors (including statements of contribution to doctoral thesis containing publications)

Contribution of Authors

Chapter	Author	Contribution to paper
'Validation' study (Chapter 3)	Kathryn Beck	Led the research, designed research including development of dietary questionnaires, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript
	Rozanne Kruger	Designed research including development of dietary questionnaires, recruited participants, conducted research, revised and approved the manuscript
	Cathryn Conlon	Assisted with development of dietary questionnaires and design of research, conducted research, revised and approved the manuscript
	Anne-Louise Heath	Reviewed dietary questionnaires, assisted with interpretation of results, revised and approved the manuscript
	Jane Coad	Assisted with design of research, revised and approved the manuscript
	Christophe Matthys	Assisted with development of dietary questionnaires and design of research, and interpretation of results, revised and approved the manuscript
	Beatrix Jones	Advised regarding the statistical analysis of data, revised and approved the manuscript
	Welma Stonehouse	Designed research, conducted research, supervised the statistical analysis of data, revised and approved the manuscript
'Dietary patterns and iron status' study (Chapter 4)	Kathryn Beck	Led the research, designed research, developed the dietary questionnaires, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript
	Rozanne Kruger	Designed research, developed the dietary questionnaires, recruited participants, conducted research, assisted with dietary pattern analysis, revised and approved the manuscript
	Cathryn Conlon	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Anne-Louise Heath	Designed research, assisted in interpretation of results related to iron status, revised and approved the manuscript
	Christophe Matthys	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Jane Coad	Assisted in interpretation of results related to iron status, revised and approved the manuscript
	Welma Stonehouse	Designed research, recruited participants, conducted research, supervised the statistical analysis of data, revised and approved the manuscript

Contribution of Authors

Chapter	Author	Contribution to paper
'Determinants of iron status' study (Chapter 5)	Kathryn Beck	Led the research, designed research, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript
	Cathryn Conlon	Study leader, designed research, recruited participants, conducted research, revised and approved the manuscript
	Rozanne Kruger	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Anne-Louise Heath	Designed research, assisted in interpretation of results related to iron status, revised and approved the manuscript
	Christophe Matthys	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Jane Coad	Assisted in interpretation of results related to iron status, revised and approved the manuscript
	Welma Stonehouse	Designed research, recruited participants, conducted research, supervised the statistical analysis of data, revised and approved the manuscript
'Well-being and fatigue' study (Chapter 6)	Kathryn Beck	Designed research, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript
	Cathryn Conlon	Study leader, designed research, recruited participants, conducted research, revised and approved the manuscript
	Rozanne Kruger	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Anne-Louise Heath	Designed research, assisted in interpretation of results, revised and approved the manuscript
	Christophe Matthys	Designed research, recruited participants, conducted research, revised and approved the manuscript
	Jane Coad	Assisted in interpretation of results related to iron status, revised and approved the manuscript
	Welma Stonehouse	Designed research, recruited participants, conducted research, supervised the statistical analysis of data, revised and approved the manuscript

Contribution of Authors

Chapter	Author	Contribution to paper
'Breakfast cereal and fruit' study (Chapter 7)	Kathryn Beck	Study leader, designed research including development of dietary questionnaires, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript
	Cathryn Conlon	Study leader, designed research, recruited participants, conducted research, supervised laboratory work, conducted research, revised and approved the manuscript
	Rozanne Kruger	Designed research including development of dietary questionnaires, recruited participants, conducted research, revised and approved the manuscript
	Jane Coad	Interpretation of results related to iron status, revised and approved the manuscript
	Welma Stonehouse	Designed research, recruited participants, conducted research, supervised the statistical analysis of data, revised and approved the manuscript

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Kathryn Beck

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Beck KL, Kruger R, Conlon C, Heath ALM, Coad J, Matthys C, Stonehouse W (2012). The relative validity and reproducibility of an iron food frequency questionnaire for identifying iron-related dietary patterns in young women in New Zealand. Journal of the Academy of Nutrition and Dietetics 112: 8, pages 1177-1187.

In which Chapter is the Published Work: Chapter 3

Please indicate either:

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Beck KL, Kruger R, Conlon C, Heath ALM, Matthys C, Coad J, Stonehouse W (accepted for publication, March 2012). Suboptimal iron status and associated dietary patterns and practices in premenopausal women living in Auckland, New Zealand. European Journal of Nutrition.

