The effect of a meat extract on iron absorption in young women

A thesis presented in partial fulfillment of the requirements for the degree of Masters of Science in Human Nutrition

At Massey University, Palmerston North Campus, New Zealand

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2007

Abstract

Iron deficiency is a global problem for which determinants and solutions need to be investigated. The first part of this study assessed the iron status and dietary intakes of 85 non vegetarian women aged 18-40 years living in the Manawatu region. Exclusion criteria included pregnancy or breastfeeding in the past 12 months, smoking, excess alcohol consumption and recent blood donation. Dietary intakes were estimated using a 24 hour recall and a non validated food frequency questionnaire. Serum ferritin (SF), haemoglobin (Hb), C-reactive protein, height, weight and supplement use were measured. Two women (2.4%) had iron deficiency anaemia (SF<12μg/L and Hb<120g/L) and 9 women (10.6%) had depleted iron stores (SF<20μg/L). All other women had normal iron stores (SF>20μg/L). The daily mean and median iron intakes were 12.7±6.2mg and 10.8mg. 71 women (83.5%) consumed less than the Recommended Dietary Intake (RDI) of 18mg iron per day and 21.2% consumed less than the Estimated Average Requirement (EAR) of 8mg iron per day. Serum ferritin was positively associated with age and total dietary iron intake. No statistically significant relationship was found between serum ferritin and Body Mass Index or exercise, or daily intakes of energy, protein, haem iron, red meat, total meat, vitamin C, vitamin A, total tea, coffee, alcohol, fibre or calcium ($p>.05$).

Eighteen women who had low iron stores (SF<30μg/L) were selected to take part in a second study to investigate the effect of a meat extract (<0.5kDa sarcoplasmic fraction) on non haem iron absorption. Each subject consumed a sodium caseinate meal, a meat meal or a sodium caseinate meal containing the meat extract. Each meal was labeled with 8.5mg ^{57}Fe and each subject received 0.5mg ^{58}Fe administered by intravenous infusion. Fourteen days later iron absorption from these meals was determined using ratios of stable isotopes of iron incorporated into the red blood cells. Iron status was significantly inversely related to iron absorption. After adjusting to a serum ferritin of 40μg/L, iron absorption was 3.8% from the sodium caseinate meal, 3.9% from the meat meal and 5.1% from the meal containing the meat extract. These values were not significantly different from one another ($p>.05$).

Acknowledgements

I would like to sincerely thank the following people for all their help and assistance provided during the completion of this thesis:

Dr Jane Coad	Senior Lecturer in Human Nutrition, Institute of Food, Nutrition and Human Health (IFNHH), Massey University, Palmerston North
Dr Roger Lentle	Associate Professor in Human Nutrition, IFNHH, Palmerston North
Mrs Chris Booth	Technician, IFNHH, Massey University, Palmerston North
Dr Patrick Morel	Senior Lecturer in Animal Science, IFNHH, Massey University, Palmerston North
Dr Brian Wilkinson	Senior Lecturer in Food Technology, IFNHH, Massey University, Palmerston North
Dr Roger Purchas	Associate Professor in Animal Science, IFNHH, Palmerston North
Dr Cath Conlon	Lecturer in Human Nutrition, IFNHH, Albany
Dr Welma Stonehouse	Associate Professor in Human Nutrition, IFNHH, Albany

Thank you to all the study participants and the Meat Biologics Consortium who provided the funding for this research.

Thank you to my husband Rob, and my parents for their support and encouragement throughout this research.

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List of Abbreviations

aa	ascorbic acid
AR	Absorption Ratio
AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Range
ANOVA	Analysis of Variance
Bd	Blood donors
BMI	Body Mass Index
BP	Bird Proof
BSA	Bovine Serum Albumin
BV	Blood Volume
Ca	Calcium
$CaCl_2$	Calcium Chloride
CCM	Calcium Citrate Malate
CRP	C-Reactive Protein
Dcytb	Duodenal cytochrome b
DMT-1	Divalent Metal Transport Protein 1
EA	Egg Albumin
EAR	Estimated Average Requirement
f	Fermented
F	Females
Fe^{2+}	ferrous
Fe^{3+}	ferric
FF	Full Fat
FFQ	Food Frequency Questionnaire
Hb	Haemoglobin
Hb incorp	Haemoglobin incorporation
HCl	Hydrochloric acid
HJV	Hemojuvelin
HMW	High Molecular Weight
HP	Hydrolyzed Soybean Proteins
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IFNHH	Institute of Food Nutrition and Human Health
ISP	Isolated Soy Protein
IV	Intravenous
KPhy	Potassium Phytate
LDB	Lyophilised Dephytinised Bran
LMW	Low Molecular Weight
LPM	Low Phytate Maize
LWB	Lyophilised Whole Bran
M	Males
mFePhy	monoferric phytate
MFP factor	Meat Fish Poultry Factor
MgPhy	Magnesium Phytate

MS	Mass Spectrometry
NAA	Neuron Activation Analysis
NaPhy	Sodium Phytate
N/A	not available
nf	non fermented
NRV	Nutrient Reference Value
ns	non significant
NTIMS	Negative Thermal Ionisation Mass Spectrometry
o/a	ovalbumin
OJ	Orange Juice
P	Phytate
PA	Phytic Acid
PAL	Physical Activity Level
Pp	Polyphenols
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
RNI	Recommended Nutrient Intake
RI	Radio Isotopes
SE	Standard Error
SF	Serum Ferritin
-SH	Sulphydryl
SI	Stable Isotopes
SLS-Hb	Sodium Lauryl Sulfate-Hb
SPI	Soy Protein Isolate
SS	Semi Synthetic
Std	Standard
TA	Tannic Acid
Tae	Tannic acid equivalent
TE	Total Energy
TIBC	Total Iron Binding Capacity
TIMS	Thermal Ionisation Mass Spectrometry
TRF2	Transferrin Receptor 2
TS	Transferrin Saturation
TSF	Textured Soy Flour
Uf	Unfermented
W	White wheat flour
WB	Whole Bran
WBC	Whole Body Counting
WHO	World Health Organisation
WTM	Wild Type Maize (normal phytate content)
YK	Yod Kratin

Introduction - Organisation of Thesis

Iron deficiency is the most common nutritional deficiency worldwide. Young women are especially vulnerable to iron deficiency both in developed and developing countries. There is a wealth of literature available covering all aspects of iron related nutrition. The first chapter of this thesis reviews the literature with a particular focus on the role of iron in the body, iron requirements, iron deficiency anaemia, excess iron and measuring iron status. The mechanism of iron absorption in the human body is covered as well as factors affecting iron absorption. Methods and issues associated with measuring non haem iron absorption in human subjects are addressed. The final part of the literature review investigates dietary factors affecting non haem iron absorption and iron status. Research that has been undertaken to identify and test the meat, fish, poultry (MFP) factor is covered in detail. Throughout the literature review there is a particular focus on the iron requirements of young women.

The aim of this study was to investigate the effect of a meat extract on non haem iron absorption in young women. Prior to this the prevalence of iron deficiency in young non vegetarian females living in the Manawatu region was investigated, including an investigation of dietary intakes and factors contributing to their iron status. This is covered in Chapter 2. From this population women were selected to take part in the meat study which was an exploratory study to investigate the effect of a meat extract on non haem iron absorption. The meat extract was identified and produced by the Institute of Food, Nutrition and Human Health (IFNHH) at Massey University and was tested using pasta based meals. Iron absorption was assessed using stable isotopes (^{57}Fe and ^{58}Fe) and the double isotope technique (Chapter 3).

Chapter 4 draws conclusions from the research undertaken and provides an indication of where future work should be directed.

Chapter 1
Review of Literature

1.1 Iron – an introduction

Iron deficiency is the most common nutritional deficiency worldwide (FAO/WHO, 1998) despite iron being one of the most abundant metals in the earth's crust (Fairweather-Tait, 1995b). It is estimated that approximately two billion people in the world suffer from anaemia, with the majority of anaemia caused by iron deficiency (UNICEF & WHO, 1999). Iron deficiency is not just confined to developing countries, but is seen in developed countries including New Zealand (Russell *et al.*, 1999; Heath *et al.*, 2001). On the other hand, iron overload is a serious clinical problem in some parts of the world.

1.2 Functions of Iron

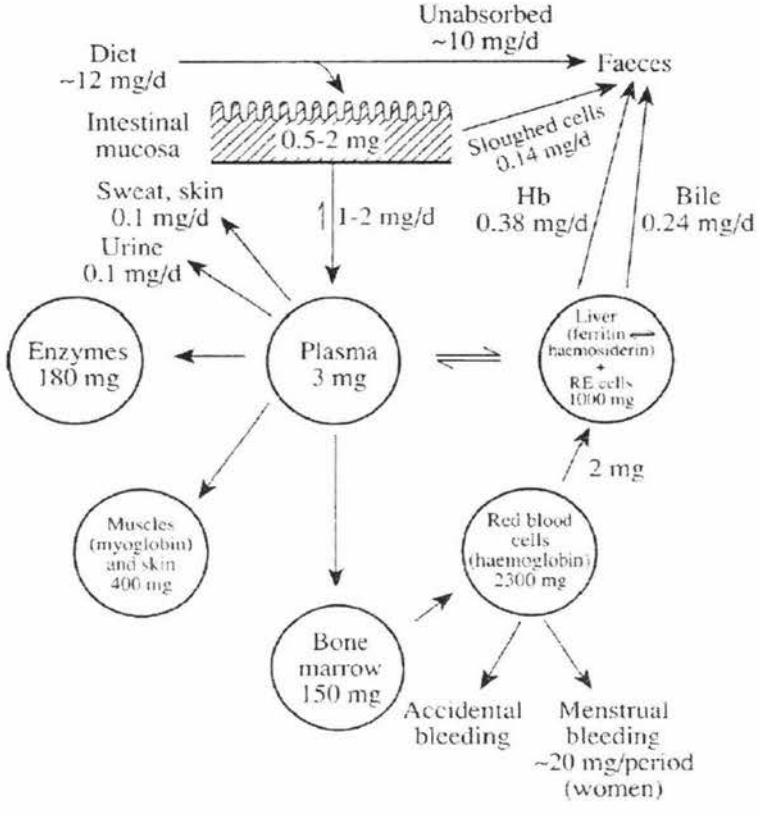
Iron (atomic weight 55.85; atomic number 26) has several vital functions in the body. Haemoglobin carries oxygen from the lungs to the body's tissues, while myoglobin stores oxygen in the muscles. Several iron containing enzymes (cytochromes) act as electron carriers transferring energy within the cell and into the mitochondria. Other roles of iron containing enzymes include the detoxification of foreign substances in the liver, synthesis of steroid hormones and bile acids, and signal controlling in some neurotransmitters (FAO/WHO, 1998). Iron switches easily between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) form, which enables iron to serve as a catalyst in redox reactions within the body.

1.3 Iron Distribution in the body

The human body contains 3-5g of iron, with an adult female having 35 to 50mg of iron per kg body weight. In women, approximately 73% of iron is found in the haemoglobin of red blood cells, 13% is stored as ferritin and haemosiderin in the liver, 8% in the myoglobin of

muscles, 6% in enzymes and a very small amount (about 4mg) in transferrin, a transport protein in the blood (Anderson, 2000). Women have lower amounts of stored iron than men. Figure 1.1 shows the distribution of iron in the body.

Figure 1.1 Iron distribution (mg) and metabolism within the body (Fairweather-Tait, 1995b)



1.4 Iron requirements for adult females

Iron is lost through the shedding of cells from the skin or the gastrointestinal tract, or through blood loss including menstruation. Iron is not readily excreted from the body through urine, bile or sweat. The total amount of iron lost is estimated at 14μg/kg body weight/day (FAO/WHO, 1998). Thus, a non-menstruating 60kg woman is calculated to lose about 0.84mg Fe/day. The range of individual losses is estimated at ±15%. Menstrual loss varies between women and over a 28 day menstrual cycle, the mean loss is about 0.56mg/day, meaning the total iron requirements for a 60kg adult menstruating women is

approximately 1.4 mg iron/day (FAO/WHO, 1998). Ten percent of women require greater than 2.27mg iron per day and 5% of women require more than 2.84mg iron/day (Hallberg & Rossander-Hulten, 1991).

1.4.1 Recommended dietary iron intakes for adult females

There are difficulties involved in developing a recommended dietary intake (RDI) for iron due to the wide range of iron bioavailability found in various diets. Individuals in most developed countries, including New Zealand, consume diets of high iron bioavailability (>2.1mg or >15% of iron absorbed daily). Diets of high iron bioavailability contain generous amounts of foods that enhance iron absorption and low levels of foods that inhibit iron absorption. In May 2006, the Nutrient Reference Values for Australia and New Zealand were published (Commonwealth Department of Health and Ageing Australia, 2006). The new Recommended Dietary Intake (RDI) for iron in women aged 19-50 years is 18mg/day. This is the same as the Recommended Dietary Allowance used by the Institute of Medicine (Food and Nutrition Board: Institute of Medicine, 2001). It is suggested that vegetarians require 1.8x this amount of iron per day due to the decreased bioavailability of iron from vegetarian diets. The estimated average requirement is 8mg/day (Commonwealth Department of Health and Ageing Australia, 2006). In the United Kingdom, the Recommended Nutrient Intake for iron for women aged 19-50 years is 14.8mg/day (Department of Health, 1991).

Table 1.1 Recommendations for iron intake for women aged 19-50 years

Females	*NRV for Australia and New Zealand (RDI), 2006*	*Institute of Medicine (RDA), 2001*	*United Kingdom, (RNI), 1991*
19-30 years	18mg	18mg	14.8mg
31-50 years	18mg	18mg	14.8mg

NRV Nutrient Reference Values
RDI Recommended Dietary Intake
RDA Recommended Dietary Allowance
RNI Reference Nutrient Intake

1.5 Iron deficiency anaemia

1.5.1 Prevalence of iron deficiency

Approximately two billion people in the world suffer from anaemia, with most anaemia attributed to iron deficiency (UNICEF & WHO, 1999). In non industrialised countries 30-60% of non pregnant women are anaemic. In developed countries, the prevalence of iron deficiency anaemia is lower and ranges from five to sixteen percent (UNICEF & WHO, 1999). However, in developed countries low iron stores are found in about 20-30% of women of reproductive age (FAO/WHO, 1998). The prevalence of iron deficiency worldwide is highest in infants, children, adolescents and women of child bearing age, especially pregnant women (FAO/WHO, 1998). Infants, children and adolescents have increased requirements due to growth. The requirements of adolescent females and women are increased due to menstruation, while pregnant women need iron to support placental and fetal development and increased blood volumes. The groups most affected by iron deficiency anaemia in developed countries are pregnant women (18% anaemic), school children (17% anaemic), non pregnant women and the elderly (both 12% anaemic) (UNICEF & WHO, 1999).

1.5.2 Stages of iron deficiency anaemia and assessment of iron status

There are three stages in the development of iron deficiency anaemia: iron depletion, iron deficient erythropoiesis and iron deficiency anaemia as seen in Table 1.2.

Table 1.2 Stages in the development of iron deficiency and overload (Herbert, 1987)

	Iron overload	*Normal*	*Iron depletion*	*Iron deficient erythropoiesis*	*Iron deficiency anaemia*
TIBC (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
Serum transferrin receptor* (mg/L)	Low	Normal	Normal	***High***	High
Free erythrocyte protoporphyrin (μg/dL RBC)	30	30	30	***>70***	>70
Erythrocytes	Normal	Normal	Normal	Normal	***Microcytic / Hypochromic***

TIBC Total iron binding capacity
* Serum transferrin receptor values vary with the assay

Iron depletion is characterised by a progressive reduction in the amount of storage iron in the liver, reflected by a fall in serum ferritin levels (<20μg/L) (Gibson, 2005b). This is followed by iron deficient erythropoiesis, characterised by a decrease in serum iron (<60μg/dL) and an elevation in total iron-binding capacity, resulting in a fall in transferrin saturation (TS) (<15%). At the same time, erythrocyte protoporphyrin concentrations will be increased (>100μg/dL), because the supply of iron is no longer adequate for haem synthesis. The third and final stage is iron deficiency anaemia, caused by exhaustion of iron stores and declining levels of circulating iron, and is characterised by a reduction of haemoglobin in the red blood cells (for females, <120g/L based on World Health Organisation (WHO) criteria. At this stage, there are decreases in serum iron (<40μg/dL) and ferritin (<10μg/L) and increases in erythrocyte protoporphyrin and total iron binding capacity (>410μg/dL) (Gibson, 2005b). In established iron deficiency anaemia, the red cells become small (microcytosis) and pale (hypochromia) and oxygen transport to the cells is compromised (MacPhail, 2002).

1.5.3 Iron status and intakes of New Zealand females

Dietary iron intake, iron bioavailability and an individual's iron requirements all contribute to an individual or populations iron status (Ministry of Health, 2003). The New Zealand

National Nutrition Survey found that low iron stores, iron deficiency and iron deficiency anaemia mainly affected women in the 15 to 44 year age group (Russell *et al.*, 1999). The percentage of New Zealand females with low iron stores, iron deficiency, iron deficiency anaemia and inadequate dietary intakes of iron can be seen in Table 1.3.

Table 1.3 Percentage of New Zealand females with low iron stores, iron deficiency, iron deficiency anaemia and inadequate dietary intakes of iron (Russell et al., 1999)

	Low iron stores[1] (%)	*Iron deficiency[2] (%)*	*Iron deficiency anaemia[3] (%)*	*Mean SF levels± SEM*	*Median dietary intake (mg)*	*Mean dietary intake (mg)*	*Inadequate intake (%)[4]*
NZ females 19-24 years	4	2	1	47±3.5	10.4	10.8	39
NZ females 25-44 years	7	3	2	62±2.6	10.1	10.5	42
NZ females total	6	3	2	80±2.6	9.9	10.3	26
NZ Maori 15-24 years	14	12	10	54±6.1	10.9	11.2	45 (15-18 yrs) 36 (19-24 yrs)
NZ Maori 25-44 years	11	8	5	73±7.8	10.6	11.0	40
NZ Maori total	11	9	6	94±13.8	10.5	10.9	32
Pacific 25-44 years	2	2	2	73±13.2	N/A	N/A	N/A
NZ European & others 15–24 years	2	1	1	40±2.9	10.1	10.4	45 (15-18 yrs) 42 (19-24 yrs)
NZ European & others 25-44 years	7	2	2	59±2.8	10.1	10.4	41
NZ European and others total	5	2	2	78±2.5	9.9	10.2	24
NZ Dep96 quartile – I[5]	5	2	2	78±4.2	10.2	10.7	#
NZ Dep96 quartile – II[5]	5	2	2	84±5.5	10.2	10.3	#
NZ Dep96 quartile – III[5]	7	3	3	75±4.1	9.7	10.1	#
NZ Dep96 quartile – IV[5]	7	4	3	84±6.7	9.7	10.2	#

[1] SF <12μg/L

[2] SF <12μg/L, zinc protoporphyrin >60μmol/mol

[3] SF <12μg/L, zinc protoporphyrin >60μmol/mol, haemoglobin <120g/L

[4] Calculated by probability analysis

[5] Quartiles – I highest quartile – IV lowest quartile

Quartiles included a range of age groups. As the requirements differ for each age group, an overall figure could not be calculated.