In which Chapter is the Published Work: Chapter 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

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- Describe the contribution that the candidate has made to the Published Work:

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Name of Candidate: Kathryn Beck

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Beck KL, Conlon C, Kruger R, Heath ALM, Matthys C, Coad J, Stonehouse W. Blood donation, Asian ethnicity and parity are stronger predictors of suboptimal iron status than dietary patterns in premenopausal women living in Auckland, New Zealand.

In which Chapter is the Published Work: Chapter 5

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Name of Published Research Output and full reference:

Beck KL, Conlon C, Kruger R, Heath ALM, Matthys C, Coad J, Stonehouse W (2012). Iron status and self-perceived health, well-being and fatigue in female university students living in New Zealand. Journal of the American College of Nutrition 31:1, pages 45-53.

In which Chapter is the Published Work: Chapter 6

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Designed research, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript

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We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Kathryn Beck

Name/Title of Principal Supervisor: Welma Stonehouse

Name of Published Research Output and full reference:

Beck KL, Conlon C, Kruger R, Coad J, Stonehouse W (2011). Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. British Journal of Nutrition 105, pages 101–109.

In which Chapter is the Published Work: Chapter 7

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Study leader, designed research including development of dietary questionnaires, recruited participants, conducted research, analysed data and performed statistical analysis, interpreted the results, main author of manuscript

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APPENDIX 4

Study protocol: The effect of gold kiwifruit consumed with an iron fortified breakfast cereal meal on iron status in women with low iron stores: A 16 week randomised controlled intervention study

This appendix describes the study protocol for the randomised control trial investigating whether consuming high versus low ascorbic acid, lutein, zeaxanthin-rich fruit (gold kiwifruit versus banana) with iron-fortified breakfast cereal and milk improved iron status in women with low iron stores.

Beck KL, Conlon C, Kruger R, Coad J, Stonehouse W (2010). Study protocol: The effect of gold kiwifruit consumed with an iron fortified breakfast cereal meal on iron status in women with low iron stores: A 16 week randomised controlled intervention study. *BMC Public Health* 10:36.

APPENDIX 5

Iron Food Frequency Questionnaire

Iron Food Frequency Questionnaire

When answering this questionnaire consider your intake of food over the past month. To help you do this, please think of an event in your life that happened one month ago and think about your eating patterns since that date.

Example

Consider if you have sugar in all your drinks during the day as well as added to other food items and indicate how many times in the day you are consuming sugar. E.g. drinking 2 cups of coffee with sugar and 4 cups of tea with sugar, one bowl of cereal with sugar and sugar on pancakes at dinner resulting in choosing the category (4 Plus times per day)

In the past month I have eaten this food….									
Food items	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Sugar									✕

Consider if you have pineapple and indicate how many times you are consuming pineapple. E.g. eating pineapple slices with a hamburger meal once a week at lunch and eating carrot and pineapple salad once a week at dinner (2 to 3 times per week)

In the past month I have eaten this food….									
Food items	**I never eat this food**	**Less than once a month**	**1 to 3 times**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Pineapple					✕				

Meat and chicken	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Beef (eg. roast, steak, chops, schnitzel, silverside, casseroles, stir fry, curry, hamburger meat, mince dishes)									
Chicken, turkey or duck (eg. roast, fried, steamed, BBQ, casseroles, stir fry, curry, fried takeaway chicken)									
Lamb, hogget or mutton (eg. roast, steak, chops, BBQ, casseroles, stir fry, curry)									
Pork (eg. roast, chops, steak, casserole, casseroles, stir fry, curry)									
Veal									
Liver, kidney, other offal (including pate)									
Ham, bacon									
Game meats (eg. venison, mutton bird, rabbit)									
Corn beef, canned									

Prepared meat	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Beef Jerky / Biltong									
Sausages, frankfurters, saveloys									
Luncheon sausage, salami, brawn, pastrami									
Black pudding									
Meat pies									

Fish and seafood	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Fresh and frozen fish (eg. snapper, tarakihi, gurnard, flounder, hoki, salmon, white bait, shark, eel)									
Battered and crumbed fish (eg. fish fingers, fish cakes)									
Canned and bottled fish (eg. tuna, salmon, herrings, sardines)									
Mussels, pipi, paua, cockles, oysters									
Scallops, crab sticks, crab, squid, crayfish, kina									
Prawns, shrimps									