N/A - not available

The prevalence of iron deficiency was higher among Maori women than other groups. A study in female Auckland high school students found iron deficiency in Maori, Pacific Islanders and Asians to be two to three times higher than that of European females (Schaaf *et al.*, 2000). No studies have investigated the reasons for these findings. In the United

States, the prevalence of iron deficiency was more than two times higher in Mexican American women than in non-Hispanic white females of childbearing age (Looker *et al.*, 1997). This disparity was unable to be explained but factors associated with household income were thought to be involved (Frith-Terhune *et al.*, 2000). In other countries, increased rates of iron deficiency anaemia are a consequence of low socioeconomic status due in part to a lack of food security, and inadequate or a lack of access to healthcare (UNICEF & WHO, 1999). In the New Zealand National Nutrition Survey, women of lower socio-economic status appeared to have a slightly higher prevalence of iron deficiency. Other factors that may contribute to a higher level of iron deficiency in Maori women include a higher number of pregnancies (Looker *et al.*, 1997; Galan *et al.*, 1998; UNICEF & WHO, 1999) or a lower frequency of oral contraceptive use (Ferguson *et al.*, 2001). Oral contraceptive agents decrease menstrual blood loss and are associated with a low risk of iron deficiency (Hallberg *et al.*, 1995).

The estimated prevalence of low iron stores (four to seven percent) in the National Nutrition Survey was lower than the number of women estimated to have an inadequate intake of dietary iron (39 and 42% for females aged 19-24 and 25-44 years respectively) based on the amount of iron required to maintain normal clinical function and supply of iron to the tissue. This may have been due to an under reporting of food intake, a better than estimated absorption of dietary iron or the estimates of blood loss in menstruation used being inappropriate for New Zealand women (therefore increasing the RDI) (Russell *et al.*, 1999).

Other New Zealand studies have produced similar findings to those of the National Nutrition Survey, with figures varying depending on the cut off criteria used. In a cross sectional study, Heath et al (2001) found that 19% of Dunedin women aged 18-40 years had mild iron deficiency (SF<20μg/L and Hb>120g/L), 4% had iron deficient erythropoiesis (SF<12μg/L, zinc protoporphyrin >40μmol/mol haem) and 2% had iron deficiency anaemia (SF<12μg/L and Hb<120g/L). The mean total iron intake for all women was 10.7mg/day (Heath *et al.*, 2001). Another Dunedin study found that the prevalence of iron deficiency anaemia (SF<12μg/L and Hb<120g/L) and iron deficiency

(SF<12μg/L and Hb >120g/L) at 21 years was 2.2 and 6.7% respectively in a study group of mainly European females (Fawcett *et al.*, 1998). 12% of women had serum ferritin values of <16μg/L and 18.7% had serum ferritin values below <20μg/L (Fawcett *et al.*, 1998). Studies in Australia have found similar results. In a cross sectional study of 265 female university students, Rangan et al (1997) found that iron deficiency anaemia affected 4.5% of participants (SF<12μg/L, TS <16%, Hb<120g/L). Iron deficiency affected 7.2% of participants (SF<12μg/L, TS <16%), and 19.8% of women had a SF<20μg/L. Another study in 920 Australian women aged 17-65 years found that the overall incidence of iron deficiency (SF<10μg/L) was 8.9% (Leggett *et al.*, 1990). The iron status of New Zealand women appears to be slightly better than that of women from other Western European countries despite lower or similar iron intakes (Hallberg, 1995; Brussard *et al.*, 1997; Looker *et al.*, 1997; Galan *et al.*, 1998; Henderson *et al.*, 2003; Ministry of Health, 2003; Ruston *et al.*, 2003). Reasons for this are unknown. However, possible explanations may include differences in the dietary intakes of foods which enhance or inhibit iron absorption, or differing levels of blood loss due to menstruation, parity, oral contraceptive use or blood donation.

The main sources of iron for women aged 19-24 years in New Zealand are bread (11%), beef and veal (11%), vegetables (11%), potatoes and kumara (8%), breakfast cereals and non alcoholic beverages (6% each). For women aged 25-44 years the main sources of iron are bread (13%), beef and veal (11%), breakfast cereals (9%), vegetables (8%), and potatoes and kumara (7%). Eight percent of women aged 19-24 years and 10% of women aged 25-44 years had used iron supplements in the past year either regularly or occasionally (Russell *et al.*, 1999).

1.5.4 Risk factors for developing iron deficiency

Iron deficiency may be caused by pathological blood loss through infection, disease or other conditions. However, an inadequate iron intake due to low levels in the diet or poor iron bioavailability from foods is the major cause of iron deficiency in most developing countries (MacPhail, 2002). In New Zealand women of child bearing age the most

important risk factors for mild iron deficiency (SF 12–20μg/L and normal haemoglobin) were recent blood donation, menstrual blood loss (including extent and duration of bleeding), nose bleeds and a low intake of meat, fish and poultry (Heath *et al.*, 2001). Other risk factors for iron deficiency in women include high parity (Looker *et al.*, 1997; Galan *et al.*, 1998; UNICEF & WHO, 1999), previous diagnosis of iron deficiency anaemia (Galan *et al.*, 1998), low iron intakes (Looker *et al.*, 1997; Galan *et al.*, 1998), the use of intra-uterine contraceptive devices which induce blood loss (Looker *et al.*, 1997; Galan *et al.*, 1998; UNICEF & WHO, 1999), and excessive menstrual bleeding (UNICEF & WHO, 1999).

1.5.5 Consequences of iron deficiency

Iron deficiency is associated with a number of consequences including anaemia. Iron deficiency anaemia results in decreased work productivity, increased child and maternal mortality, slowed child development (Stolzfus, 2001), decreased ability to maintain and regulate body temperature when exposed to the cold (UNICEF & WHO, 1999) and possible increased susceptibility to infection (Stolzfus, 2001). Iron deficiency without anaemia has been linked to delayed cognitive development in children and adolescents (Grantham-McGregor & Ani, 2001) and may be associated with reduced work capacity (Hass & Brownlie, 2001).

1.5.6 Treatment of iron deficiency

Iron deficiency at a population level can be treated through iron supplementation, iron fortification of certain foods or through education to increase the intake and iron bioavailability of the diet (FAO/WHO, 1998). Different strategies or combinations of strategies may be used depending on their effectiveness and feasibility in different populations.

1.6 Iron excess

Excess levels of iron may be seen in alcoholics, in individuals given repeated blood transfusions or increased parental or oral iron and in patients with certain chronic anaemias such as thalassaemia major, where an increased, ineffective erythropoiesis causes increased absorption of dietary iron (Hallberg *et al.*, 2001).

Iron toxicity can occur with the acute ingestion of large amounts of iron. Overdoses of iron medication can cause severe poisoning with ingestion of doses greater than 600mg iron and death after 1g of iron (MacPhail, 2002). Large doses of iron supplements may cause gastrointestinal distress and inhibit zinc absorption when taken in the fasted state (Food and Nutrition Board: Institute of Medicine, 2001). The relationship between high iron stores and cancer and vascular disease is not clear (Food and Nutrition Board: Institute of Medicine, 2001). Iron may act as a prooxidant, leading to free radical formation and DNA damage, or may promote the oxidation of lipids which in turn leads to arterial inflammation, blockage and vascular disease (Anderson, 2000).

The majority of cases of iron overload are caused by an autosomal recessive disorder, haemochromatosis. In haemochromatosis, the absorption of dietary iron is increased resulting in serious organ damage, in particular cirrhosis of the liver. Haemochromatosis, is thought to be caused by a defect in iron release from the mucosal cell and reticuloendothelial system (MacPhail, 2002). Up to 1 in 300 individuals of Northern European descent are affected by haemochromatosis (The UK Haemochromatosis Consortium, 1997). Most patients with haemochromatosis are homozygous for the C282Y mutation of the HFE gene (The UK Haemochromatosis Consortium, 1997), however many other mutations in the HFE coding sequence have been identified, the most common being the H63D mutation (Roe *et al.*, 2005).

Only one example of dietary overload has been identified. It has been suggested that the high prevalence of iron overload in African populations is associated with the consumption large amounts of beer brewed in iron pots (Bothwell *et al.*, 1964). There may however also

be a genetic basis to the iron overload seen in this population (MacPhail, 2002). In most individuals a high dietary intake or increased bioavailability of iron will lead to increased absorption, but only up to a certain level (Hulthen *et al.*, 1995).

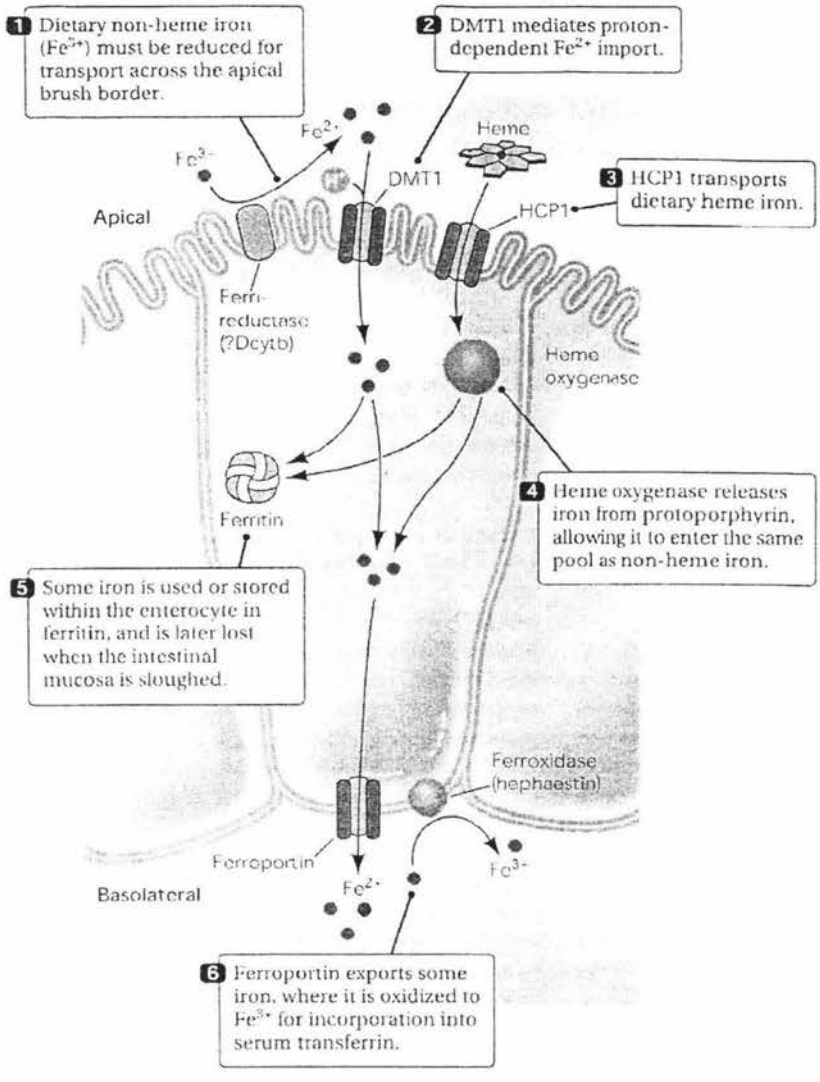
1.7 Mechanism of iron absorption

Iron homeostasis is tightly regulated by the body, primarily through iron absorption. The prime sites of iron absorption are in the duodenum and upper jejunum (Donovan *et al.*, 2005). Iron absorption involves the uptake of iron into the intestinal mucosal cell (enterocyte), the movement of iron through the enterocyte and the release of iron from the enterocyte into circulation (Fairweather-Tait, 1995a). In recent years, a number of proteins involved in iron absorption have been identified (Donovan *et al.*, 2005; Shayeghi *et al.*, 2005; Crichton, 2006).

Membrane extensions at the enterocyte's surface form a brush border increasing the surface area available for iron absorption (Donovan *et al.*, 2005). Haem and non haem iron enter the enterocyte by two independent pathways. Haem iron is absorbed as an intact porphyrin iron complex into the enterocyte (Miret *et al.*, 2003) via a haem carrier protein 1 (HCP1) (Shayeghi *et al.*, 2005). Inside the enterocyte haem iron is degraded by haem oxygenase to release ferrous (Fe^{2+}) iron, which is processed similarly to non haem iron.

Non haem iron is transported across the brush border membrane by the divalent metal transport protein 1 (DMT1). DMT1 also transports other divalent metals including Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}. Because DMT1 only transports divalent metals, Fe^{3+} must be converted to Fe^{2+} (Donovan *et al.*, 2005). Duodenal cytochrome b (Dcytb) was recently identified (Donovan *et al.*, 2005) and may play a role in reducing non haem iron from ferric (Fe^{3+}) to the more soluble (Fe^{2+}) form. Hydrochloric acid from the stomach and dietary factors also play a role in reducing ferric to ferrous iron.

Figure 1.2 Iron absorption in normal subjects (Donovan et al., 2005)



Within the enterocyte, Fe^{2+} derived from both haem and non haem iron enters a low molecular weight pool. Ferritin appears to have a regulatory role, directing iron to either be stored as ferritin in the enterocyte or to be transported across the basolateral membrane by ferroportin to reach the interstitial fluid/plasma. Iron stored as ferritin is lost from the body through the gastrointestinal tract when the intestinal mucosa is sloughed. The lifespan of the intestinal mucosa is approximately five to six days (Anderson, 2000)

Iron released from the enterocyte binds to the transport protein, transferrin (MacPhail, 2002). The incorporation of iron into transferrin may be facilitated by the oxidation of Fe^{2+} to Fe^{3+}, either by hephaestin, a membrane bound protein or by ceruloplasmin, the principal copper-containing protein of serum (Crichton, 2006). Transferrin has a high affinity for iron and is able to bind two ferric iron molecules. Transferrin delivers iron to the liver for storage or to the bone marrow where it is incorporated into haemoglobin in red blood cells. Approximately 80% of transferrin iron is delivered to erythroid precursors (Crichton, 2006) due to their high content of transferrin receptors (TfR1 and TfR2). The transferrin-iron complex is taken into the cell and the iron is released. The released iron is probably reduced to Fe^{2+} and transported across the endocytic membrane into the cytosol by DMT-1 (Crichton, 2006). The transferrin receptor returns to the cell surface, ready to receive more iron.

The typical lifespan of a red blood cell is 120 days. After this time the red blood cells are engulfed by cells of the reticuloendothelial system and the iron is recovered by the action of haem oxygenase. The released iron is either transported by transferrin to the bone marrow for new red cell production or excess iron is stored as ferritin or hemosiderin in the liver (MacPhail, 2002; Sharp, 2005).

1.8 Factors regulating iron absorption

Iron bioavailability can be defined as the proportion of iron in a food or diet that is utilised for normal body functions (Fairweather-Tait, 1992) including digestion, absorption and metabolism. Iron absorption is affected by both host and diet related factors.

1.8.1 Host related factors

The most significant determinants of iron absorption include a person's iron status and level of erythropoiesis (red blood cell production) (Finch, 1994). People with low iron stores absorb increased amounts of iron, with an inverse relationship observed up to a serum ferritin level of 60µg/L (Hallberg *et al.*, 1997). During iron deficiency cells produce

more DCytb, ferroportin and DMT-1 (Fleming & Bacon, 2005). The up-regulation of DMT-1 is only effective if the iron is accessible at the brush border in a reduced, soluble form, which is more likely with a highly bioavailable diet. Transferrin receptors on the cell surface also increase in iron deficiency increasing the potential uptake of iron (Hulthen *et al.*, 1995).

An increased rate of erythropoiesis is positively correlated with iron absorption (Skikne & Cook, 1992). Erythropoiesis increases in conditions of iron deficiency anaemia and hypoxia and decreases following blood transfusions, a return to sea level from high altitude and starvation (Conrad & Umbreit, 2002).

Hepcidin, an antimicrobial peptide produced by the liver appears to be a major regulator of iron homeostasis. Hepcidin binds to ferroportin reducing dietary iron absorption. During iron deficiency anaemia and hypoxia, hepcidin expression is decreased meaning more iron is available for erythropoiesis (Donovan *et al.*, 2005). In contrast, hepcidin expression is increased in response to inflammation and non genetic iron overload, minimising the availability of iron. In haemochromatosis, hepcidin is lacking or unavailable, resulting in increased ferroportin and increased transfer of iron out of the erythrocyte (Donovan *et al.*, 2005). The factors regulating hepcidin production have not been identified. However, the protein products of haemochromatosis disease genes possibly act as regulators of hepcidin expression as mutations in each result in inadequate hepcidin production (Donovan *et al.*, 2005). These include HFE, hemojuvelin (HJV) and transferrin receptor 2 (TRF2). A soluble cleavage product of HJV (sHJV) has also been shown to inhibit hepcidin production (Donovan *et al.*, 2005).

Iron absorption increases under physiological conditions such as growth and pregnancy (Whittaker *et al.*, 2001). Iron binding medications and some disease states (eg. Coeliac Disease) may reduce iron uptake into the enterocytes. In contrast, other disease states (eg. β-thalassaemia) may cause an increased iron uptake (Heath & Fairweather-Tait, 2002). A positive correlation between iron absorption and gastric juice, bile, pancreatic and mucous secretions has been described (Fairweather-Tait, 1995a). A decreased rate of gastric

emptying increases iron absorption as more iron is solubilised, with food exposed to the stomach's acidic environment for a longer time period (Fairweather-Tait, 1995a). Recent iron intake may affect mucosal absorptive cells so that absorption is decreased following a large dose of iron (Fairweather-Tait, 1995a).

1.8.2 Diet related factors

Dietary factors have a major influence on iron absorption, including the amount and type of iron in the diet, and the effect of dietary enhancers and inhibitors on iron absorption. There are two main types of iron in food – haem iron and non haem iron. Haem iron is derived from haemoglobin and myoglobin in meat, poultry, fish and seafood. Haem iron tends to contribute up to 15% of the total iron intake (Hallberg, 2002). The average absorption of haem iron from meat containing meals is about 25% (Hallberg *et al.*, 1979), but can vary from 10% during iron repletion to about 40% during iron deficiency (Hallberg *et al.*, 1997). Few dietary factors affect the absorption of haem iron. 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 (FAO/WHO, 1998; Baech *et al.*, 2003b).

Non haem iron is the main form of dietary iron and is found in both animal and plant foods such as meat, cereals, pulses, legumes, eggs, vegetables and fruit. On average 2-20% of non haem iron is absorbed (Gibson *et al.*, 1997). The absorption of non haem iron is affected by a person's iron status and by dietary factors such as the amount of iron and composition of the meal. Bioavailability can vary more than ten times in meals containing the same content of iron (Hallberg, 1981). Dietary factors can enhance non haem iron absorption by changing ferric (Fe^{3+}) iron to the more soluble ferrous (Fe^{2+}) form (Fairweather-Tait, 1995a) or by maintaining iron released from food during digestion in a soluble form. Other foods may inhibit iron absorption by forming insoluble ferric complexes reducing iron bioavailability (Miret *et al.*, 2003). Ascorbic acid and meat are strong enhancers of non haem iron absorption (Cook & Monsen, 1975; Cook & Monsen,

1977). Other enhancers of non haem iron absorption include organic acids, alcohol and fermented foods (Gillooly *et al.*, 1983; Baynes *et al.*, 1990; Cook *et al.*, 1995). Vitamin A may have an enhancing effect on non haem iron absorption but evidence is conflicting (Layrisse *et al.*, 1997; Garcia-Casal *et al.*, 1998; Layrisse *et al.*, 1998; Walczyk *et al.*, 2003).

Ascorbic and other organic acids chelate with iron in the intestinal lumen, and promote acidic conditions in the stomach so that dietary iron is solubilised, preventing iron from binding to inhibitory ligands (Allen & Ahluwalia, 1997) Ascorbic acid also enhances iron absorption by reducing ferric to ferrous iron and helping to maintain the solubility of iron when it enters the small intestine's alkaline environment (Allen & Ahluwalia, 1997). There is still considerable controversy about how meat enhances non haem iron absorption with research being carried out to identify the factor in meat that enhances non haem iron absorption. This will be discussed in detail later in the chapter. Alcohol may increase iron absorption by enhancing gastric acid secretion, thereby lowering the pH of the small intestine and increasing iron solubility (Cook *et al.*, 1995). The enhancing effect of fermented products on non haem iron absorption may be due to the hydrolysis of soy protein leading to the release of amino acids and peptides (Baynes *et al.*, 1990), a high lactic acid content in fermented foods (Gillooly *et al.*, 1983) or through the stimulation of gastric acid secretions.

Inhibitors of non haem iron absorption include polyphenols, proteins, calcium, phytates, and oxalate. These bind non haem iron, forming insoluble complexes which are poorly absorbed and unable to be taken up into the enterocyte (Oke, 1969; Disler *et al.*, 1975a). It is thought that calcium's inhibition occurs within the enterocyte because calcium inhibits the absorption of both haem and non haem iron (Hallberg *et al.*, 1991; Hallberg, 1998). High doses of organic elements (zinc, cobalt, cadmium, manganese and copper) interfere with non haem iron absorption (Solomons, 1986; Monsen, 1988; Rossander-Hulten *et al.*, 1991) by competing for transport on the Divalent Metal Transport Protein-1. However the amounts of these substances found in the diet are unlikely to inhibit iron absorption. Dietary factors and their relative effects on iron absorption will be discussed in detail.

1.9 Methods for estimating non haem iron absorption

Several techniques have been developed to investigate factors that influence non haem iron absorption (Fairweather-Tait, 2001). These include in vitro and in vivo animal and human studies.

In vitro methods are useful as screening tools to predict potential availability for iron absorption (Latunde-Dada *et al.*, 1998). The amount of soluble, ionisable or dialysable iron has been measured under stimulated gastrointestinal conditions as a measure of iron bioavailability (Miller *et al.*, 1981; Kane & Miller, 1984; Hazell & Johnson, 1987). It has however been suggested that these methods are not adequate for assessing iron bioavailability (Glahn *et al.*, 1996).

Caco-2 cells, a human colon adenocarcinoma cell line has been used in recent years to measure iron bioavailability (Garcia *et al.*, 1996a; Latunde-Dada *et al.*, 1998; Au & Reddy, 2000). Caco-2 cells resemble the cells lining the inner surface of the small intestine which absorb iron (Au & Reddy, 2000). Food and digestive enzymes are placed on the upper chamber of a culture well, where a dialysis membrane (representing mucous protecting the cells of the digestive tract) prevents digestive enzymes reaching the Caco-2 cells in the lower chamber. Iron passes through the membrane to the Caco-2 cells and iron uptake can be measured by uptake of radio labeled iron or by ferritin formation by the Caco-2 cells.

Some groups report similar responses between rodents and humans, while other have found major discrepancies in iron absorption (Reddy & Cook, 1991; Wienk *et al.*, 1999). For example, rats are able to absorb ferrous iron and ferric iron, and haem iron and non haem iron to equal extents, compared with humans who preferentially absorb ferrous iron and haem iron. In addition, rodents have intestinal phytase activity and are able to synthesise ascorbic acid which humans are unable to do (Wienk *et al.*, 1999). Reddy and Cook (1991) found that rodents were less sensitive than humans to dietary factors affecting non haem iron absorption. The piglet may be useful as a model as the gastrointestinal tract of pigs

resembles that of humans (Van Campen & Glahn, 1999). Few studies however, have measured iron absorption in piglets.

Algorithms have also been used to estimate iron bioavailability in foods (Monsen *et al.*, 1978; Hallberg & Hulthen, 2000; Reddy *et al.*, 2000). Algorithms are based on data from human studies on the effects of enhancers and inhibitors of various foods on iron absorption. Their accuracy depends on the quality and availability of food composition data for iron and dietary factors that enhance or inhibit of iron absorption. At present there are no extensive lists available of phytate and polyphenol contents of food. Algorithms often do not account for degradations in enhancers and inhibitors that occur due to storage, cooking and food preparation methods (Yun *et al.*, 2004).

The various methods used to determine iron absorption in human subjects will be discussed. The haemoglobin incorporation technique using stable isotopes will be discussed in detail.

Table 1.4 In vivo methods for measuring iron absorption

Methods that do not use isotopes	***Methods using isotopes***
Chemical balance studies	Haemoglobin incorporation
Rate of haemoglobin repletion	Faecal monitoring
Changes in serum ferritin/haemoglobin concentration	Whole body counting
Plasma appearance/disappearance	Plasma appearance/disappearance

1.10 Methods that do not use isotopes

1.10.1 Chemical balance technique

Early human studies used this technique with the difference between dietary iron intake and the faecal excretion of iron representing iron absorption (van den Heuvel *et al.*, 1997). This technique can be useful in determining the bioavailability of more than one nutrient or to

determine iron absorption from the whole diet over time (Wienk *et al.*, 1999). However, the technique demands considerable compliance from subjects with regard to the diet and collection of faeces. It is a time consuming, insensitive and imprecise means of measuring iron absorption (Fairweather-Tait & Dainty, 2002).

1.10.2 Rate of haemoglobin repletion

This method is rarely used in humans to measure iron absorption. Iron absorption is studied by observing the increase in haemoglobin over time in anaemic subjects or subjects who have iron deficiency induced by phlebotomy (Fairweather-Tait, 2001). In humans, this method is invasive and insensitive and with large individual variation between subjects (Wienk *et al.*, 1999). The method has been used by the Institute of Food, Nutrition and Human Health (IFNHH) at Massey University to investigate the effects of various diets on iron bioavailability in semi-anaemic piglets (Morel & Purchas, 2006).

1.10.3 Changes in serum ferritin / haemoglobin

Examining changes in serum ferritin or haemoglobin over time provides an indirect estimate of the amount of iron absorbed and is useful for measuring the impact of dietary interventions in improving iron absorption at the community level. Limitations include the ability to measure only one intervention in each subject and the need to correct any changes in serum ferritin or haemoglobin for initial iron status. Other micronutrient deficiencies may also affect serum ferritin or haemoglobin levels (Allen & Ahluwalia, 1997). Haemoglobin and serum ferritin plateaus once iron stores are repleted (Viteri *et al.*, 1995) meaning this method is less useful in a population who are iron replete. Changes in serum ferritin concentrations are more reflective of changes in iron status, than changes in haemoglobin. However, serum ferritin levels are falsely elevated by inflammation and infection (Hulthen *et al.*, 1998).

1.10.4 Plasma appearance / disappearance

Large doses of oral iron (25-100mg) can be given and iron absorption calculated from the plasma appearance of iron in the hours following. This method is only suitable for large amounts of iron (and not the level of iron found in food) due to large fluctuations in plasma iron (Fairweather-Tait & Dainty, 2002). This method has been validated using whole body counting, however is best used to measure relative rather than absolute iron absorption (Ekenved *et al.*, 1976).

1.11 Methods that use isotopes

An isotope is one of two or more species of atoms of a chemical element with the same atomic number and position in the periodic table and nearly identical chemical behaviour but with different atomic masses and physical properties (Wong & Abrams, 2003).

The radioisotopes of iron ^{55}Fe and ^{59}Fe have been used extensively to measure iron absorption in humans (Hallberg, 1981). However, stable isotopes are increasingly being used to measure iron absorption due to ethical concerns regarding the exposure of subjects to ionising radiation (Fairweather-Tait, 2001). Radioisotopes of iron are less costly and easier to measure than stable isotopes, and only a tracer amount is required to label test meals. There are usually no background levels present to allow for when doing enrichment calculations (Fairweather-Tait & Dainty, 2002). However, radioisotopes of iron require specialised disposal techniques and decay with time, meaning they cannot be stored for long periods of time.

Small amounts of iron occur naturally as stable isotopes in the environment. Iron has four stable isotopes of iron. These are ^{54}Fe (5.8%), ^{56}Fe (91.8%), ^{57}Fe (2.2%) and ^{58}Fe (0.3%). Three (^{54}Fe, ^{57}Fe, ^{58}Fe) of these isotopes have a natural abundance low enough to be added to test meals and used in iron bioavailability studies (Fairweather-Tait *et al.*, 2001). High levels of stable isotopes are required to ensure adequate enrichment of red blood cells.

Stable isotopes are extremely expensive, limiting their use to studies with small numbers of subjects (Fairweather-Tait *et al.*, 2001). Samples collected using stable isotope methodologies are able to be stored indefinitely.

Isotopes of iron may be used to intrinsically label foods by biosynthetic incorporation of the iron into live plants or animals, or added to food prior to consumption as an extrinsic label. The isotope must be in the same form as the iron in food to monitor its absorption and metabolism (Fairweather-Tait & Dainty, 2002). The extrinsic labeling technique was first validated for non haem iron using radioisotope tracers (Bjorn-Rasmussen *et al.*, 1972; Bjorn-Rasmussen *et al.*, 1973) and assumes that complete isotopic exchange takes place between the added isotope and iron in the food by the time it reaches the site of absorption (Fairweather-Tait & Dainty, 2002).

1.11.1 Whole body counting (^{59}Fe)

Using this method, the subject is given a ^{59}Fe labeled iron test meal and radioactivity is counted 1-5 hours and 10-14 days later. The difference between these two values equals the amount of iron absorbed, once allowances are made for isotope decay and geometry (Fairweather-Tait, 2001). An initial baseline count is made to measure background activity from ^{40}K and any activity from previous studies using radioisotopes. Whole body counting was once considered the gold standard for measuring iron absorption (Fairweather-Tait, 2001), as it does not rely on estimates of blood volume or efficiency of iron utilisation. Whole body counting is rarely used today as it requires access to a whole body counter and specialist techniques. Because ^{59}Fe is gamma emitting, it can only be given to adult males (Heath & Fairweather-Tait, 2002). Only one test food can be investigated at any one time in a subject.

1.11.2 Faecal monitoring (radio and stable isotopes)

As losses of absorbed iron in faeces and urine are minimal, iron absorption can be measured by giving subjects a meal containing a known amount of isotope and collecting

their faeces for 3-14 days. Iron absorption is calculated as the difference between intake and faecal excretion of the isotope (Fairweather-Tait, 2001). This method mainly uses stable isotopes and the doses given are less than required for other methods such as the haemoglobin incorporation method (Fairweather-Tait, 2001). Again, assumptions do not need to be made about blood volume or efficiency of utilisation of absorbed iron. This method is time consuming and there is a risk of incomplete collection of iron (due to short collection periods, incomplete faecal collections or prolonged transit times in the gut) leading to an overestimate of iron absorption (Fairweather-Tait, 2001). Non absorbable markers such as rare earth elements can be used to test for completeness of collections (Fairweather-Tait *et al.*, 1997).

1.11.3 Plasma appearance / disappearance

This method uses stable isotopes, with an intravenous dose of iron given at the same time as an oral dose. Plasma samples are taken over 10 hours and the areas under the oral and intravenous plasma concentrations versus time curves are measured and dose corrected to determine iron absorption (Fairweather-Tait, 2001). This method has been validated against absorption of ^{59}Fe measured by whole-body counting (Barrett *et al.*, 1994). The main disadvantage of this method is the large volumes of blood required which may affect body iron stores and limit the number of absorption tests that can be undertaken in any individual (Heath & Fairweather-Tait, 2002).

1.11.4 Haemoglobin incorporation (radio and stable isotopes)

This is the preferred method for determining iron absorption and is a direct measure of iron bioavailability (Fairweather-Tait, 2001). Iron absorption can be determined by the amount of an oral dose of isotopically labeled iron found in haemoglobin, as most newly absorbed iron is incorporated in the immature red blood cells in order to make haemoglobin (Fairweather-Tait, 2001). A test meal with a known amount of isotope is fed to fasting subjects on one or more occasions and the percentage of incorporation of the isotope into red blood cells is determined from a blood sample taken 14-28 days later (Fairweather-Tait

& Dainty, 2002). The percentage iron absorption is calculated on the basis of blood volume and red cell incorporation of the absorbed dose. It is assumed that 80-100% of absorbed iron is incorporated into red blood cells depending on the subjects level of serum ferritin (Cook *et al.*, 1991a). Blood volume is usually estimated using calculations made from height and weight. ^{59}Fe and ^{55}Fe have both been used extensively to investigate iron absorption using this method. Iron absorption can be compared from two different sources of iron, or between a test meal and a well absorbed reference dose (Hallberg *et al.*, 1997).

Alternatively, a double isotope method can be used (Fairweather-Tait & Dainty, 2002). This avoids making assumptions about the percentage of iron incorporated into red blood cells. One isotope is given intravenously (^{55}Fe) and the other given orally with the meal (^{59}Fe). Absorption is calculated 14 days later by relating the ratio of the two isotopes in the red cells to the ratio of administered isotopes (Fairweather-Tait, 2001).

More recently, the haemoglobin incorporation technique has used stable isotopes to measure iron absorption. Stable isotopes are used similarly to radioiron isotopes, including the use of the double isotope method. In recent years, the double isotope technique has been used to compare iron absorption in different groups of women given ^{57}Fe orally and ^{58}Fe intravenously (Barrett *et al.*, 1992; Whittaker & Barrett, 1992; Barrett *et al.*, 1994; van den Heuvel *et al.*, 1998a; Whittaker *et al.*, 2001). One study has compared iron absorption from two different types of meals in the same subjects. These meals were labelled with ^{57}Fe and ^{54}Fe and ^{58}Fe was given intravenously (Roe *et al.*, 2005). Only one study has used the double isotope method to compare iron absorption from two different meals fed to different groups of subjects (Tondeur *et al.*, 2004).

Stable isotope doses must be large enough to produce a measurable enrichment of red blood cells (Fairweather-Tait, 2001). This is achieved more easily in infants due to their smaller blood volumes (Fairweather-Tait, 2001). As mass spectrometry techniques improve, the use of stable isotopes to measure iron absorption is becoming more feasible (Fairweather-Tait & Dainty, 2002). Isotopes can also be given with more than one meal (multiple dosing) to ensure that adequate quantities of stable isotopes are received as seen in the

study by Roe et al (2005). As stable isotopes make a significant contribution to the total iron intake this may be a confounding factor (van den Heuvel *et al.*, 1997; Fairweather-Tait *et al.*, 2001). Increased sensitivity may be achieved by measuring the stable isotopes in young red blood cells (ie. 4-7 days after administration of the isotopes) where the newly absorbed iron is located, reducing the oral isotope dose by up to one–third (van den Heuvel *et al.*, 1998a). Another potential problem with the double isotope method is that the incorporation of the isotope given orally into the red blood cells may differ from that of the intravenous isotope (Fairweather-Tait & Dainty, 2002).

1.12 Analytical methods for stable isotope analysis

Methods for the analysis of stable isotopes must be accurate and precise and are based on either neuron activation analysis (NAA) or mass spectrometry techniques (MS) (Janghorbani, 2001). With all methods, corrections need to be made for the natural abundance of the stable isotope. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Thermal Ionisation Mass Spectrometry (TIMS) are the primary analytical instruments for stable isotope research with trace elements (Woodhouse & Abrams, 2001). In mass spectrometry the ions in the sample are separated on the basis of their mass to charge ratio, and a detector receives an ion signal proportional to their concentration.

1.12.1 Thermal Ionisation Mass Spectrometry (TIMS)

This has been the method of choice for stable isotope analysis due to its high accuracy and precision (Woodhouse & Abrams, 2001). It uses either magnetic-sector machines which separate masses in a magnetic field or quadrupole machines which separate masses by applying alternating and constant voltages to parallel rods (Woodhouse & Abrams, 2001). The magnetic sector instruments yield the best precision (<0.1%). The use of TIMS is extremely expensive (Chen *et al.*, 2005), requiring 0.5-1.0mL red blood cells, extensive sample preparation and high levels of expertise (Abrams, 1999). Sample throughput is very slow and it is difficult to analyse more than 10-15 samples per day (Abrams, 1999). Stable

isotope absorption studies of iron have been limited by the high cost and limited availability of isotope ratio analysis using TIMS (Chen *et al.*, 2005).

1.12.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is the most recent of MS techniques and offers a reasonably rapid method for analyses of ^{54}Fe, ^{57}Fe and ^{58}Fe. Samples are introduced through a nebuliser into a high temperature argon plasma produced by electrical discharge, where the solids are volatised and ionised. The plasma is sampled at atmospheric pressure through a differentially pumped interface, and ions are usually separated by mass with a quadrupole MS (Woodhouse & Abrams, 2001) . ICP-MS has a faster sample throughput and lower costs. However, larger amounts of isotopes must be given to allow for the lower precision of ICP-MS (Griffin, 2002). In addition, some elements are unable to be analysed easily due to interferences from argides, polyatoms and doubly charged interference isotopes (Chen *et al.*, 2005).

In 2005, Chen et al developed a high resolution double focusing ICP-MS (HR-ICP-MS). This showed excellent agreement with TIMS in measuring iron isotope ratios and calculating iron absorption from red blood cell samples from human subjects previously given ^{58}Fe and ^{57}Fe. The HR-ICP-MS required <2μL red blood cells and the analytical time was less than 5 minutes (Chen *et al.*, 2005). HR-ICP-MS was also able to account for nickel contamination (currently not available for TIMS) which may offset any differences in precision between HR-ICP-MS and TIMS (Chen *et al.*, 2005)

HR-ICP-MS is becoming increasingly available (Griffin, 2002). It appears to offer high sensitivity (so lower sample volumes are needed), high precision, high resolution (reducing interference and the need for sample preparation) and rapid sample throughput compared with TIMS (Griffin, 2002; Chen *et al.*, 2005). It is likely that the use of HR-ICP-MS will increase and be used rather than the more expensive TIMS when accurate precision is needed at a reasonable cost (Griffin, 2002).