Eggs	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Eggs – boiled, fried, poached, scrambled, raw and egg based dishes including quiche, soufflés, frittatas, omelettes									

Nuts	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Peanuts, mixed nuts, macadamias, pecan, hazelnuts, brazil nuts, walnuts, cashews, pistachios									
Almonds									
Pumpkin seeds, sunflower seeds, pinenuts									
Sesame seeds, tahini									

Legumes	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Tofu, soybeans, tempeh									
Beans in sauce (eg. baked beans, chilli beans)									
Beans (canned or dried) (eg. black beans, butter beans, haricot beans, red kidney beans, white kidney beans, refried beans)									
Lentils									
Peas (eg. chick peas, hummus, falafels, split peas, cow peas)									
Dahl (all varieties)									

Dairy products	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Cheese (eg. Cheddar, Colby, Edam, Tasty, blue vein, camembert, parmesan, gouda, processed)									
Cottage cheese, ricotta cheese									
Cream, sour cream, cream cheese, cheese spreads, fromage frais (all varieties)									
Milk (cow's milk) **as a drink** (eg. flavored milk, milk shakes)									
Milk (cow's milk) (all varieties) **added to drinks** (eg. in tea, coffee)									
Milk (cow's milk) (all varieties) **added to food** (eg. cereals, dishes such as macaroni cheese, milk puddings such as rice pudding, custard, semolina, instant puddings, dairy food)									
Soy Milk									
Coconut milk									
Yoghurt									
Ice cream									

Fruit	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Apples									
Bananas, green bananas									
Citrus fruits (eg. orange, tangelo, tangerine, mandarin, grapefruit, lemon)									
Green kiwifruit									
Zespri gold kiwifruit									
Pears, nashi pears									
Stone fruit (eg. apricots, nectarines, peaches, plums, lychees)									
Avocadoes, olives									
Feijoas, persimmon, tamarillos									
Grapes									
Mango									
Watermelon									
Pawpaw (papaya), other melons (eg. honey dew, rock melon)									
Pineapple									
Rhubarb									
Fruit salad, canned									
Strawberries, blackberries, cherries, blueberries, boysenberries, loganberries, cranberries, gooseberries, raspberries									
Sultanas, raisins, currants, figs									
Dried apricots, prunes, dates, mixed dried fruit									

Vegetables	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Potato (eg. boiled, mashed, baked, roasted, fried, chips)									
Kumara (eg. boiled, mashed, baked, roasted, fried, chips)									
Green beans, broad beans, runner beans, asparagus									
Broccoli (all varieties)									
Red cabbage									
Cabbage (all varieties), brussel sprouts									
Capsicum, peppers (all varieties)									
Carrots									
Cauliflower									
Corn (all varieties)									
Courgette, zucchini, cucumber, gerkins or marrow (all varieties)									
Beetroot									
Radishes (all varieties)									
Lettuce									
Mushrooms									
Onions (all varieties), leeks, celery									
Tomatoes (all varieties)									
Peas, green									
Spinach, silver beet, swiss chard (all varieties)									
Other green leafy vegetables (eg. watercress, puha, Whitloof, chicory, kale, chard, collards,									

Chinese kale, Bok Choy)									
Pumpkin, squash, yams									
Parsnip									
Taro leaves (palusami)									

Breakfast cereals or porridge	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Porridge, rolled oats, oat bran, oat meal									
Muesli (all varieties)									
Weetbix (all varieties)									
Cornflakes or rice bubbles									
Bran based cereals (all varieties eg. All Bran, Sultana Bran)									
Light and fruity cereals (eg. Special K, Light and tasty)									
Chocolate based cereals (eg. Milo cereal, CocoPops)									
Sweetened cereals (eg. Nutrigrain, Fruit Loops, Honey Puffs, Frosties)									
Breakfast drinks (eg. Up and Go)									

Grains	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
White rice									
Brown rice									
Instant noodles									
Pasta, noodles (white)									
Pasta, noodles (whole wheat)									
Couscous, polenta									
Bulgur wheat (eg. tabbouleh)									
Wheat germ, wheat bran (flakes)									