1.13 Issues related to the measurement of iron absorption

Several factors need to be considered when investigating iron absorption. These factors include the iron status of subjects, the use of single meals versus whole diets, and the composition of the meal(s) being tested (Heath & Fairweather-Tait, 2002).

1.13.1 Iron status of subjects

Iron status has a marked effect on the absorption of non haem iron, meaning that individual absorption values need to be corrected to a common reference point before the results of different studies can be compared. This can be achieved by three different methods. The reference dose method compares iron absorption from the test meal with a well absorbed reference dose (usually 3mg ferrous sulphate plus 30mg ascorbic acid). The bioavailability of the iron in the test meal can be expressed as a ratio of ferrous sulphate to test iron, or corrected to a mean reference value of 40% by multiplying absorption by 40/R, where R is the reference dose absorption (Magnusson *et al.*, 1981). The serum ferritin method uses an individual's absorption from the test meal and their serum ferritin concentration, to calculate a predicted absorption based on that individual having a serum ferritin level of 40μg/L (Cook *et al.*, 1991a). Several values of serum ferritin should be used to determine an individual's average serum ferritin concentration when using this method (Cook *et al.*, 1991a). Both of the above methods assume that the inverse relationship between iron status and iron absorption occurs in a linear manner (Van Campen & Glahn, 1999). Several studies have shown a straight line relationship between log iron absorption from reference doses of iron and log serum ferritin (Cook *et al.*, 1974; Taylor *et al.*, 1988; Hallberg, 2002). In one study higher correlations were found when comparing iron absorption with absorption from a reference dose than when comparing it to serum ferritin (Taylor *et al.*, 1988). However, Hallberg (1981) found that adjusting each individual absorption value to a constant serum ferritin was more reliable than the reference dose absorption correction in correcting for iron status. The standard test meal approach expresses iron absorption in relation to iron absorption from a standard test meal (Cook & Monsen, 1976). This approach allows various additions of foods to be added to a meal for comparison of iron

absorption relative to the standard meal and avoids potential errors made through the use of calculated predictions (Heath & Fairweather-Tait, 2002).

1.13.2 Single meals versus whole diets

A typical Western diet consists of several dietary factors that enhance or inhibit iron absorption spread over several meals. Single meal studies may overestimate iron absorption compared to iron absorption from a daily diet (Cook *et al.*, 1991a; Hulthen *et al.*, 1995; Tidehag *et al.*, 1995; Reddy & Cook, 1997). For example, in one study the difference in iron absorption between high and low bioavailability diets was 6 fold when the foods were consumed as single meals, but only 2.5 fold when the total diet was studied (Cook *et al.*, 1991a). These differences may be due to the 10-12 hour fast prior to the single meal, which exaggerates the effects of dietary factors on iron absorption (Heath & Fairweather-Tait, 2002).

1.13.3 Composition of meals being tested

The composition of the test meal will influence iron absorption. For example, the effects of ascorbic acid on iron absorption are less when added to meals of high iron bioavailability compared to meals of low iron bioavailability (Cook & Monsen, 1977). Meal composition must be considered when evaluating the effects of different foods on iron absorption.

1.14 Dietary factors affecting non haem iron absorption

The remaining part of the literature review will investigate dietary factors that enhance or inhibit iron absorption. Each section will focus on studies that have been undertaken in human subjects only. However, the section on meat and the MFP factor will also consider research undertaken using in vitro techniques. Studies investigating the effect of dietary components on iron status have focused on those studies undertaken in pre menopausal adult women in Western countries.

Each section includes a table which summarises the literature on iron absorption from single meal studies. The tables describe the author and method of analysis, the number of subjects, gender, age and serum ferritin levels (when available), the meals tested and the iron content of these meals, the reference dose absorption (if used), actual non haem iron absorption, the adjusted absorption to account for iron status (if applicable), and the absorption ratio (AR) of the test meal compared with the control meal. An AR of 2.0 means the intervention doubled non haem iron absorption, while an AR of 0.50 indicates the intervention halved iron absorption. Only studies that have adjusted for subject iron status by one of the methods described previously have been included in the tables. In some cases not all of the information is available so has not been included. In some studies the iron content of the meals under investigation are standardised. In others, the iron levels are not standardised, meaning it is more difficult to determine if changes in iron absorption are due to the iron content of the meals or to other dietary factors.

1.15 Meat, fish and poultry

1.15.1 Studies using single meals

Meat has two major roles in improving iron absorption. As well as containing approximately 40% haem iron and 60% non haem iron (Monsen *et al.*, 1978), meat contains a factor that enhances the absorption of both haem and non haem iron. This is commonly known as the meat, fish and poultry (MFP) factor.

Early intrinsic tag studies found that total iron absorption increased significantly when corn, beans and maize were served with fish, veal and liver (Layrisse *et al.*, 1968; Martinez-Torres *et al.*, 1974). These studies did not differentiate between haem and non haem iron absorption. More recent studies have shown that meals containing meat promote an increased level of non haem iron absorption compared with meals which do not contain meat (Hallberg & Rossander, 1982b; Acosta *et al.*, 1984). Several single meal studies have demonstrated the enhancing effect of beef on non haem iron absorption. Iron absorption was five times higher from a standard meal containing beef compared to a semi synthetic

meal of the same macronutrient composition without beef (Cook & Monsen, 1975), while adding 80g beef to a standard meal (bun, fries, milkshake) doubled iron absorption (Reddy & Cook, 1991). Replacing ovalbumin with 100g beef in a semi synthetic meal increased iron absorption by 196% (Cook & Monsen, 1976). Halving the beef content in a 82g hamburger meal reduced non haem iron absorption by 25% in one study (Hallberg & Rossander, 1982d) and by 30% in another study (Hallberg & Rossander, 1982a). Adding 90-92g of beef to a low ascorbic acid meal and a maize gruel increased non haem iron absorption by two (Hallberg & Rossander, 1982a) and three times (Hurrell *et al.*, 1988) respectively. However, the same amount of beef had no effect on non haem iron absorption when added to a wheat bread meal. This effect could not be explained (Hurrell *et al.*, 1988). Using stable isotopes to measure iron absorption in infants, it was observed that 25g beef added to vegetables increased iron absorption significantly (Engelmann *et al.*, 1998).

Meat improves non haem iron absorption even in the presence of inhibitors of iron absorption (Morck *et al.*, 1982; Hallberg & Rossander, 1984; Hallberg *et al.*, 1989; Reddy *et al.*, 1996). Adding 75g meat to a Latin American meal high in phytates increased non haem iron absorption 2.5 times (Hallberg & Rossander, 1984). The addition of 50g meat to wheat rolls containing 25 and 250mg phytate increased iron absorption by 12% (non significant) and 81% respectively (Hallberg *et al.*, 1989). 39.4g of cooked freeze-dried beef added to a semi synthetic meal and a semi synthetic meal containing 300mg phytic acid increased iron absorption by 150 and 214% (Reddy *et al.*, 1996). Adding 100g beef to a semi synthetic meal containing egg albumin or isolated soy protein increased iron absorption by 26 and 290% respectively (Morck *et al.*, 1982). Meat's effect on iron absorption appears to be greatest when more or stronger inhibitors of iron absorption are present in a meal.

The effects of different types of meat on non haem iron absorption has been investigated (Cook & Monsen, 1976; Bjorn-Rasmussen & Hallberg, 1979; Rossander *et al.*, 1979; Hurrell *et al.*, 2006). When beef in a standard meal was substituted with pork, lamb, liver and chicken iron absorption remained similar. Only fish had a significant effect and reduced iron absorption by 18% when compared with beef. When these meat products

replaced ovalbumin in a semi synthetic meal the increase in iron absorption ranged from 111% with fish to 277% with liver (Cook & Monsen, 1976). Bjorn–Rasmussen & Hallberg (1979) observed that beef, fish, chicken and calf thymus all increased iron absorption to similar extents when protein equivalent quantities were added to a maize meal. The addition of 50g bacon (and an egg) to a Western breakfast meal increased iron absorption significantly while the addition of an egg alone had no effect (Rossander *et al.*, 1979). Replacing egg albumin with chicken and beef in protein equivalent quantities increased iron absorption by 95 and 181% respectively (Hurrell *et al.*, 2006).

Meat has a dose dependent effect on non haem iron absorption (Cook & Monsen, 1975; Layrisse *et al.*, 1984; Baech *et al.*, 2003a). This effect has been shown with up to 300g of beef added to a corn meal (Layrisse *et al.*, 1984). In two studies, the addition of 25g of beef or pork to a semi synthetic and rice meal increased iron absorption but not significantly. Fifty grams of beef or pork led to a significant increase in iron absorption in both studies (Cook & Monsen, 1975; Baech *et al.*, 2003a). Layrisse *et al* (1984) found that 50 and 100g of beef increased iron absorption when added to a corn meal. However, the increase in iron absorption when 50g beef was added to this meal was not significant.

Table 1.5 summarises the single meal studies that have investigated the effect of meat, fish, or poultry on non haem iron absorption using the extrinsic tag method.

Table 1.5 Effect of meat on iron absorption (single meals studies)

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Layrisse et al (1974)	n=14M+F, 41(18-64)yrs; Hb 138 (88-162)g/L	100g cooked maize	Meal alone	?	36.9	4.55	5.0	-
			Meal + 150g papaya (66mg aa)	?		24.69	26.8	5.36
RI – Hb incorp				?				
			Meal + 150g papaya + 100g fish			23.01	24.9	4.98
Cook & Monsen (1975)	n=8F, 24 (20-26)yrs, SF 20 (7-43)µg/L	SS meal (egg albumin, dextrimaltose, corn oil)	SS meal alone	4.1, 0	22.1	3.4	N/A	-
			SS meal +25g beef	4.1, ?		3.9		1.14 (ns)
	n = 8F, 25 (21-29)yrs, SF 28 (12-130)µg/L		SS meal alone	4.1, 0	18.2	1.3		-
RI – Hb incorp		Standard meal (lean beef, cornmeal, potatoes, bread, margarine, peaches, icemilk)	SS meal+50g beef	4.1, ?		2.4		1.88 (p<0.02)
	n = 8F, 23 (19-25)yrs, SF 12 (6-64)µg/L		SS meal alone	4.1, 0	40.9	2.0		-
			SS meal+75g beef	4.1, ?		4.2		2.11 (p<0.02)
	n = 8F, 23 (19-28)yrs, SF 27 (8-74)µg/L		SS meal alone	4.1, 0	19.9	1.2		-
			SS meal+100g beef	4.1, ?		4.3		3.55 (p<0.02)
	n=32F, 18-30yrs, SF 20 (7-130)µg/L		SS meal	4.1, 0	-	1.8		-
			Standard meal	2.9, 1.2		10.0		5..56
Batu et al (1976)	n=6M, 26 (25-27)yrs, Hb 150 (136-160)g/L	200g rice, 65g water spinach + beans, spices	Meal alone	7.6, 0	46	1.4	1.2	-
RI – Hb incorp	n=9M, 26 (24-31)yrs, Hb 153 (124-179)g/L		Meal + 40g fish	7.6, 1.2	36	6.4	7.1	5.9
	n=11F, 25 (23-27)yrs, Hb 127 (114-140)g/L		Meal + 40g fish	7.6, 1.2	71	11.9	6.7	5.6

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Cook & Monsen	n=7F, 25 (21-28)yrs, SF 35 (12-116)µg/L	Standard meal (beef, potatoes, corn, bread, margarine, ice milk, peaches)	Standard meal	4.1, ?	N/A	9.7	N/A	-
			Std + 100g pork (replaces beef)	3.5, 0		9.3		0.96 (ns)
RI – Hb incorp			SS meal	4.1, 0		1.6		-
			SS + 100g pork (replaces o/a)	4.1, ?		5.2		3.21 (p<0.001)
	n=7F, 22 (18-26)yrs, SF 40 (13-92)µg/L	SS meal (dextrimaltose, corn oil, ovalbumin (o/a))	Standard meal	4.1, ?		7.6		-
			Std + 102g lamb (replaces beef)	3.3, ?		7.8		1.03 (ns)
		Replacements made on basis of protein content (20.2g)	SS meal	4.1, 0		1.6		-
			SS + 102g lamb (replaces o/a)	4.1, ?		5.2		2.23 (p<0.001)
	n=8F, 22 (19-29)yrs, SF 33 (9-76)µg/L		Standard meal	4.1, ?		3.8		-
			Std + 102g liver (replaces beef)	7.7, ?		3.9		1.01 (ns)
			SS meal	4.1, 0		1.4		-
			SS + 102g liver (replaces o/a)	9.9, ?		5.4		3.77 (p<0.001)
	n=7F, 28 (20-37)yrs, SF 34 (13-110)µg/L		Standard meal	4.1, ?		11.5		-
			Std + 98g chicken (replaces beef)	2.6, ?		10.4		0.90 (ns)
			SS meal	4.1, 0		1.4		-
			SS + 98g chicken (replaces o/a)	4.1, ?		3.4		2.43 (p<0.01)
	n=8F, 21 (18-23)yrs, SF 21 (11-58)µg/L		Standard meal	4.1, 0		14.8		-
			Std + 115g fish (replaces beef)	2.0, ?		12.2		0.82 (p<0.05)
			SS meal	4.1, 0		1.9		-
			SS + 115g fish (replaces o/a)	4.1, ?		3.9		2.11 (p<0.05)
	n=7F, 26 (19-32)yrs, SF 22		Standard meal	4.1, ?		10.0		-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	(19-49)µg/L		Std + 25g o/a (replaces beef)	1.9, 0		3.9		0.39 (p<0.01)
			SS meal	4.1, 0		1.7		-
			SS + 100g beef (replaces o/a)	4.1, ?		5.1		2.96 (p<0.001)
Bjorn-Rasmussen & Hallberg (1979)	n=6M+F	50g maize flour as a porridge	Meal alone	5.0, 0	N/A	2.20	N/A	-
			Meal + 75g beef	?		7.17		3.80 (p<0.05)
	n=6M+F		Meal alone	5.0, 0		2.42		-
RI – Hb incorp + WBC			Meal + 95g chicken	?		5.69		3.24 (p<0.05)
	n=9M+F		Meal alone	5.0, 0		0.44		-
			Meal + 134g fish	?		1.26		4.37 (p<0.01)
	n=8M+F (3bd)		Meal alone	5.0, 0		2.24		-
			Meal + 67g fish (10g protein)	?		3.68		1.52 (p<0.05)
	n=8M+F		Meal alone	5.0, 0		0.90		-
	All 29-42yrs		Meal + 127g calf thymus	?		2.60		2.97 (p<0.01)
			All meat = 20g protein					
Rossander et al (1979)	n=12M, 29yrs, Hb 142g/L + 9F, 29yrs, Hb 129g/L	Western breakfast meal (2 wheat rolls + 12g	Meal alone	2.8, 0	52.0	7.6	5.8	-
	n= 6M, 24yrs, Hb 145g/L	margarine + 10g orange	Meal alone	4.2, 0.03		6.6	5.1	-
RI – Hb incorp + WBC	+ 6F, 25yrs, Hb 128g/L	marmalade + 15g cheese)	Meal + egg +50g bacon			7.1	5.5	1.08 (p<0.01)
	n=10M, 25yrs, Hb 149g/L	+ 8g coffee in 150ml	Meal alone	4.1, 0		9.3	7.2	-
	+ 2F, 20yrs, Hb 135g/L	water	Meal + egg			7.6	5.8	0.81 (ns)
Cook et al (1981)	n=11M, 23 (18-37)yrs, SF 48 (27-70)µg/L	Hamburger bun, 55g fries, 180ml vanilla milkshake	70g beef + 30g TSF	?	N/A	1.51	N/A	-
			100g beef + 30g TSF	?		1.24		0.82 (p<0.001)
RI – Hb incorp								
Hallberg & Rossander (1982a)	n=47M, 40yrs, Hb 148g/L + 12F, 23yrs, Hb133g/L	Hamburger bun, 60g string beans, 150g mashed	Meal + 82g beef	3.0, 0.5	36.4	10.9	12.0	-
	n=9M, 24yrs, Hb 141g/L +	potatoes	Meal + 41g beef	2.3, 0.2	46.9	9.9	8.4	0.7

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp + WBC	2F, 23yrs, Hb134g/L n=7M, 25yrs, Hb145g/L + 3F, 21yrs, Hb126g/L	Low ascorbate meal (45g dried navy beans, 45g	Meal alone	5.8, 0	43.2	2.5	2.3	-
	n=5M, 24yrs, Hb143g/L + 5F, 24yrs, Hb136yrs	brown rice, bread (25g cornflour, 25g wheat flour), 14g margarine, 55g apples, 8g walnuts, 225g yoghurt)	Meal + 90g beef	6.8, 1.2	26.4	1.7	2.6	1.13
Hallberg & Rossander (1982d)	n=47M(8), 40yrs, Hb 148g/L + 12F, 23yrs, Hb 133g/L	60g string beans, 150g mashed potatoes, hamburger patty	82g meat patty (19g protein)	3.0, 0.5	36.4	10.9	11.2	-
RI – Hb incorp + WBC	n=9M (6bd), 24 yrs, Hb 141g/L + 2F, 23 yrs, Hb 134g/L		42g meat patty (12g protein)	2.35, 0.25	46.9	9.9	8.4	0.75
Morck et al (1982)	n=7M, 22 (19-28)yrs, SF 55 (18-94)µg/L	SS drink (corn syrup solids, corn oil) + 16.9g isolated soy protein	Meal alone	5.6, 0	N/A	0.36	N/A	-
			Meal + 100g beef	5.6, 1.0		1.44		3.90 (p<0.0001)
RI – Hb incorp		SS drink (corn syrup solids, corn oil) + 18.4g egg albumin	Meal alone	5.6, 0		5.94		-
			Meal + 100g beef	5.6, 1.0		7.47		1.26 (p<0.05)
Hallberg & Rossander (1984)	n=4M(4bd), 34yrs, Hb 151g/L + 5F(1bd), 25 yrs, Hb 139g/L	Latin American meal (80g dry maize chapattis, 31g dry black beans, 50g polished rice cooked)	Meal alone	5.3, 0	38.9	3.5	3.2	-
RI – Hb incorp + WBC			Meal + 75g beef (15g protein)	5.3, 0.7		8.0	8.4	2.63 (p<0.01)
Layrisse et al (1984)	n=12M+F, SF 23µg/L	300g corn meal	Meal alone	2.0, 0	N/A	4.4	N/A	-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp	n=20M+F, SF 10µg/L		Meal + 50g beef	?		6.2		1.42 (ns)
			Meal alone	2.0, 0		3.3		-
			Meal + 100g beef	?		6.0		1.83 (p<0.001)
			Meal + 200g beef	?		6.7		2.02 (p<0.001)
			Meal + 300g beef	?		8.0		2.43 (p<0.001)
Hurrell et al (1988)	n=8M, 26 (22-34)yrs, SF 50 (19-118)µg/L	60g corn meal, 4.1g butter	Meal alone	4.2, 0	N/A	0.49	N/A	-
			Meal + 92g beef	4.2, 1.92		1.59		3.22 (p<0.05)
RI – Hb incorp	n=3F + 2M, 26 (23-29)yrs, SF 30 (14-76)µg/L	60g baladi bread (82% extraction wheat flour), 4g butter	Meal alone	4.0, 0		3.04		-
			Meal + 92g beef	4.0, 1.92		3.23		1.07 (ns)
Hallberg et al (1989)	n=4M (2bd) + 4F (1bd)	Wheat roll + 20g margarine + 150ml water + 25mg phytate	Meal alone	4.1, 0	28.5	4.8	8.0	-
			Meal + 50g beef	?		5.1	8.4	1.12 (ns)
RI – Hb incorp +WBC	n= 1M (1bd) + 9F(3bd)	Wheat roll + 20g margarine + 150ml water + +250mg phytate	Meal alone	4.1, 0	35.9	4.0	3.6	-
			Meal + 50g beef	?		6.9	6.8	1.86 (p<0.001)
	All 19-47yrs							
Reddy & Cook (1991)	n=7M, 24 (19-37)yrs, SF 76 (65-88)µg/L	75g bun, 68g fries, 50g milkshake	Meal alone	1.9, 0	N/A	1.96	N/A	-
			Meal + 80g beef	4.1mg total Fe		3.90		1.99 (p<0.01)
RI – Hb incorp								
Reddy et al (1996)	n=4F+6M, 19-37yrs, SF 30µg/L	SS meal (67g hydrolysed corn starch, 36g corn oil, 12ml vanilla extract)	Meal alone	6.2mg total Fe in all meals	N/A	10.77	N/A	-
			Meal + 39.4g cooked freeze dried beef (30g protein)			26.73		2.50 (p<0.0001)
RI – Hb incorp		SS meal (as above) + 300mg phytic acid	Meal alone			1.83		-
			Meal + 39.4g cooked freeze			5.76		3.14 (p<0.01)