Breads, cakes, biscuits and crackers	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
White bread and rolls (including specialty breads such as foccacia, panini, pita, naan, crumpets, pizza bases, tortilla's, burrito, roti)									
Brown bread and rolls (including multigrain, wholegrain, whole meal breads)									
Breads fortified with iron (eg. Mighty White Tip Top bread)									
Fruit and currant bread / buns									
White flour muffins (all varieties)									
Whole meal muffins (all varieties)									
Cakes (all varieties excluding chocolate and fruit cake)									
Chocolate cake									
Fruit cake									
Biscuits, plain sweet									
Biscuits, chocolate or chocolate covered									
Crackers (eg. crisp bread, water crackers, rice cakes, cream crackers, Cruskits, Mealmates									
Iron fortified crackers (eg. Vita wheat)									

Miscellaneous foods and snacks	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Marmite									
Chocolate spread (eg. Nutella)									
Peanut butter									
Butter or margarine									
Cooking oil (all varieties)									
Soup, vegetable based, homemade or canned									
Soup, meat based, homemade or canned									
Sugar (all varieties) added to food / drinks									
Jam, marmalade, honey or syrups									
Muesli or cereal bar (all varieties)									
Chocolate covered Muesli or cereal bar (all varieties)									
Potato crisps									
Milk chocolate									
Dark chocolate									
White chocolate									

Alcohol	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Beer, cider (all varieties)									
Red wine									
White wine									
Spirits (all varieties)									
Ready to drink alcoholic beverages									

Non – alcoholic beverages	**I never eat this food**	**Less than once a month**	**1 to 3 times a month**	**Once per week**	**2 to 3 times per week**	**4 to 6 times per week**	**Once per day**	**2 to 3 times per day**	**4 plus times per day**
Complan, Sustagen (all varieties)									
Milo									
Hot chocolate, drinking chocolate, Cocoa, Ovaltine, Nesquik									
Coffee (all varieties)									
Black tea									
Herbal tea, fruit tea									
Cordials (including syrups, powders) (eg. Blackcurrant, orange)									
Fruit and vegetable juices (all varieties)									
Sports drinks (eg. Powerade)									
Energy drinks (eg. Red Bull, V)									
Water (including tap water or bottled water)									

APPENDIX 6

Dietary Practices Questionnaire

Dietary practices questionnaire

When answering this questionnaire please consider your intake of food over the past month. To help you do this, please think of an event in your life that happened one month ago and think about your eating patterns since that date.

1. At which of the following time periods do you eat or drink usually?

We realise that everyone has different eating patterns. Feel free to decide for yourself which eating occasion you choose to call breakfast, etc no matter what time it happens in the day.

	Always (5 to 7 times per week)	Sometimes (2 to 4 times per week)	Never (0 to 1 times per week)
Before breakfast			
Breakfast			
Between breakfast and lunch			
Lunch			
Between lunch and evening meal			
Evening meal			
After evening meal			
Other eg. midnight snack			

2. Do you usually eat breakfast?

YES	
NO	

3. If you usually eat *breakfast* what types of food do you *usually* eat? (tick as many as apply)

Breakfast cereals	
Porridge	
Bread or toast	
Noodles or rice	
Milk products (eg. milk on cereal, yoghurt, dairy food, cheese)	
Fruit	
Meats (eg. bacon, sausages)	
Baked beans or eggs	
Vegetables	

4. Do you *usually* have a drink *up to 1 hour before, with, or up to 1 hour after* your breakfast?

YES	
NO	

5. If yes, what drinks do you *usually* have with your breakfast? (tick as many as apply)

Fruit or vegetable juices	
Milk or milk-based drinks (all varieties)	
Soy-based drinks	
Chocolate-based drinks (eg. Milo)	
Coffee (all varieties)	
Tea (all varieties)	
Water (all varieties)	
Other	

6. Do you usually eat lunch?

YES	
NO	

7. If you usually eat *lunch*, what types of food do you *usually* eat? (tick as many as apply)

Bread (eg. sandwiches, rolls)	
Starchy food (eg. pasta, rice, potato)	
Milk products (eg. yoghurt, dairy food, cheese)	
Fruit	
Meats (eg. meat, fish, chicken, seafood, ham, salami)	
Legumes, nuts, eggs	
Vegetables (including salad)	

8. Do you *usually* have a drink *up to 1 hour before, with, or up to 1 hour after* your lunch?

YES	
NO	

9. If yes, what drinks do you *usually* have with your lunch? (tick as many as apply)

Fruit or vegetable juices	
Milk or milk-based drinks (all varieties)	
Soy-based drinks	
Chocolate-based drinks (eg. Milo)	
Coffee (all varieties)	
Tea (all varieties)	
Water (all varieties)	
Other	

10. Do you usually eat an evening meal?

YES	
NO	

11. If you usually eat an *evening meal*, what types of food do you *usually* eat? (tick as many as apply)

Bread (eg. sandwiches, rolls, etc)	
Starchy food (eg. pasta, rice, potato)	
Milk products (eg. yoghurt, dairy food, cheese, custard, ice cream)	
Meats (eg. meat, fish, chicken, seafood, ham)	
Legumes, nuts, eggs	
Vegetables (including salad)	
Fruit	

12. Do you *usually* have a drink *up to 1 hour before, with, or up to 1 hour after* your evening meal?

YES	
NO	

13. If yes, what drinks do you *usually* have with your evening meal? (tick as many as apply)

Fruit or vegetable juices	
Milk or milk-based drinks (all varieties)	
Soy-based drinks	
Chocolate-based drinks (eg. Milo)	
Coffee (all varieties)	
Tea (all varieties)	
Alcohol	
Water (all varieties)	
Other	

14. Do you usually eat or drink between meals?

YES	
NO	

15. If you usually eat or drink *between meals*, what types of food or drink do you *usually* have? (tick as many as apply)

Bread based snacks (eg. sandwiches)	
Breakfast cereal	
Porridge	
Biscuits or cakes	
Crackers	
Milk products (eg. yoghurt, dairy food, cheese, ice cream)	
Fruit	
Potato crisps	
Cereal bars, muesli bars	
Chocolate or sweets	
Vegetables	
Fruit or vegetable juices	
Milk or milk-based drinks (all varieties)	
Soy-based drinks	
Chocolate-based drinks (eg. Milo)	
Coffee (all varieties)	
Tea (all varieties)	
Water (all varieties)	
Other	

16. Do you usually eat or drink after your evening meal?

YES	
NO	

17. If you usually eat or drink *after your evening meal* what types of food or drink do you *usually* have? (tick as many as apply)

Bread based snacks (eg. sandwiches)	
Breakfast cereal	
Porridge	
Biscuits or cakes	
Crackers	
Milk products (eg. yoghurt, dairy food, cheese, ice cream)	
Fruit	
Potato crisps	
Cereal bars, muesli bars	
Chocolate or sweets	
Vegetables	
Fruit or vegetable juices	
Milk or milk-based drinks (all varieties)	
Soy-based drinks	
Chocolate-based drinks (eg. Milo)	
Coffee (all varieties)	
Tea (all varieties)	
Alcohol	
Water (all varieties)	
Other	

18. Some foods and drinks have iron added to them (eg. some breakfast cereals). When you are choosing foods and drinks, how often do you choose the product with added iron instead of the product without?

Whenever I can	
Usually	
Sometimes	
Never	
I don't know or I don't consider whether foods have iron added to them	

19. How often do you use a *cast-iron* fry pan, wok or pot when preparing your meals?

Never or less than once a month	
1 to 3 times per month	
Once per week	
2 to 3 times per week	
4 to 6 times per week	
Once per day	
2 to 3 times per day	
4 plus times per day	
I don't know	

APPENDIX 7

Demographics/medical history questionnaire

Demographics and medical history questionnaire

General instructions

Please fill in Section 1 of this questionnaire. If you require any help any please ask one of the researchers

Section 1

	Yes	No
1) Are you aged between 18 to 44 years?	☐	☐
If the answer is no please speak to a researcher		
2) Are you a student at Massey University Auckland Campus?	☐	☐

3) Which country were you born in?

- ☐ New Zealand → go straight to question 5.
- ☐ Australia
- ☐ England
- ☐ Scotland
- ☐ China (People's Republic of)
- ☐ South Africa
- ☐ Samoa
- ☐ Cook Islands
- ☐ Other *Please state which country*

_____

4) If you live in New Zealand but were not born here, when did you first arrive to live in New Zealand?

Month (eg. February) _____

Year (eg. 2000) _____

5) Which ethnic group do you belong to? Tick whichever applies to you (you may tick more than one box).