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Abs (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		(84.6mg phytate)	dried beef (30g protein)					
Engelmann et al (1998) SI – Hb incorp - TIMS	n=8M+F; 45.5 (43-49) weeks	125g vegetable puree	Meal alone Meal + 25g beef	0.9, 0 09, 0.4	N/A	9.9 15.0	N/A	- 1.52 (p<0.005))
Baech et al (2003a) RI – Hb incorp + WBC	n=45F, 24 yrs n=15F, SF 16.9µg/L n=15F, SF 20.7µg/L n=15F, SF 21.0µg/L *All SF 8-35µg/L, Hb>120g/L*	100g cooked rice, 100g tomato sauce, 50g pea puree, 50g wheat roll, 200ml water	Basic meal Basic meal +25g pork Basic meal + 50 g pork Basic meal + 75g pork	2.3, 0 2.5, 0.06 2.5, 0.12 2.3, 0.19	- 36.4 27.1 25.0	4.3 5.1 6.3 6.7	5.9 7.1 8.6 9.2	- 1.15 (ns) 1.44 (p<0.001) 1.57 (p<0.001)
Hurrell et al (2006) RI – Hb incorp	n=3M+5F, 24(20-27)yrs, 25(8-121) µg/L n=6M+4F, 26(22-29)yrs, 19(6-69) µg/L	SS meal (67g hydrolysed corn starch, 36g corn oil, 12ml vanilla extract)	SS meal + 35.2g egg albumin SS meal + 30g freeze-dried chicken muscle SS meal + 17.6g egg albumin SS meal + 15g freeze-dried beef muscle	4.1, 0 4.1, ? 2.05, 0 2.05, ?	N/A	8.41 16.43 11.21 31.52	N/A	- 1.95 (p<0.01) - 2.81 (p<0.01)

1.15.2 In vitro studies

Iron dialysability under simulated gastrointestinal conditions can be used as a measure of iron bioavailability (Kane & Miller, 1984). Dialysability was high for beef and bovine serum albumin and low for soy flour, gelatin, casein, soy protein isolate and gluten (Kane & Miller, 1984), while all forms of meat enhanced non haem dialysability significantly when compared with egg albumin (Mulvihill & Morrissey, 1998b). Kapsokefalou & Miller (1991) found that beef reduced high amounts of ferric iron (Fe^{3+}), whereas inhibitors of iron absorption (casein, bovine serum albumin and egg albumin) were ineffective at reducing ferric iron (Fe^{3+}) during in vitro digestions.

Caco-2 cells have also been used to investigate meat's effect on iron absorption. Using radio labeled iron to measure iron absorption in Caco-2 cells, meat increased iron bioavailability five times that of non meat protein sources (Garcia *et al.*, 1996a), while iron uptake from digests of beef, chicken and fish were three to four times the iron intake from a digest containing casein (Glahn *et al.*, 1996). Au and Reddy (2000) found that beef's effect on iron bioavailability measured using Caco-2 cells was similar to that found in human studies (Reddy *et al.*, 1996). Greater iron bioavailability was found from beef and fish digests compared with digests of corn and green beans, when measured by ferritin formation in Caco-2 cells (Glahn *et al.*, 1998).

1.15.3 Studies using complete diets

Two studies have found meat to increase non haem iron absorption when consumed as part of a complete diet. Non haem iron absorption was 70% less from a lacto-ovo-vegetarian diet than from a non vegetarian diet (1.1% versus 3.8%) over an 8 week period using a cross over design (Hunt & Roughead, 1999). Iron absorption was significantly higher when 60g of pork was added to main meals over a five day period compared with a vegetarian diet containing similar contents of vitamin C and phytic acid (Kristensen *et al.*, 2005).

1.15.4 Effect of meat intake on iron status – intervention studies

Experimental studies have shown varied results with regard to the effect of meat on iron status. An increased intake of meat better compensated for initial decreases in iron status in women who exercised over a 12 week period when compared to women who ingested a 50mg iron supplement (Lyle *et al.*, 1992). The women in the meat group consumed 11.8±2.8mg iron (1.8±0.4mg haem iron) per day while the women in the iron supplement group consumed 57.8±2.4mg iron (0.6±0.6mg haem iron) per day. This shows the importance of the type of iron consumed rather than total dietary iron intake in determining iron status. In contrast, no change in iron status was observed when subjects ate either a lacto-ovo-vegetarian diet or a non vegetarian diet for 8 weeks despite an observed difference in dietary iron absorption (Hunt & Roughead, 1999). Hunt et al (1995) found a high meat (289g meat/day; 12.1mg total iron) diet to be unexpectedly associated with lower iron status than a low meat (38.5g meat/day; 8.8mg total iron) and a low meat with supplement (38.5g meat/day; 12.3mg total iron) diet in women who consumed each of the three diets for 7 weeks in random order. Iron balance (an insensitive measure of iron absorption) was similar for the three diets. The three diets consisted of similar amounts of ascorbic acid, dietary fibre and phytic acid, but coffee intake was individualised to subject's preferences. The low meat diets contained only 10% protein compared with the meat diet which contained 20% protein. The reduction in protein in the low meat diets may have enhanced iron bioavailability (Hunt *et al.*, 1995). In addition time periods of 7-8 weeks may not be long enough to observe a significant change in iron status.

1.15.5 Effect of meat intake on iron status - observational studies

In observational studies of free living subjects, meat appears in most cases to have a beneficial effect on iron status. In New Zealand, Heath et al (2001) found that pre menopausal women with mild iron deficiency had a significantly lower intake of meat, fish and poultry (86g versus 111g) compared with subjects who had normal iron stores. This was the only dietary factor associated with mild iron deficiency. A low haem iron intake was associated with low iron stores in Australian women aged 15-30 years (Rangan *et al.*,

1997), while several European studies have found iron status in women to be positively correlated with haem iron or meat intake (Takkunen & Seppanen, 1975; Brussard *et al.*, 1997; Galan *et al.*, 1998).

A recent study in the United Kingdom found that the consumption of a lacto-ovo-vegetarian diet was not associated with a lower iron status (Harvey *et al.*, 2005). Surprisingly, serum ferritin levels were significantly lower in women who ate red meat compared with those who ate fish and poultry. Total dietary iron intake was lower in the red meat group compared with the group who ate fish and poultry (Harvey *et al.*, 2005). In contrast, pre menopausal American women who consumed red meat as their predominant protein source showed a higher iron status than lacto-ovo vegetarians or those consuming fish / poultry as their main protein source, with total iron intakes similar between all three groups (Worthington-Roberts *et al.*, 1988).

Several studies have compared the iron status of vegetarian women with those of non vegetarians. Most studies show that female vegetarians have lower serum ferritin concentrations than non vegetarian females (Helman & Darnton-Hall, 1987; Reddy & Sanders, 1990; Alexander *et al.*, 1994; Ball & Bartlett, 1999; Hua *et al.*, 2001). In Britain, iron deficiency anaemia was less prevalent (6%) among high consumers of red and processed meat compared with women who consumed average amounts of meat (14%) and those who consumed no meat (38%) (Gibson & Ashwell, 2003). However, in most studies vegetarians and vegans do not appear to have a higher incidence of iron deficiency anaemia compared with non vegetarians (Armstrong *et al.*, 1974; Sanders *et al.*, 1978; Worthington-Roberts *et al.*, 1988; Alexander *et al.*, 1994; Ball & Bartlett, 1999). A study in New Zealand Seventh Day Adventists found that vegetarians and non vegetarians had similar haemoglobin and serum ferritin concentrations, however the vegetarian group had more individuals with serum ferritin levels at the lower end of the reference range (Harman & Parnell, 1998).

1.15.6 The New Zealand situation

Meat and meat products provide on average 30-31% of dietary iron for females aged 19-44 years. Ninety two percent of females aged 19-24 years and 94% of females aged 25–44 years consume a regular omnivorous diet. The most common types of meat consumed by females at least once per week included beef, veal and chicken (Russell *et al.*, 1999).

1.16 Meat, fish, poultry factor (MFP factor)

Research to identify the MFP factor and the mechanism by which it increases non haem iron absorption has resulted in considerable controversy. The effect of the MFP factor relates specifically to muscle protein and not to animal protein in general (Cook & Monsen, 1976; Bjorn-Rasmussen & Hallberg, 1979). It has been suggested that meat may enhance non haem iron absorption by stimulation of gastric acid secretion, which is known to increase non haem iron absorption (Bjorn-Rasmussen & Hallberg, 1979). However, meat has been shown to increase iron absorption in patients with achlorhydia, therefore an increase in gastric acid secretion is not a major explanation for the MFP factor effect (Bjorn-Rasmussen & Hallberg, 1979).

No studies have been able to clearly define the MFP factor. It is thought that several factors may contribute to meat's enhancing effect on iron absorption. The MFP factor may work by increasing the transfer of iron into the intestinal mucosal cells or by acting on the contents of these cells (Bjorn-Rasmussen & Hallberg, 1979). The MFP factor may increase non haem iron absorption by reducing ferric (Fe^{3+}) iron to the more soluble ferrous (Fe^{2+}) iron, through the release of amino acids or polypeptides which chelate to non haem iron during the digestion of meat allowing iron to be taken into the intestinal cell or by preventing the formation of iron compounds which are unavailable for absorption.

Both the sulphydryl (-SH) content of meat and the molecular weight of meat's protein degradation products appear to be important in determining the effect of the MFP factor,

while recent studies have suggested that carbohydrate fractions may contribute to the MFP factor (Huh *et al.*, 2004; Hurrell *et al.*, 2006; Wolber *et al.*, 2006).

Early studies suggested that meat's enhancing property was found in the tissue and not in the water-soluble extract of meat (Bjorn-Rasmussen & Hallberg, 1979; Slatkavitz & Clydesdale, 1988). Protein digestion products, including the sulphydryl (-SH) content of muscle tissue may be responsible for the MFP factor. The –SH group includes cysteine and cysteine containing peptides such as glutathione.

These protein digestion products have been investigated using radioiron absorption techniques in human subjects (Martinez-Torres & Layrisse, 1970; Bjorn-Rasmussen & Hallberg, 1979; Martinez-Torres *et al.*, 1981; Layrisse *et al.*, 1984; Taylor *et al.*, 1986). Martinez-Torres and Layrisse (1970) found that a mixture of amino acids equal to those present in 100g of fish enhanced iron absorption to the same extent as 100g of fish when added to black beans. Further experiments found that only the sulphur containing amino acids increased iron absorption from black beans and cysteine was the only amino acid capable of increasing iron absorption to the same extent as fish (Martinez-Torres & Layrisse, 1970). Martinez-Torres et al (1981) found that cysteine given during the ingestion of food doubled non haem iron absorption from black beans, soy beans and maize. However, no enhancing effect was seen when cysteine was mixed with the food prior to the final cooking (Bjorn-Rasmussen & Hallberg, 1979; Martinez-Torres *et al.*, 1981). Cysteine in contact with food may be rapidly oxidised and transformed into cystine, which does not have any effect on iron absorption (Martinez-Torres & Layrisse, 1970). Taylor et al (1986) found iron absorption to be two times greater from an untreated cysteine extract compared with an oxidised cysteine extract prepared from 100g beef when added to maize. At doses calculated to contain the same amount of cysteine; meat, glutathione, and cysteine all increased iron absorption from maize to a similar extent (Layrisse *et al.*, 1984). The amount of glutathione in meat (3% of total cysteine) was too low to fully account for the MFP factor (Layrisse *et al.*, 1984). Because other animal proteins have a similar amino acid composition to meat but no enhancing effect on non haem iron absorption, peptide digestion products rather than free amino acids are more likely to be responsible for the

MFP factor (Hurrell *et al.*, 2006). Hurrell et al (2006) found that isolated beef protein (94% protein content), isolated haem free beef protein (98% protein content), and isolated chicken muscle protein (94% protein) increased iron absorption to the same extent as native beef and chicken muscle when added to a semisynthetic meal in protein equivalent quantities, suggesting that these meats contained no other factors that enhanced iron absorption. However, iron absorption from a meal containing isolated haem free chicken protein (98% protein) further enhanced iron absorption when compared with that of native chicken muscle, indicating that other non protein components in muscle tissue may influence iron absorption (Hurrell *et al.*, 2006).

Studies have found that the MFP factor is not affected by heat (Garcia *et al.*, 1996b; Baech *et al.*, 2003b). Increasing the cooking temperature of meat to 95 and 120°C did not impair non haem iron absorption in humans compared with cooking meat at 70°C (Baech *et al.*, 2003b). Because the cysteine content of the meat decreased with an increase in cooking temperature, this finding does not support a role for the –SH group in meat in promoting non haem iron absorption (Baech *et al.*, 2003b).

In vitro studies have been undertaken to determine the factor in meat that enhances non haem iron absorption. Animal proteins with a high –SH content were found to be more powerful enhancers of iron bioavailability than proteins with a low –SH content (Mulvihill *et al.*, 1998; Mulvihill & Morrissey, 1998a; Mulvihill & Morrissey, 1998b). Significant relationships have been found between the –SH content of meat proteins and their ability to reduce ferric iron (Fe^{3+}), and dialyse ferrous iron (Fe^{2+}) during vitro digestions (Mulvihill & Morrissey, 1998a). Kapsokefalou and Miller (1991) found that cysteine, glutathione and beef all reduced ferric iron compared with inhibitors of iron absorption, which were ineffective at reducing ferric iron. Incorporation of the –SH blocking agent, N-ethylmaleimide (NEM) significantly inhibited ferric iron (Fe^{3+}) reduction (Mulvihill & Morrissey, 1998a) and ferrous iron (Fe^{2+}) dialysability in a dose related manner (Mulvihill *et al.*, 1998; Mulvihill & Morrissey, 1998a; Mulvihill & Morrissey, 1998b). Approximately 65% of the –SH groups are present in myosin and ~29% present in actin (myofibrillar proteins) of meat (Hofmann & Hamm, 1978). Mulvihill et al (1998) found

that myosin had a greater enhancing effect on non haem iron absorption than actin, and within myosin, the enhancing effect was greatest for the heavy meromyosin fraction. The enhancement was related to the distribution of cysteine residues in the myofibrillar proteins (Mulvihill *et al.*, 1998). Several other in vitro studies using iron dialysability methods support the hypothesis that the MFP factor is contained within the peptide digestion products of muscle protein (Kane & Miller, 1984; Politz & Clydesdale, 1988; Slatkavitz & Clydesdale, 1988; Kapsokefalou & Miller, 1991).

The extent and degree of protein digestion appears to be the other important factor in determining the MFP factor. High molecular weight (HMW) peptides formed due to partial or non digestion of proteins may bind iron, preventing its transport through the intestinal mucosa, while low molecular weight (LMW) peptides that form may facilitate iron transport and enhance non haem iron absorption by solubilising iron (Kane & Miller, 1984; Berner & Miller, 1985; Slatkavitz & Clydesdale, 1988). Mulvihill and Morrissey (1998b) found that LMW peptides (<6,000 to 8,000 kDa) significantly enhanced, whereas the HMW peptides (>6,000 to 8,000 kDa) significantly reduced iron dialysability compared with a complete fraction of beef protein in a simulated gastrointestinal in vitro digestion. This was despite the LMW fraction containing a significantly lower proportion of –SH groups than the HMW fraction (Mulvihill & Morrissey, 1998b). Carpenter and Mahoney (1989) found similar results.

Caco-2 cells have also been used to attempt to identify the MFP factor. Cysteine and reduced cysteinyl glycine enhanced iron uptake by Caco-2 cells when the pH was similar to that of the intestinal lumen (pH ~6), while the addition of glutathione and histidine had no effect on uptake from iron complexes, nor did it affect iron solubility (Glahn & Van Campen, 1997). Using separated peptides from beef muscle proteins, Swain et al (2002b) found that LMW peptides ranging from 1-7kDa increased iron solubility at pH 6 and promoted iron uptake, and peptides rich in histidine residues increased iron uptake (Swain *et al.*, 2002b). However, histidine did not appear to enhance iron absorption in a study undertaken in humans (Layrisse *et al.*, 1984). Huh et al (2004) examined the enhancing effect of acidic digests of cooked fish prepared without digestive enzymes on non haem

iron bioavailability. The most active fractions contained negligible amounts of proteins and amino acids but were enriched with low molecular weight carbohydrates (1-10kDa) thought to be oligosaccharides from glycosaminoglycans (GAGs) (Huh *et al.*, 2004). GAGs are part of the connective tissue between the muscle fibres and represent approximately 0.1% of muscle tissue (Pedersen *et al.*, 1999). They have sulphate and carboxylic side chains which could bind iron (Hurrell *et al.*, 2006). These differing conclusions regarding the MFP factor may be due to differences in the active component in meat and fish muscle, differences in Caco-2 cell uptake methodology or due to differences in the way the tissue digests were prepared (Reddy *et al.*, 2006). A recent study, however in humans using stable isotopes found the purified GAGs, sodium hyaluronate and chondroitin sulphate had no effect on iron absorption from a semisynthetic meal (Storcksdieck genannt Bonsmann *et al.*, 2007).