- ☐ New Zealand European
- ☐ Maori
- ☐ Samoan
- ☐ Cook Island Maori
- ☐ Tongan
- ☐ Niuean
- ☐ Chinese
- ☐ Indian
- ☐ Other *Please state which ethnicity*_____

_____

6) What is your first language? _____

7) Children: If you have children:

- How many children do you have? _____

- When was your youngest child born? __ / __ / ____ (DD/MM/YYYY)

	Yes	**No**
8) Are you currently taking any contraception such as?		
Oral contraception?	☐	☐
Or		
Patch contraception?	☐	☐
Or		
Contraception by injection (Depo Provera)?	☐	☐
Or		
Intra-uterine device?	☐	☐

If yes, how long have you been using this contraceptive method?

_____

_____

	Yes	No
9) Are you currently pregnant?	☐	☐

If yes, how many weeks pregnant are you?

_____

	Yes	No
10) Have you been pregnant within the last year?	☐	☐

If yes, did the pregnancy result in any significant blood loss that you are aware of? Please comment.

_____

_____

_____

11) Further Studies

Please tick if you are interested in finding out more about a follow up study and about other nutrition studies ☐

Even if we contact you about further studies you would not be obligated to participate. Your contact details will be confidential

The next section will be completed by a researcher

Section 2: Lifestyle

(Note for interviewer: Please check section 1)

	Yes	No
1) Do you smoke?	☐	☐

If *yes* how many cigarettes do you smoke a day _____

2) Do you live?

At home with parents ☐

At home with a partner/family ☐

On your own ☐

Shared accommodation ☐

University accommodation ☐

Home stay ☐

Other ☐

*Please describe*_____

_____

3) At home (where you live now) who prepares <u>most</u> of the food?

I do ☐

My mother ☐

My partner ☐

Other ☐

Please state who _____

4) **Who does most of the food shopping for your household?**

I do ☐

My mother ☐

My partner ☐

Other ☐

Please state who _____

5) How would you describe your eating pattern?

Eat a variety of all foods, including animal products ☐

Eat eggs, dairy, fish and chicken but avoid other meats ☐

Eat eggs and dairy products but avoid all meats and fish ☐

Eat eggs but avoid dairy products, all meats and fish ☐

Eat no animal products ☐

Other ☐

Please specify _____

	Yes	**No**
6) Do you follow any diet for cultural or religious reasons?	☐	☐

If yes, what type of diet do you follow? _____

_____

	Yes	**No**
7) Have you dieted strictly in the last year?	☐	☐

Please comment_____

_____

Section 3: Health

	Yes	No
1) Do you have or have you ever suffered from any acute or chronic illness?	☐	☐

Diagnosis *Date* *Diagnosed by* *Any further details*

_____

_____

_____

_____

	Yes	No
2) Do you have or have you ever suffered from any acute or chronic illness which may affect your iron status?	☐	☐

Diagnosis *Date* *Diagnosed by* *Any further details*

_____

_____

_____

_____

	Yes	No
3) Have you ever suffered from low iron stores, iron deficiency or iron deficiency anaemia?	☐	☐

Diagnosis *Date* *Diagnosed by* *Any further details*

_____

_____

_____

_____

	Yes	No
4) Have you ever been treated for iron deficiency or iron deficiency anaemia?	☐	☐

Type of treatment Duration Any further details

_____

_____

_____

	Yes	No
5) Do you have or have you had any medical condition which has resulted in blood loss?	☐	☐

If yes, please describe and give approximate dates

_____

_____

_____

	Yes	No
6) Have you had a blood transfusion in the last year?	☐	☐

If yes, do you know why you received the transfusion?

_____

	Yes	No
7) Have you had any blood loss (other than your periods or nose bleeds) such as wounds, regular scratches from contact sports, blood in stools or urine in the past year?	☐	☐

If yes, please describe

_____

_____

_____

	Yes	No
8) Are you currently taking any medication (excluding nutritional supplements)?	☐	☐

If yes, please state what medication you are taking and why

_____

_____

_____

_____

_____

Do not ask the following question if participant has had a miscarriage (check Section 1)

	Yes	No
9) Have you breastfed a baby within the last year?	☐	☐

Section 4: Supplements

	Yes	No
1) Did you take any vitamin and/or mineral capsules/tablets at any time during the past year?	☐	☐

If yes, please list the brand name of the supplement, the type of supplement, the number taken and the frequency of intake and the dose (including units)?