Research has been undertaken in the IFNHH, Massey University, Palmerston North to identify the MFP factor and its effects on non haem iron absorption. Numerous beef meat extracts have been identified, produced and tested using iron binding characteristic procedures, Ussing Chambers to measure in vitro iron absorption, haemoglobin repletion in piglets and mice and Caco-2 cell methodology. Wilkinson et al (2006) used a screening procedure to identify the ability of different extracts to bind iron. This approach assumes that the meat extract binds iron within the stomach and then releases it to the epithelial cells in the duodenum where there is a more neutral pH. This however could be a negative outcome as iron absorption is known to be reduced when iron binds to dietary inhibitors of iron absorption as well as HMW peptides. A number of extracts from meat were found to be capable of binding iron, including extracts containing protein and carbohydrate, extracts associated with the water soluble sarcoplasmic fractions and others which appeared to be hydrolysis products of digestion (Wilkinson *et al.*, 2006).

Wolber et al (2006) tested the extent to which various preparations enhanced the uptake of iron into Caco-2 cells, and the transport of iron across Caco-2 cells. Extracts containing proteins of HMW had little bioactivity. A carbohydrate rich meat extract containing proteins <0.5kDa induced significant up regulation of iron uptake and transport in Caco-2

cells without disrupting epithelial tight junction integrity. This finding is similar to that of Huh et al (2004). While Caco-2 cells are useful as screening models, they are not representative of the true physiological conditions of the gut. Therefore a preparation comprising intact small intestinal tissues dissected from iron deficient mice and mounted in Ussing chambers was used to study the movement of substances across gastrointestinal epithelia. Using this method, the <0.5kDa sarcoplasmic fraction extract was found to increase iron absorption in the jejunum of the small intestine but not the duodenum. The reason for this was unknown (Reynolds *et al.*, 2006a). The addition of different sarcoplasmic meat extracts to non meat diets improved iron retention efficiency by 50-90% in semi anaemic piglets using the haemoglobin repletion method (Morel & Purchas, 2006). Finally a mouse model was used to measure iron absorption from the <0.5kDa sarcoplasmic fraction extract. This extract had a positive effect on iron absorption as measured by the tracer ^{59}Fe, and no difference was found in absorption between mice fed diets containing meat and a meat extract (Reynolds *et al.*, 2006b).

In summary, earlier studies found the sulphydryl content and the molecular weight of protein products to be important aspects of the MFP factor. Recent studies indicate that the MFP factor may be contained in the carbohydrate fractions of meat. Within the IFNHH, the <0.5kDa sarcoplasmic fraction has been shown to be effective in increasing iron absorption in Caco 2 cells, Ussing chambers and in mice. In order to test for MFP factor activity in any preparation the ultimate test has to be in humans. In addition, further isolation and identification of these products is necessary to improve our understanding of how the MFP factor works and what the structure of the MFP factor is.

1.17 Ascorbic acid

1.17.1 Studies using single meals

Numerous single meal studies have consistently shown ascorbic acid to be a strong enhancer of non haem iron absorption (Layrisse *et al.*, 1974; Rossander *et al.*, 1979; Hallberg & Rossander, 1982a; Hallberg & Rossander, 1982c; Gillooly *et al.*, 1983; Gillooly

et al., 1984b; Hallberg & Rossander, 1984; Lynch *et al.*, 1985; Hallberg *et al.*, 1986; Ballot *et al.*, 1987; Reddy & Cook, 1991). Ballot et al (1987) observed that fruits with a high ascorbic acid content (orange, pawpaw, guava) had a greater effect on iron absorption compared with fruits containing less ascorbic acid. Adding cauliflower (high in ascorbic acid) to a meal low in ascorbic acid increased iron absorption, while removing cauliflower from a high ascorbic acid meal decreased iron absorption (Hallberg & Rossander, 1982a). The addition of 150ml of orange juice (70mg ascorbic acid) to a breakfast meal (Rossander *et al.*, 1979) and 250ml orange juice (110mg ascorbic acid) to a hamburger meal doubled iron absorption (Hallberg & Rossander, 1982c). Both the ascorbic acid found naturally in foods and synthetic ascorbic acid enhance iron absorption (Hallberg *et al.*, 1986). Layrisse et al (1974) found that the addition of 70mg ascorbic acid or 150g papaya (containing 70mg ascorbic acid) to a maize meal increased iron absorption by 472 and 537% respectively, while the addition of 50mg dry ascorbic acid and 125g cauliflower (65mg ascorbic acid) to a Latin American meal increased iron absorption by 133 and 170% (Hallberg & Rossander, 1984). Adding 100 and 1000mg of vitamin C to a hamburger meal increased non haem iron absorption by 90 and 133% (Lynch *et al.*, 1985). Recent studies using stable isotopes have all demonstrated ascorbic acid's enhancing effect on non haem iron absorption when added to meals and drinks of varying composition in both children (Davidsson *et al.*, 1998; Davidsson *et al.*, 2001b) and adults (Davidsson *et al.*, 2001a; Fidler *et al.*, 2004).

Several studies across a wide range of meals have shown the enhancing effect of ascorbic acid on iron absorption to be dose related (Bjorn-Rasmussen & Hallberg, 1974; Cook & Monsen, 1977; Hallberg *et al.*, 1986; Ballot *et al.*, 1987; Tuntawiroon *et al.*, 1990; Siegenberg *et al.*, 1991). Adding 12.5mg ascorbic acid to a maize porridge meal increased iron absorption non significantly by 28%, with ascorbic acid increasing iron absorption in a dose dependent manner up to 508% (200mg ascorbic acid) (Bjorn-Rasmussen & Hallberg, 1974). Cook and Monsen (1977) found that the increase in iron absorption was directly proportional to the amount of ascorbic acid added. The addition of 25mg ascorbic acid to a semi synthetic meal increased iron absorption by 65%, while 1000mg ascorbic acid increased iron absorption by 857%. Siegenberg et al (1991) observed a dose dependent

relationship when increasing amounts of ascorbic acid were added to meals high in phytate and tannic acid.

Ascorbic acid improves iron absorption even in the presence of inhibitors such as polyphenols, phytates, calcium and soy proteins (Derman *et al.*, 1977; Rossander *et al.*, 1979; Gillooly *et al.*, 1984b; Hallberg *et al.*, 1989; Deehr *et al.*, 1990; Siegenberg *et al.*, 1991; Tuntawiroon *et al.*, 1991). The inhibitory effects of tea when consumed with maize meal porridge was overcome by large amounts of ascorbic acid (250 or 500mg) (Derman *et al.*, 1977), while 150ml orange juice (70mg ascorbic acid) counteracted the negative effect of tea on iron absorption from a Western breakfast meal (Rossander *et al.*, 1979). The addition of 50 and 100mg of ascorbic acid increased iron absorption significantly in wheat rolls containing 25 and 250mg phytate, with approximately 80mg ascorbic acid necessary to fully counteract 25mg phytate's inhibitory effect (Hallberg *et al.*, 1989). Thirty milligrams of ascorbic acid overcame the inhibitory effect of 58mg phytate phosphorus in a wheat bread meal and adding 25mg ascorbic acid to bread containing 420mg tannic acid restored iron absorption to half of that expected from tannin free bread (Siegenberg *et al.*, 1991). Siegenberg et al (1991) concluded that approximately 50mg of ascorbic acid is necessary to restore iron bioavailability to normal values from any meal containing >100mg tannic acid. In another study, 25 and 50mg ascorbic acid restored iron absorption from a bread and nut meal and a bread and peanut meal to an amount similar to that from a bread meal alone (MacFarlane *et al.*, 1988b). The inhibitory effect of a 500mg calcium supplement on iron absorption from a breakfast meal was overcome when 450ml orange juice was consumed with the meal (Deehr *et al.*, 1990). The addition of 80 but not 40mg of ascorbic acid to a soy infant formula significantly increased iron absorption in adult females (Gillooly *et al.*, 1984b). One hundred milligrams of ascorbic acid was unable to fully overcome the inhibition of iron absorption by Yod Kratin (the leaves of the lead tree, *Leucana glauca*, a vegetable containing iron binding phenolic groups and commonly eaten in Thailand) (Tuntawiroon *et al.*, 1991). The greater the level of inhibitors in a meal, the greater the amount of ascorbic acid required to overcome them. Ascorbic acid's greatest effect occurs when added to meals containing high levels of inhibitors (Hallberg *et al.*, 1986).

Similarly, the effects of ascorbic acid on iron absorption appear to be less when added to meals of high iron bioavailability. Adding 100mg ascorbic acid to a semi synthetic meal of low iron bioavailability increased iron absorption by 277%, but when added to a standard meal containing beef (high iron bioavailability) iron absorption increased by only 67% (Cook & Monsen, 1977). When 100mg ascorbic acid was added to semi synthetic meals containing either isolated soy protein or egg albumin, iron absorption increased by 469% in the isolated soy protein meal and by only 120% in the more highly bioavailable egg albumin meal (Morck *et al.*, 1982).

Hallberg et al (1986) suggested that approximately 50mg ascorbic acid is needed to optimally enhance non haem iron absorption from a meal. Lower levels of ascorbic acid (<25mg) are less likely to increase iron absorption significantly when added to meals (Bjorn-Rasmussen & Hallberg, 1974; MacPhail *et al.*, 1981; Gillooly *et al.*, 1984b; Fairweather-Tait *et al.*, 2000) while 25-50mg ascorbic acid promotes a measurable effect on iron absorption (Hallberg *et al.*, 1986; Tuntawiroon *et al.*, 1991). Table 1.6 summarises the single meal studies that have investigated the effect of ascorbic acid on non haem iron absorption.

Table 1.6 Effect of ascorbic acid on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Cook et al (1972)	n=18M+F, 30yrs	100g maize meal	Maize alone	?	48.7	5.0	4.1	-
	n=14M+F, 41yrs		Maize + 500mg aa	?	36.5	22.5	24.7	6.02
RI – Hb incorp	n=8M+F, 45yrs		Maize + veal	?	47.0	11.6	9.9	2.41
Bjorn-Rasmussen & Hallberg (1974)	n= 1M+ 5F	50g maize flour as porridge	Meal alone	5; 0	N/A	8.50	N/A	-
			Meal + 12.5mg aa	5; 0		9.32		1.28 (ns)
	n= 1M+ 5F		Meal alone	5; 0		5.75		-
RI – Hb incorp + WBC			Meal + 25mg aa	5; 0		13.05		2.95 (p<0.05)
	n= 4M+ 2F		Meal alone	5; 0		6.75		-
			Meal + 50mg aa	5; 0		19.50		2.65 (p<0.05)
	n= 4M+ 2F		Meal alone	5; 0		4.78		-
			Meal + 100mg aa	5; 0		20.83		4.61 (p<0.05)
	n= 4M+ 2F		Meal alone	5; 0		2.63		-
	All 19-35yrs		Meal + 200mg aa	5; 0		9.83		6.08 (p<0.05)
Layrisse et al (1974)	n=13M+F, 38 (18-65)yrs, Hb 140g/L	100g cooked maize	Maize alone	?	19.86	1.38	2.8	-
			Maize + 70mg aa	?		7.90	15.9	5.72
RI – Hb incorp			Maize + 150g papaya (70mg aa)	?		8.79	17.7	6.37
	n=14M+F, 41 (19-63)yrs, Hb 138g/L		Maize alone	?	36.93	4.55	4.9	-
			Maize + 150g papaya (66mg aa)	?		24.69	26.7	5.45
			Maize + 150g papaya +100g fish	?		23.01	24.9	5.08
Cook & Monsen (1977)	n=12M, 23 (19-32)yrs, SF 43 (9-91)µg/L	SS meal (dextrimaltose, corn oil, ovalbumin)	Meal alone	4.1, 0	N/A	0.77	N/A	-
			Meal + 25mg aa	4.1, 0		1.27		1.65 (p<0.01)
			Meal + 50mg aa	4.1, 0		1.94		2.53

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp			Meal + 100mg aa	4.1, 0		3.19		4.15
	n=13M, 21 (18-28)yrs, SF 74 (10 -198)µg/L	SS meal (as above)	Meal alone	4.1, 0		0.74		-
			Meal + 250mg aa	4.1, 0		3.48		4.70
			Meal + 500mg aa	4.1, 0		4.59		6.19
			Meal + 1000mg aa	4.1, 0		7.10		9.57
	n=13M, 20 (18-23)yrs, SF 45 (10-168)µg/L	SS meal (as above)	Meal alone	4.1, 0		2.08		-
			Meal + 100mg aa	4.1, 0		7.86		3.77
	n=13M, 20 (18-25)yrs, SF 76 (36-215)µg/L	Standard meal (beef, potatoes, corn meal, peaches, ice milk, bread, margarine)	Meal alone	4.1, 0		4.05		-
			Meal + 100mg aa	4.1, 0		6.78		1.67 (p<0.002)
	n=12M, 24 (22-30)yrs, SF 74 (29-308)µg/L	SS meal (as above)	Meal alone	4.1, 0		1.0		-
			Meal + 500mg aa	4.1, 0		5.86		5.86
Derman et al (1977)	n=8F, SF 20.8µg/L	150g cooked maize meal porridge + tea (5g tea leaves/150ml + 10ml milk)	Meal alone	?	58.7	1.5	1.0	-
			Meal + 100mg aa	?		9.4	6.4	6.27 (p<0.02)
RI – Hb incorp	n=8F, SF 14.6µg/L		Meal alone	?	30.4	3.7	4.9	-
			Meal + 250mg aa	?		21.6	28.4	5.84 (p<0.01)
	n=11F, SF 17.3µg/L		Meal alone	?	24.0	2.0	3.3	-
			Meal + 500mg aa	?		21.3	35.5	10.65 (p<0.005)
Rossander et al (1979)	n=12M, 29yrs, 142g/L + 5F, 29yrs,129g/L	Western breakfast meal (2 wheat rolls + 12g margarine + 10g orange marmalade + 15g cheese) + 8g coffee in 150ml water	Meal alone	2.8, 0	52.0	3.7	0.1	-
	n=5M, 32yrs, 148g/L + 7F,		Meal + 150ml orange juice (70mg aa)	3.1, 0		8.0	0.3	1.96 (p<0.05)
RI – Hb incorp + WBC	26yrs,127g/L							
	n=5M, 29yrs, 136g/L + 7F,	Western breakfast meal (as	Meal alone	2.8, 0		5.6	4.3	-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	25yrs,131g/L n=5M, 28yrs, 144g/L + 7F, 31yrs,130g/L	above) + 2.5g tea in 150ml water	Meal + 150ml orange juice (70mg aa)	3.1, 0		6.8	5.2	1.09 (p<0.05)
MacPhail et al (1981)	n=12F, SF 19µg/L	250g maize porridge	Meal alone	5.0, 0	25.4	6.4	8.5	-
			Meal + 25mg aa	5.0, 0		6.9	8.9	1.05 (ns)
RI – Hb incorp	n=11F, SF 33µg/L		Meal alone	5.0, 0	20.4	6.3	12.0	-
			Meal + 50mg aa	5.0, 0		6.0	12.0	1.0 (ns)
	n=9F, SF 21µg/L		Meal alone	5.0, 0	30.5	6.1	8.0	-
			Meal + 100mg aa	5.0, 0		12.0	15.6	1.95 (p<0.01)
Hallberg & Rossander (1982a)	n=7M, 25yrs, Hb 145g/L+ 3F, 21yrs, Hb 126g/L	Vegetarian low ascorbate meal (45g dried navy beans, 45g brown rice, bread (25g cornflour, 25g wheat flour), 14g margarine, 55g apples, 8g walnuts, 225g yoghurt)	Meal alone (7mg aa)	5.8, 0	43.2	2.5	2.3	-
	n=5M, 29yrs, Hb 143g/L + 5F, 29yrs, Hb 130yrs		Meal + cauliflower (67mg aa)	6.5, 0	39.9	5.1	5.1	2.21
RI – Hb incorp + WBC	n=8M, 27yrs, Hb 144g/L+ 4F, 25yrs, Hb 130yrs	Vegetarian high ascorbate meal (42g red kidney beans, 30g tomato sauce, bread roll, 15g margarine, 125g cauliflower, 55g cottage cheese, 125g pineapple, 37g banana)	Meal alone (74mg aa)	5.8, 0	31.5	13.5	17.1	-
	n=7M, 23yrs, Hb 151g/L + 3F, 23yrs, Hb 137yrs		Meal less cauliflower (14mg aa)	5.1, 0	33.6	5.3	6.3	0.37
	n= 47M, 40yrs, Hb 148g/L	Hamburger meal	Meal alone (2mg aa)	3.0, 0.5	36.4	10.9	12.0	-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	+ 12F, 23yrs, Hb133g/L n=7M, 24yrs, Hb 152g/L + 3F, 23yrs, Hb 129g/L	(hamburger bun, 60g string beans, 150g mashed potatoes)	Meal + salad (47mg aa)	3.6, 0.5	27.6	12.7	18.4	1.53
Hallberg & Rossander (1982c)	n=7M, 25yrs, Hb 156g/L + 5F, 27yrs, Hb 139g/L	110g hamburger (82g meat), 60g string beans, 150g potatoes	Meal + 250ml water	3.0, ?	35.5	8.7	9.8	-
			Meal + 250ml orange juice (110mg ascorbic acid)	3.5, ?		16.1	18.1	2.10 (p<0.01)
RI – Hb incorp + WBC								
Morck et al (1982)	n=11M, 27 (19-50)yrs, SF 31 (2-130)µg/L	SS drink (corn syrup solids, corn oil) + 16.9g isolated soy protein	Meal alone	4.0, 0	N/A	0.56	N/A	-
			Meal + 100mg aa	4.0, 0		3.20		5.69 (p<0.001)
RI – Hb incorp		SS drink (corn syrup solids, corn oil) + 18.4g egg albumin	Meal alone	4.0, 0		5.05		-
			Meal + 100mg aa	4.0, 0		10.19		2.20 (p<0.001)
Gillooly et al (1983)	n=25F, SF 14.7µg/L	215g rice pudding (rice, sucrose, margarine)	Meal alone	3.0, 0	32.0	N/A	3.1	-
	n=9F, SF 42.6µg/L		Meal + 15mg aa	3.0, 0	24.2		8.1	2.61
RI – Hb incorp	*All 21-76 yrs*							
Hallberg & Rossander (1984)	n=9M, 29yrs, Hb 146g/L + 1F, 46yrs, Hb 150g/L	Latin American meal (80g dry maize chapattis, 31g dry black beans, 50g polished rice cooked)	Meal alone	4.4, 0	30.4	2.7	4.0	-
			Meal + 125g cauliflower (65mg aa)	5.4, 0		7.7	10.8	2.7 (p<0.01)
RI – Hb incorp + WBC	n=4M, 30 yrs, Hb 157g/L + 6F, 24 yrs, Hb 132g/L		Meal alone	4.3, 0	36.0	1.2	1.2	-
			Meal + 50mg dry aa	4.3, 0		3.4	2.8	2.3 (p<0.01)
Gillooly et al (1984a)	n=13F, SF 16.1µg/L	200g malted sorghum porridge	Sorghum + 9.5mg aa	3.0-4.2mg	45.0	N/A	2.4	-
	n=18F, Hb 139g/L		Sorghum + 50mg aa	total iron	30.5		9.4	3.92 (p<0.005)
RI – Hb incorp								
Gillooly et al (1984b)	n=11F, SF 20.6µg/L	50g soy infant formula	Formula alone	3.0, 0	31.0	2.6	3.35	-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Formula + 20mg aa	3.0, 0		3.4	4.39	1.31 (ns)
	n= 9F, SF 34.8µg/L		Formula alone	3.0, 0	35.0	0.6	0.69	-
			Formula + 40mg aa	3.0, 0		4.5	5.14	7.45 (p<0.002)
	n=12F, SF 16.4µg/L		Formula + 40mg aa	3.0, 0	35.0	4.9	5.60	-
			Formula + 80mg aa	3.0, 0		5.2	5.94	1.06 (ns)
	n=12F, SF 12.1µg/L	50g soy infant formula	Formula + 20mg aa	3.0, 0	34.0	2.4	2.82	-
		50g milk infant formula	Formula + 20mg aa	3.0, 0		5.3	6.24	2.21 (p<0.02)
	n=7F, SF 20.5µg/L	50g soy infant formula	Formula + 40mg aa	3.0, 0	32.2	7.2	8.94	-
		50g milk infant formula	Formula + 40mg aa	3.0, 0		19.5	24.2	2.71 (p<0.02)
Lynch et al (1985)	n=10M, 23yrs, SF 63µg/L	Quarter pound hamburger	Meal + water	?	N/A	6.62	N/A	-
		(white bun, fries, vanilla	Meal + 100mg Vit C	?		12.58		1.90 (p<0.01)
RI – Hb incorp	n=11M, 28yrs, SF 92µg/L	milkshake)	Meal + water	?		3.60		-
			Meal + 1000mg Vit C	?		8.39		2.33 (p<0.01)
Hallberg et al (1986)	n=7M (2bd), 24yrs + 3F, 23yrs	Hamburger meal (110g	Meal alone	3.0, 0.5	27.4	6.0	7.6	-
		hamburgers, 60g string	Meal + salad (45mg aa)	3.6, 0.5		9.1	12.0	1.56
RI – Hb incorp + WBC	n=8M (6bd), 31yrs + 2F (1bd), 21yrs	beans, 150g potatoes, 66g	Meal alone	2.0, 0.5	39.1	14.2	16.4	-
		meat, 50g lettuce, 35g	Meal + cauliflower (70mg aa)	4.0, 0.5		25.8	28.4	1.92
	n=7M (3bd), 25yrs + 5F, 27 yrs	tomatoes, 35g cucumbers,	Meal alone	3.0, 0.5	35.5	8.7	10.0	-
		25g green pepper)	Meal + 250ml OJ (105mg aa)	3.5, 0.5		16.1	19.2	2.10
	n=8M (3bd), 27yrs + 4F (1bd), 25yrs	Vegetarian 'low ascorbate'	Meal alone	5.1, 0	33.6	5.3	5.6	-
		meal (45g dried navy	Meal + cauliflower (70mg aa)	5.8, 0	31.5	13.5	16.6	2.96
		beans, 45g brown rice,						
		bread (25g cornflour + 25g						
		wheat flour), 14g						
		margarine, 55g apples, 8g						

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		walnuts, 225g yoghurt)						
	n=5M (3bd), 29yrs + 5F (1bd), 29yrs	Vegetarian 'high ascorbate' meal (42g dried red kidney beans, 30g tomato sauce, 15g margarine, 55g cottage cheese, 125g pineapple, 37g banana)	Meal alone	5.8, 0	39.9	1.9	2.0	-
			Meal + cauliflower (70mg aa)	6.5, 0		5.1	6.4	3.57
	n=14F (2bd), 20yrs	Rice meal (50g rice, 20g string beans, 20g cabbage, 20g collard)	Meal alone	1.8, 0	30.6	6.0	8.4	-
			Meal + 150g papaya (75mg aa)	2.3, 0		20.7	30.4	3.61
	n=1M (1bd), 26yrs + 9F (3bd), 23yrs	Breakfast meal (150ml coffee, 1 wheat roll, 12g margarine, 10g orange marmalade, 15g cheese)	Meal alone	2.8, 0	41.7	5.7	5.2	-
			Meal + 12.5mg aa	2.8, 0		7.7	6.4	1.12
	n=1M, 23yrs + 9F, 21yrs		Meal alone	2.8, 0	41.3	5.4	4.4	-
			Meal + 25mg aa	2.8, 0		7.7	6.8	1.52
	n=1M (1bd), 26yrs + 9F (2bd), 22yrs		Meal alone	2.8, 0	34.7	5.7	6.8	-
			Meal + 50mg aa	2.8, 0		9.1	10.4	1.64
	n=4M (3bd), 34yrs + 6F (1bd), 26yrs		Meal alone	2.8, 0	34.7	5.9	7.6	-
			Meal + 75mg aa	2.8, 0		8.6	10.8	1.61
	n=5M (2bd), 26yrs + 5F (1bd), 24yrs		Meal alone	2.8, 0	39.6	5.4	4.4	-
			Meal + 500mg aa	2.8, 0		15.3	11.6	2.34
	n=1M (1bd), 28yrs + 8F (2bd), 27yrs	Breakfast meal (as above, wheat roll unfortified)	Meal alone	0.4, 0	44.4	6.0	5.6	-
			Meal + 50mg aa	0.4, 0		9.2	8.4	1.68

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	n=4M (3bd), 34yrs + 6F (1bd), 24yrs	Wheat bran rolls (27g unfortified wheat flour, 12.5g bran) + 20g margarine + 150ml water	Meal alone	2.9, 0	30.8	3.9	5.2	-
			Meal + 50mg aa	2.9, 0		8.6	10.8	2.43
	n=5M (2bd), 27yrs + 5F (2bd), 27yrs	Hamburger meal (as above)	Meal alone	3.0, 0.5	27.0	9.2	10.4	-
			Meal + 25mg aa	3.0, 0.5		12.8	15.2	1.63
	n=7M (3bd), 29yrs + 3F (1bd), 20yrs		Meal alone	3.0, 0.5	30.0	10.8	15.2	-
			Meal + 50mg aa	3.0, 0.5		18.2	24.8	1.61
	n=4M (2bd), 26yrs + 6F (1bd), 24yrs		Meal alone	3.0, 0.5	39.3	11.0	10.0	-
			Meal + 500mg aa	3.0, 0.5		30.1	32.0	3.47
	n=3M (2bd), 30yrs + 6F (1bd), 24yrs	Latin American meal (as above)	Meal alone	4.3, 0	34.7	2.5	2.7	-
			Meal + 25mg aa	4.3, 0		4.3	4.8	2.05
	n=4M (2bd), 30yrs + 6F (1bd), 24yrs		Meal alone	4.3, 0	36.0	1.2	1.2	-
			Meal + 50mg aa	4.3, 0		3.4	2.8	2.44
	n=6M (3bd), 28yrs + 3F (1bd), 29yrs		Meal alone	4.3, 0	37.3	1.3	1.2	-
			Meal + 500mg aa	4.3, 0		8.4	9.2	6.52
	n=4M (4bd), 26yrs + 6F (1bd), 23yrs	Pizza meal (84g wheat flour, 40g tomato puree, 25g black olives, 30g anchovies, 100g cheese)	Meal alone	4.2, 0	33.9	6.4	6.8	-
			Meal + 50mg aa	4.2, 0		13.3	14.8	2.64
	n=11M, 26yrs + 9F, 25yrs	Rice meal (as above)	Meal + 25mg aa	1.8, 0	31.7	10.1	10.8	1.24
	n=8M, 28yrs + 8F, 24yrs		Meal + 50mg aa	1.8, 0	16.0	6.5	16.8	1.93

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	n=25F, 25yrs	Rice, dried chili, 50g fish, fish sauce, 300ml soup	Meal alone Meal + 50mg aa	1.3, 0 1.3, 0	34.8	14.7 29.2	19.2 38.0	- 2.19
Ballot et al (1987)	n=29F, SF 14.7µg/L	200g Rice meal (includes margarine + sucrose)	Meal alone	3.4, 0	41.2	1.9	2.5	-
RI – Hb incorp	n=14F, SF 25.4µg/L		Meal + 100ml apple juice (1.7mg aa)	3.4, 0	42.8	3.8	3.5	1.4
	n=10F, SF 5.3µg/L		Meal + 100ml grape juice (1.4mg aa)	3.4, 0	41.0	4.8	4.0	1.6
	n=14F, SF 25.4µg/L		Meal + 100ml guava nectar (15mg aa)	3.4, 0	42.8	9.9	9.2	3.68
	n=10F, SF 10.7µg/L		Meal + 100ml apple juice (24mg aa)	3.4, 0	48.9	12.9	11.1	4.44
	n=8F, SF 19.1µg/L		Meal + 100ml pineapple juice (5.2mg aa)	3.4, 0	36.6	9.7	12.1	4.84
	n=9f, SF 7.3µg/L		Meal + 100ml orange juice (30mg aa)	3.4, 0	51.0	16.6	12.3	4.92
	n=8F, SF 19.1µg/L *All 21-74 years*		Meal + 100ml pear juice (30mg aa)	3.4, 0	36.6	13.8	15.0	6.0
MacFarlane et al (1988a) RI – Hb incorp	n=13F, SF 19.5µg/L n=11F, SF 7.2µg/L	Lupines (7g protein)	Lupines alone Lupines + 30mg aa Lupines alone Lupines + 60mg aa	3.0, 0 3.0, 0 3.0, 0 3.0, 0	67.1 52.1	1.0 5.3 0.7 6.9	0.6 3.2 0.5 5.3	- 5.30 (p<0.0001) - 9.95 (p<0.0001)
MacFarlane et al (1988b)	n=14F, SF 14.3µg/L n=11F, SF 16.1µg/L	60g white bread + margarine + 50g nuts + 10g honey + 40ml water	Bread + peanut alone Bread + peanut + 25mg aa Bread + brazil nut alone	?	60.4 60.9	3.1 5.2 2.6		- 1.68 (p<0.005) -

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp			Bread + brazil nut + 50mg aa			6.9		2.65 (p<0.001)
Hallberg et al (1989)	n=1M (1bd), 6F (1bd)	Wheat roll (WR) (80g white wheat flour, 60% extraction) + 20g margarine +150ml water	Roll alone	4.1, 0	36.6	16.7	22.4	-
			Roll + 50 mg aa	4.1, 0		27.3	37.6	1.75 (p<0.01)
RI – Hb incorp + WBC	n=4M (2bd) + 6F		Roll + 25mg phytate	4.1, 0	36.3	7.1	7.2	-
			Roll + 25mg P + 50 mg aa	4.1, 0		14.2	14.4	2.17 (p<0.005)
	n=8F (2bd)		Roll + 25mg P	4.1, 0	40.6	6.5	6.8	-
			Roll + 25mg P + 100mg aa	4.1, 0		19.5	19.6	3.56 (p<0.005)
	n=3M (2bd) + 5F		Roll + 250mg P	4.1, 0	36.4	6.2	5.6	-
			Roll + 250mg P + 50 mg aa	4.1, 0		15.0	15.2	2.84 (p<0.001)
	n=3M (1bd) + 7F (2bd)		Roll +250mg P	4.1, 0	30.7	3.2	4.0	-
	All 19-47 yrs		Roll + 250mg P + 100mg aa	4.1, 0		10.0	12.4	3.43 (p<0.001)
Deehr et al (1990)	n=19 F, 63 (52-72)yrs, SF 111 (30-216)µg/L	Breakfast meal (12g Corn Chex, 14g cracker, 11g dried milk, 10g margarine, 317g formula beverage) (238mg Ca)	Meal alone	3.18, 0	N/A	8.3	N/A	-
			Meal + 500mg CCM	3.18, 0		6.0		0.72 (p<0.05)
RI – WBC			Meal + 500mg CCM + 450ml orange juice (193mg aa)	3.18, 0		7.4		0.89 (ns)
Fairweather-Tait et al (2000)	n=20F, 40 (24-50)yrs, SF 44.7 (19.4-103.5)µg/L	30g cornflakes, 125g milk, 300ml tea with milk	Meal alone	3.75, 0	N/A	14.1	N/A	-
			Meal + 15mg aa (aa:Fe 1.3:1)	3.75, 0		17.5		1.24 (ns)
SI – faecal monitoring								
Tuntawiroon et al (1990)	n=9	70g pork, 100g cabbage, 100g rice (4:1 polished: unpolished rice)	Meal alone	?	N/A	N/A	11.7	-
			Meal + green collard (25mg aa)	?			14.1	1.20 (ns)
RI – Hb incorp			Meal + green collard (50mg aa)	?			15.1	1.29 (p<0.05)

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	n=11	70g pork, 100g cabbage, 100g rice (0:5 polished: unpolished rice)	Meal alone	?			6.4	-
			Meal + green collard (25mg aa)	?			8.8	1.37 (ns)
			Meal + green collard (50mg aa)	?			13.7	2.14 (p<0.05)
Reddy & Cook (1991) RI – Hb incorp	n=13, 20 (18-23)yrs, SF 45 (38-54)µg/L	SS meal (egg albumin, dextrimaltose, corn oil)	Meal alone Meal + 100mg aa	4.1, 0 4.1, 0	N/A	2.08 7.86	N/A	- 3.77 (p<0.01)
Siegenberg et al (1991)	n=11F, SF 6.6µg/L	80g wheat bread (58mg phytate) + 15g potato + margarine	Bread alone	3.0, 0	46.7	6.7	5.7	-
			Bread + 30mg aa	3.0, 0		12.6	10.8	2.08 (p<0.01)
	n=12F, SF 20.7µg/L		Bread alone	3.0, 0	52.7	3.8	2.9	-
RI – Hb incorp			Bread + 50mg aa	3.0, 0		10.4	7.9	2.97 (p<0.01)
	n=14F, SF 6.2µg/L		Bread alone	3.0, 0	76.3	10.4	5.5	-
			Bread + 150mg aa	3.0, 0		27.4	14.4	3.21 (p<0.0001)
	n=12F, SF 21µg/L	80g wheat bread (420mg tannic acid) + 15g potato + margarine	Bread alone	3.0, 0	61.7	1.5	1.0	-
			Bread + 25mg aa	3.0, 0		3.9	2.5	2.90 (p<0.01)
	n=16F, SF 18.9µg/L		Bread alone	3.0, 0	63.1	2.0	1.3	-
			Bread + 100mg aa	3.0, 0		7.9	5.0	5.51 (p<0.001)
	n=13F, SF 8.1µg/L		Bread alone	3.0, 0	44.7	3.6	3.2	-
			Bread + 500mg aa	3.0, 0		18.0	16.1	7.11 (p<0.001)
Tuntawiroon et al (1991) RI – Hb incorp	n=9M	100g rice, 50g fried fish, 55g curry, 150ml water, 5g Yod Kratin (146mg tae)	Meal alone Meal + 50mg aa Meal + 100mg aa	5.20mg total iron	N/A	2.8 4.5 6.4	3.4 5.4 7.6	- 1.59 (ns) 2.24 (p<0.05)
	n=11M	100g rice, 50g fried fish,	Meal alone			2.8	2.8	-

Author / Method	*Subjects*	*Test meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non Haem Iron Absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	All 20-40 yrs	55g curry, 150ml water, 10g Yod Kratin (293mg tae)	Meal + 50mg aa Meal + 100mg aa			4.2 4.9	4.2 4.9	1.50 (p<0.05) 1.75 (p<0.01)
Davidsson et al (1998) SI – Hb incorp – NTIMS	n=10, SF 25µg/L n=10, SF 22 µg/L *6-7 year old M + F*	Chocolate flavoured milk drink (25g chocolate drink powder, 220g water, 30g condensed milk)	Drink alone Drink + 25mg aa Drink + 25mg aa Drink + 50mg aa	6.3, 0 6.3, 0 6.3, 0 6.3, 0	N/A	1.6 5.1 5.4 7.7	N/A	- 3.19 (p<0.0001) - 1.43 (p<0.05)
Davidsson et al (2001b) SI – Hb incorp - NTIMS	n=9, SF 10 (6-18)µg/L *6-7 year old M + F*	42g white wheat bread + 50g drink powder (16% rice flour, 8% wheat flour, 16% milk powder, 16% soy flour) + 200g water	Meal + 27mg aa (0.6:1) Meal + 70mg aa (1.6:1) (molar ratio aa: Fe)	14.0, 0 14.0, 0	N/A	5.1 8.2	N/A	- 1.61 (p<0.01)
Davidsson et al (2001a) SI – Hb incorp - NTIMS	n=10F , 20-28yrs, SF 10 (6-27)µg/L	217g pea-protein isolate infant formula	1:2.1 (molar ratio Fe:aa) 1:4.2 (molar ratio Fe:aa)	? ?	N/A	14.8 22.1	N/A	- 1.49 (p<0.0001)
Fidler et al (2004) SI – Hb incorp - NTIMS	n=10F, 20-26yrs, SF 13.0µg/L	50g wheat based infant cereal + 8g milk powder + 75ml water	Meal alone Meal + 63mg aa (molar ratio aa:Fe 4:1)	5.0, 0 5.0, 0	N/A	4.1 11.7	N/A	- 2.90 (p<0.001)

Ascorbic acid only appears to be effective in enhancing iron absorption when taken with meals. Cook and Monsen (1977) found that ascorbic acid taken in the morning does not influence iron absorption from meals eaten 4 and 8 hours later.

Cooking may completely or partially destroy ascorbic acid (Hallberg *et al.*, 1982). Iron absorption was significantly lower from meals kept warm at 75°C for 4 hours compared with freshly prepared meals (Hallberg *et al.*, 1982). Most of the reduction in ascorbic acid occurred during the first hour.

1.17.2 Ascorbic acid versus meat

A few studies have compared ascorbic acid and meat's enhancing effect on iron absorption. Iron absorption from a maize meal given with papaya and fish was similar to a maize meal given with papaya alone (Layrisse *et al.*, 1974). Adding 125g cauliflower (60mg ascorbic acid) to a vegetarian meal increased iron absorption more than the addition of 90g meat (Hallberg & Rossander, 1982a) while 50mg of ascorbic acid improved iron absorption to the same extent as 75g beef when added to a Latin American meal (Hallberg & Rossander, 1984). Hallberg et al (1989) found adding 50mg ascorbic acid to a wheat roll meal (25mg phytate) increased iron absorption by 117%, while 50g meat increased iron absorption by 12%. The same amount of ascorbic acid or meat added to a wheat roll meal containing 250mg phytate increased iron absorption by 184 and 86% respectively. Allen and Ahluwalia (1997) suggested that 1mg of ascorbic acid equates to 1 to 1.5g meat in its ability to promote non haem iron absorption from a meal. Meat contains no ascorbic acid, so it is other factors in meat which contribute to its enhancing effect on non haem iron absorption.