Note, it is important to obtain the amounts and types of iron, vitamin C and calcium in any supplement if that information is available

eg. Healtheries Iron & vitamin C, 1 taken every 2nd day, ferrous gluconate (170mg) providing elemental iron (20mg) and vitamin C (40mg).

If participant is not able to remember details please ask them to send us an email with the details

Email request ☐ **Email received** ☐

_____

_____

_____

_____

_____

_____

_____

_____

_____

	Yes	No
2) Did you take any other any other dietary supplements such as plain wheat bran (unprocessed bran, not 'All Bran' , or breakfast cereal), fibre tablets, Noni juice, lecithin, evening primrose oil, performance enhancers, protein supplements, etc at any time during the past year?	☐	☐

If yes, please list the brand name of the supplement, the type of supplement, the number taken and the frequency of intake and the dose (including units)?

eg. Nutralife Evening Primrose Oil, 3 capsules taken per day, contains 1000mg evening primrose oil,

_____

_____

_____

_____

_____

_____

_____

_____

_____

APPENDIX 8

Compliance diary and instructions for eating the breakfast cereal

KIWI STUDY

Your Diary

Information about the study

KIWI Women's Iron Study

Your details

Your first appointment was on _____

Your second appointment will be on _____

Fruit will be delivered to your house on a _____

Don't forget if you change your contact details, please let us know as soon as possible.

Please feel free to contact us at anytime and for any reason – we appreciate your help with this study and we want to support you.

Contacting us
Telephone numbers:
Kathryn Beck on (09) 443 9649 or 021 441 021 outside of normal working hours.
Dr Cath Conlon on (09) 443 9748

Email:
kiwi@massey.ac.nz

The Study

Each breakfast meal consists of 1 individually packed serving of cereal (60g), 150ml of milk and either 2x Zespri kiwifruit or 1x banana.

You will be given all the cereal and milk you need for 16 weeks at the beginning of the study. You will also be provided with a measuring jug to measure your milk each morning to add to the cereal.

You will be given your fruit for the first week of the study. The remaining fruit will be delivered to your home on a weekly or two weekly basis.

Storage of the foods

The cereal can be kept in it's packets at room temperature until ready for consumption.
The milk can be stored at room temperature. Once opened, refrigerate at 2-4ºC and consume within 7 days.
Store the bananas at room temperature. Eat the ripest bananas first.
If possible store the kiwifruit in the fridge. This is not essential but will keep them in a better condition for longer.

Instructions for eating breakfast

1. Shake the packet of cereal thoroughly before opening.
2. Place the cereal into a serving bowl.
3. Measure 150ml of milk using the plastic jug.
4. Add the milk to the cereal.
5. Please do not add any other foods to your breakfast cereal (with the exception of the fruit you have been given)

For the banana group

1. Peel the banana
2. The banana may be sliced and placed on top of the cereal or eaten from the skin. Whichever method is used, it is important that the banana is eaten at the same time, or immediately before or immediately after eating the cereal.

For the kiwifruit group

1. The kiwifruit may be eaten in a number of ways (see below). Only the flesh should be eaten. Discard the skin. Whichever method is used, it is important that the kiwifruit is eaten at the same time, or immediately before or immediately after eating the cereal.
    - Method 1: Use the skive (small knife / spoon provided) to slice the kiwifruit in half. Scoop out the contents, place on the cereal and consume.
    - Method 2: Use the skive to slice the kiwifruit in half. Eat the flesh using the skive.

- Method 3: Cut the kiwifruit into quarters and eat directly from the peel.
- Method 4: Peel and slice the kiwifruit.

For both groups

Consume your breakfast cereal, milk and fruit. It is important that all of the cereal and milk is consumed. There may be iron in the milk at the bottom of the bowl. It is important that all of this milk is consumed.

It is important not to consume any fluids or eat any other foods with or within 1 hour prior to or 1 hour after consuming the breakfast cereal. Other food and fluids are extremely likely to interfere with iron absorption and affect the results of the study. You may drink water with your breakfast.

Completing the compliance questionnaire

Please take a moment each day to complete the compliance questionnaire. This is a really important aspect of the KIWI Study.

Follow up

We will aim to contact you throughout the study to ensure everything is going well with the study.

If you have any problems or concerns, please contact Kathryn Beck on (09) 443 9649 or Cath Conlon on (09) 443 9748.

Your Diary

We very much appreciate your participation in this research project. By committing time and effort to this project we assume that it is just as important to you as it is to us that this project is a success. We need reliable data to be able to make recommendations for improving iron status. In order to do this it is extremely important that you consume the breakfast we have provided to you according to the guidelines given (pages 2-3) and that you do not make major changes to your habitual daily routine for the duration of the study. Any changes can affect the results of the study and therefore the reliability of the results. We realise that changes are sometimes inevitable, for example illness. Therefore, we would appreciate it if you could supply information about your breakfast intake and indicate any changes from your daily routine or with regard to the intake of breakfast by completing the included compliance diary. Please complete one sheet for each week of the study – if you run out of room please find blank sheets at the end of the diary which you can use.

Thank you again for taking part in this study.

Kind regards,

Kathryn & Cath

KiWI Study – COMPLIANCE DIARY | Subject Number:

Weekly Compliance Diary

Week 1 **Date starting:**_____

Did you consume your breakfast everyday this week?
Please tick if all of the breakfast provided was consumed and record the time of day the breakfast was eaten.
Please comment on any food not consumed including amounts or any other food eaten with the breakfast or consumed within 1 hour either side of the breakfast.

	Tick if you ate all the breakfast (cereal, milk and fruit)	Record the time breakfast was eaten	Any other comments (eg. comment on any food not consumed including amounts; comment on any other food eaten with the breakfast or consumed within 1 hour either side of the breakfast)
Mon			
Tues			
Wed			
Thurs			
Fri			
Sat			
Sun			

Were you ill this week? O Yes O No

If yes, what was the nature of your illness?_____

Did you consume any medication for the illness? O Yes O No
If yes, please provide details of the medication used:

_____

Did you have any of the following symptoms during the week: abdominal discomfort, bloating, constipation, diarrhoea, nausea, vomiting, headache, fatigue?

O Yes O No
If yes, please provide details such as the duration of the symptom and any treatment required:

_____

_____

Have there been any changes in your normal daily routine this week, e.g. eating habits, physical activity, alcohol consumption, smoking, etc?

O Yes O No
If yes, please provide more detail:_____

_____

Did you consume any supplements this week? O Yes O No

If yes, please provide more detail: (brand name, dosage, reason for taking the supplement)

_____

_____

Are you experiencing any practical problems with eating the breakfast?

O Yes O No
If yes, please provide more detail:

_____

_____

Do you have anything else you would like to report?

_____

_____

_____

Please email or ring one of the researchers if you would like to discuss any issues no matter how small you think they are – it would be great to hear from you.

Additional space

Please use this page to continue if you run out of space or would like to any other comments

Common questions

My banana or kiwifruit supply for the week hasn't arrived and I have run out of kiwifruit or bananas.
Contact the researchers immediately.

I have been invited out for brunch? Can I go?
Yes, you can. If possible try and have your breakfast at least 1 hour prior to brunch. If this is not possible eat your breakfast later in the day.

I am going away for the weekend? What should I do?
If possible take your cereal, milk and fruit with you. It is important that all people consume the same milk during the study due to the varying levels of calcium in milk. Calcium has an effect on iron absorption.

I've missed breakfast. What should I do?
If possible try and eat the breakfast meal later in the day. Remember to record what time you ate breakfast in your diary

I've run out of milk
Contact the researchers.

I feel hungry after eating the breakfast
Wait one hour and then you may eat again.

I'm feeling really full and finding it hard to eat all of the breakfast.
It is really important that you consume all the breakfast. Take your time eating the breakfast. A breakfast like this will often leave you feeling full, but you tend to feel fuller for longer and will be less likely to fill up on other food during the morning.

During the study
It is important that do not consume any iron, vitamin C or calcium supplements (or any supplements that contain these nutrients) for the entire duration of the study.

Do not donate blood during the study as this will affect your iron levels.

Let the researcher know if you become pregnant, ill or are unable to complete the study for any reason.

During the study it is important that you maintain your normal daily routine. Eg eating patterns, physical activity, alcohol consumption and smoking habits. Don't aim to lose weight during the study.

We would like to thank you for taking part in this research. We appreciate the time and the effort that it takes. Without you it wouldn't be possible.

We hope you enjoy the study