1.17.3 Studies using complete diets

Four studies have investigated the effect of ascorbic acid on the absorption of iron from complete diets. The addition of 25mg ascorbic acid as limeade to 2 meals per day for 2 weeks increased iron absorption by 246% in women in rural Mexico (Diaz *et al.*, 2003).

Hunt et al (1994) found that ascorbic acid (500mg 3 times per day for 5½ weeks) improved iron absorption using the balance method. In both studies women were iron depleted and consumed diets of poor iron bioavailability. In contrast, two studies have found ascorbic acid to have no effect on iron absorption when added to a complete diet (Hunt *et al.*, 1994; Cook & Reddy, 2001). Cook and Reddy (2001) found no significant difference in mean iron absorption among 3 diets (self selected, high ascorbic acid (247mg/day) and low ascorbic acid (51mg/day)) in subjects who ate each diet for 5 days. This lack of significant effect may have been due to the inclusion of subjects with normal iron stores and the consumption of diets of high iron bioavailability. However, Hunt et al (1994) found that ascorbic acid supplementation (500mg 3x/day over 5 weeks) did not affect apparent iron absorption (diet-faeces) by women with low iron stores who consumed either a poor iron bioavailability diet or a typical Western diet (Hunt *et al.*, 1994). The effect of vitamin C on iron absorption from complete diets appears to be lower than that from single meals. This may explain why prolonged supplementation with vitamin C does not appear to affect iron status (Cook & Reddy, 2001).

1.17.4 Effect of ascorbic acid on iron status – intervention studies

Prolonged supplementation with Vitamin C appears to have little or no effect on iron status. Two grams of ascorbic acid per day with meals for 16 weeks failed to increase serum ferritin levels in healthy volunteers eating self selected diets. This was not caused by adaptation to the ascorbic acid intake because iron absorption from single meals was still increased by ascorbic acid at the end of 16 weeks. No significant effect was seen in serum ferritin levels of 5 iron replete and 4 iron deficient subjects who continued the study 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). Increasing dietary ascorbic acid intake by 25mg at 2 meals per day (using limeade) for 8 months did not improve iron status in iron deficient Mexican women consuming diets high in phytate and non haem iron. Groups consumed similar amounts of haem and non haem iron. It is likely that these women may have had high menstrual blood losses (leading to low iron stores), meaning that the improved iron absorption seen with the addition of limeade to meals (Diaz *et al.*, 2003) was insufficient to increase iron stores (Garcia *et al.*, 2003). When 25 healthy

women with low iron stores consumed either a diet with poorly bioavailable iron or a typical Western diet and were given 500mg ascorbic acid 3 times daily with meals for 10 weeks, serum ferritin levels increased slightly (Hunt *et al.*, 1994).

1.17.5 Effect of ascorbic acid on iron status - observational studies

The majority of studies in young women have not any association between total daily ascorbic acid intake and iron status (Galan *et al.*, 1985; Bairati *et al.*, 1989; Preziosi *et al.*, 1994; Rangan *et al.*, 1997; Galan *et al.*, 1998). A New Zealand study found that Vitamin C intake was not related to the risk of female subjects having mild iron deficiency (Heath *et al.*, 2001). Razagui et al (1991) found that the total daily intake of ascorbic acid was less important than the amount of ascorbic acid consumed at meal times in determining the iron status of long stay mentally handicapped menstruating women. This supports the finding that ascorbic acid is effective in enhancing iron absorption only when taken with meals (Cook & Monsen, 1977), and may explain why total daily ascorbic acid intake has been shown to have minimal effect on iron status.

1.17.6 The New Zealand situation

The mean and median intakes of Vitamin C for female New Zealanders aged 19-24 years was 108 and 96mg/day; and 105 and 92mg/day for women aged 25-44 years (Russell *et al.*, 1999) both which are above the RDI of 45mg/day (Commonwealth Department of Health and Ageing Australia, 2006). No data is available on whether this ascorbic acid intake is consumed at meal times or not. Six percent of females aged 19-24 and 4% aged 25-44 years had used Vitamin C supplements in the past year (Russell *et al.*, 1999) The vitamin C intake of New Zealand women was described as moderate in a Dunedin study when compared to premenopausal women in other countries (Heath *et al.*, 2001).

1.18 Organic acids

The effects of organic acids on non haem iron absorption have not been widely studied (Table 1.7). Citric, malic, tartaric, lactic and erythorbic acid all appear to enhance non haem iron absorption. Citrus fruits contain a minimum of 1g citric acid/100g and high quantities of citric acid are found in vegetables and in milk (Gillooly *et al.*, 1983). The addition of 1g but not 36mg citric acid to a rice meal increased iron absorption significantly (Gillooly *et al.*, 1983). Mean iron absorption from a rice meal and solution containing 33mg ascorbic acid increased significantly with the addition of 750mg citric acid (Ballot *et al.*, 1987). Adding 4g of citric acid to a rice meal and 100ml drink of orange juice increased iron absorption significantly; while iron absorption from 100ml orange juice containing 28mg ascorbic acid was better than that from 100ml plain water containing the same amount of ascorbic acid when added to a rice meal, indicating that citric acid's effect is additive to that of ascorbic acid (Ballot *et al.*, 1987). Derman et al (1987) demonstrated a dose related but limited enhancing effect of 1-4g citric acid on iron absorption from an isolated soy protein drink. One study, however observed significant inhibition of non haem iron absorption when 1g citric acid was added to a meal containing rice, maize and black beans (Hallberg & Rossander, 1984).

Both 1g of malic and tartaric acid increased mean iron absorption significantly when added to a rice meal (Gillooly *et al.*, 1983). In contrast, a significant negative correlation was found between the malic acid content of fruit and iron absorption. However, fruits with higher malic acid contents tended to also have lower levels of ascorbic acid (Ballot *et al.*, 1987). Malic acid is found in deciduous plants such as plums, peaches and apples in concentrations ranging from 0.3g to greater than 1g/100g. Tartaric acid is found in white wines in concentrations ranging from 163-234mg/100g (Gillooly *et al.*, 1983).

Lactic acid is thought to be responsible for promoting iron absorption from sorghum and maize derived beers (Derman *et al.*, 1980). The addition of lactic acid to a maize gruel increased iron absorption significantly when compared with hydrochloric acid (Derman *et al.*, 1980). The addition of 340mg lactic acid to a rice meal increased iron absorption but

not significantly (Baynes *et al.*, 1990). An oat gruel fermented with the probiotic *Lactobacillus plantarum 299v* enhanced non haem iron absorption significantly compared with non fermented oat gruels. This effect was attributed to lactic acid as well as a specific effect of the probiotic itself (Bering *et al.*, 2006).

Erythorbic acid is a stereoisomer of ascorbic acid and a food additive. The addition of erythorbic acid at molar ratios of 2:1 and 4:1 relative to added iron increased iron absorption by 163 and 358% respectively. Iron absorption from a meal with added erythorbic acid (molar ratio 4:1) was higher than that from a meal fortified with ascorbic acid at the same molar ratio (Fidler *et al.*, 2004).

Table 1.7 Effect of organic acids on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Derman et al (1980)	n=21M+F, SF 50µg/L	Maize gruel (200g solids/L)	Gruel + hydrochloric acid	3.0, 0	N/A	0.4	N/A	-
			Gruel + lactic acid (2ml/L solution)	3.0, 0		1.2		3.00 (p<0.001)
Gillooly et al (1983)	n=7F, SF 6.7µg/L	215g rice pudding (rice, sucrose, margarine)	Meal alone	3.0, 0	31.8	N/A	4.9	-
			Meal + 36mg citric acid	3.0, 0			9.9	2.02 (ns)
RI - Hb incorp	n=7F, SF 18.2µg/L		Meal alone	3.0, 0	27.2		2.8	-
			Meal + 1g citric acid	3.0, 0			8.5	3.04 (p<0.001)
	n=8F, SF 11.5µg/L		Meal alone	3.0, 0	36.7		4.8	-
			Meal + 1g L-malic acid	3.0, 0			9.5	1.98 (p<0.05)
	n=11F, SF 18.8µg/L		Meal alone	3.0, 0	32.1		4.1	-
			Meal + 1g tartaric acid	3.0, 0			9.6	2.34 (<0.05)
	All 21-76 yrs							
Hallberg & Rossander (1984)	n=5M, 30yrs, Hb 149g/L + 5F, 24yrs, Hb 129g/L	Latin American meal (80g dry maize chapattis, 31g dry black beans, 50g polished rice cooked)	Meal alone	4.3, 0	36.9	2.4	2.0	-
			Meal + 1g citric acid	4.3, 0		1.1	0.8	0.40 (p<0.05)
RI - Hb incorp + WBC								
Ballot et al (1987)	n=13F, SF 15.9µg/L	200g rice + 100ml water + 33mg aa	Meal alone	3.0, 0	N/A	11.4	N/A	-
			Meal + 750mg citric acid	3.0, 0		17.0		1.49 (p<0.005)
RI - Hb incorp	n=13F, SF 15.8µg/L	200g rice	Meal + 100ml water + 28mg aa	3.0, 0		9.8		-
			Meal + 100ml orange juice (28mg aa)	3.0, 0		13.9		1.42 (p<0.025)
	n=9F, SF 7.3µg/L	200g rice + 100ml orange juice (30mg aa + 700mg	Meal alone	3.0, 0		16.6		-
			Meal + 4g citric acid	3.0, 0		22.6		1.36 (p<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	All 21-74yrs	citric acid)						
Derman et al (1987)	n=12F, SF 23.8µg/L	10g isolated soy protein drink	Drink alone	3.1, 0	45.1	1.1	0.98	-
			Drink + 1g citric acid	3.1, 0		2.6	2.3	2.36
RI - Hb incorp	n=10F, SF 31.9µg/L		Drink + 2g citric acid	3.1, 0	65.2	4.8	2.9	-
	All 21-63yrs		Drink + 4g citric acid	3.1, 0		6.8	4.2	1.42 (p<0.05)
Baynes et al (1990)	n=13F, SF 27µg/L	100g cooked rice	Rice alone	3.0, 0	33.3	2.2	2.6	-
			Rice + 340mg lactic acid	3.0, 0		3.1	3.7	1.41 (ns)
RI - Hb incorp								
Fidler et al (2004)	n=10F, 20-26yrs, SF 13.0µg/L	50g wheat based infant cereal + 8g milk powder + 75ml water	Meal alone	5.0, 0	N/A	4.1	N/A	-
			Meal + 31.5mg erythorbic acid (2:1 EA:Fe)	5.0, 0		10.8		2.63 (p<0.0001)
SI – Hb incorp - NTIMS			Meal + 63mg erythorbic acid (4:1 EA:Fe)	5.0, 0		18.8		4.58 (p<0.0001)
			Meal + 63mg aa (4:1 aa:Fe)	5.0, 0		11.7		2.85 (p<0.001)

1.19 Alcohol

1.19.1 Studies using single meals

Alcohol appears to increase non haem iron absorption although evidence is limited (Table 1.8). Wine tends to have a reasonably high iron content (Cook *et al.*, 1995) and the tartaric acid in white wines may also contribute to an increased iron bioavailability (Gillooly *et al.*, 1983). Iron absorption increased but not significantly when 30ml ethanol was added to a thick gruel mixture (Derman *et al.*, 1980). Hallberg and Rossander (1982c) observed a significant 23% increase in iron absorption from a hamburger meal when consumed with distilled alcohol but not beer or wine. Iron absorption from a dinner roll was significantly reduced when served with red but not white wine. This decrease in iron absorption was attributed to the polyphenol content of the red wine. It is well known that polyphenols reduce non haem iron absorption (Hurrell *et al.*, 1999). When the alcohol content of the wines was reduced, there was a significant decrease in iron absorption with red wines but no effect was seen with white wines (Cook *et al.*, 1995).

Table 1.8 Effect of alcohol on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption*	*Non haem iron absorption (%)*		*Absorption Ratio*
						Actual	*Adjusted*	
Derman et al (1980)	n=7M+F; Hb 13.2g/L	Maize gruel (40g maize meal, 25g sorghum)	Gruel alone	?	N/A	1.2	N/A	-
			Gruel + 30ml ethanol	?		2.3		1.92 (ns)
RI – Hb incorp								
Hallberg & Rossander (1982c)	n=4M, 25yrs, 162g/L + 6F, 45 yrs, 133g /L	110g hamburger (82g meat), 60g string beans, 150g potatoes	Meal + 250ml water	3.0, ?	34.3	9.1	10.6	-
			Meal + 250ml red wine	6.9, 0		10.9	12.7	1.09 (ns)
	n=7M, 31yrs, Hb 145g/L + 2F, 31yrs, Hb 137g/L		Meal + 250ml water	3.0, ?	45.3	14.1	12.5	-
RI – Hb incorp + WBC			Meal + alcohol - 60ml (40%) + 190ml water	3.0, ?		17.1	15.1	1.23 (p<0.05)
	n=10M, 26 yrs, Hb 151g/L		Meal + 250ml water	3.0, ?	28.8	6.3	8.8	-
			Meal + 250ml beer (2.2% alcohol)	3.0, ?		6.8	9.4	1.04 (ns)
Cook et al (1995)	n=7M + 1F, 24yrs, SF 49 (36-66)µg/L	70g dinner roll	Roll + water	0.7-1.0mg in all meals	N/A	15.89	N/A	-
			Roll + 120ml aramon (red wine)			5.05		0.32 (p<0.005)
RI – Hb incorp			Roll + 120ml pinot noir (red wine)			4.05		0.25 (p<0.005)
			Roll + 120ml white wine			11.43		0.72 (ns)
	n=2M + 6F, 26yrs, SF 29 (20-42)µg/L		Roll + water			14.12		-
			Roll + 120ml low alcohol aramon (red wine)			3.31		0.23 (p<0.005)
			Roll + 120ml low alcohol pinot noir (red wine)			3.08		0.22 (p<0.001)
			Roll + 120ml low alcohol white wine			11.66		0.83 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption*	*Non haem iron absorption (%) Actual Adjusted*	*Absorption Ratio*
	n=7M+1F, 24yrs, SF 37 (27-53)µg/L		Roll + 120ml low alcohol pinot noir (red wine)			2.39	-
			Roll + 120ml pinot noir (red wine)			3.32	1.39 ($p<0.01$)
			Roll + 120ml low alcohol white wine			10.38	-
			Roll + 120ml white wine			9.43	0.91 (ns)

1.19.2 Effect of alcohol intake on iron status – observational studies

Several studies in elderly subjects have found alcohol intake to be positively associated with serum ferritin concentration (Jacques *et al.*, 1989; Fleming *et al.*, 1998; Liu *et al.*, 2003; Milman *et al.*, 2004). These findings were also seen in a younger population of men (Leggett *et al.*, 1990). Most studies in young women have not investigated the effect of alcohol on iron status. Brussard et al (1997) found a positive relationship between alcohol intake and iron status, while no association was found between alcohol intake and iron status in Australian females aged 15-30 years (Rangan *et al.*, 1997). In a large study of Australian adult twins, alcohol consumption was found to be positively associated with serum ferritin levels, with beer having a greater influence than wine or spirits (Whitfield *et al.*, 2001). Using data from the third National Health and Examination Survey, Ioannou et al (2004) concluded that consumption of up to 2 alcoholic drinks/day is associated with reduced risk of iron deficiency and iron deficiency anaemia without any increase in the risk of iron overload.

1.19.3 The New Zealand situation

In New Zealand beer is consumed at least once per week by 15% of women aged 15-24 years and by 11% of women aged 25-44 years. White wine is consumed at least once per week by 9% of women aged 15-24 years, and 5% of women aged 25-44 years, with red wine being consumed at least once per week by 1% of women aged 15-24 years and by 5% of women aged 25-44 years. Twelve percent of women aged 15-24 years and 9% of women aged 25-44 years consume spirits/liqueurs at least once per week (Russell *et al.*, 1999). No further data is available on the amounts of alcohol consumed weekly and the manner in which this alcohol is consumed.

1.20 Vitamin A and β-Carotene

The first studies on vitamin A and iron found that vitamin A increased iron absorption when added to breakfast meals of varying compositions (Layrisse *et al.*, 1997; Layrisse *et al.*, 1998). When vitamin A was added to a breakfast meal containing tea or coffee, iron absorption from that breakfast meal was not significantly different to a breakfast meal containing no tea or coffee, indicating that vitamin A can overcome tea and coffee's inhibitory effects on iron absorption (Layrisse *et al.*, 1997). Garcia-Casal et al (1998) found the addition of Vitamin A increased iron absorption two times for rice, 0.8 times for wheat and 1.4 times for corn, while β-Carotene increased iron absorption more than three times for rice and 1.8 times for wheat and corn. Increasing the doses of Vitamin A or β-Carotene did not further significantly increase iron absorption. In contrast to these results Walczyk et al (2003) observed that the addition of Vitamin A did not significantly change iron absorption from corn bread in five separate studies using radioisotope and stable isotope methods. An analysis of variance (ANOVA) of all 5 of these studies showed that iron absorption from corn bread to which Vitamin A was added was significantly lower (5%) than that from corn bread without added Vitamin A (Walczyk *et al.*, 2003). It was suggested that this negative effect may have been the result of systematic errors or the population group studied. Walczyk et al's (2003) study was conducted in a young, healthy European population, while earlier studies were conducted in Venezuelan populations of lower socio-economic status (Layrisse *et al.*, 1997; Garcia-Casal *et al.*, 1998; Layrisse *et al.*, 1998). One hypothesis is that Vitamin A may only enhance iron absorption in subjects with impaired Vitamin A status (Walczyk *et al.*, 2003). This however needs to be investigated further. Table 1.9 summarises the single meal studies that have investigated the effect of Vitamin A and β-Carotene on non haem iron absorption.

Table 1.9 Effect of vitamin A and β-Carotene on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%)*		*Absorption Ratio*
						Actual	*Adjusted*	
Layrisse et al (1997)	n=1M+17F, SF 13µg/L	Maize bread + ferrous fumerate + 8g espresso coffee	Meal alone	6.0, 0	N/A	2.0	N/A	-
			Meal + 1000 IU Vit A			8.5		4.25 (p<0.05)
RI – Hb incorp								-
	n=5M+10F, SF 32µg/L	Maize bread + ferrous fumerate	Meal alone	6.0, 0		3.2		1.96 (p<0.05)
			Meal + 1000 IU Vit A			6.3		-
		Maize bread + ferrous fumerate + 1g tea	Meal alone			2.0		1.80 (p<0.05)
			Meal + 1000 IU Vit A			3.6		-
	n=2M+18F, SF 28µg/L	White wheat flour bread + 8g espresso coffee	Meal alone	3.0, 0		2.0		0.90 (ns)
			Meal + 1000 IU Vit A			1.8		-
	n=2M+12F, SF 27µg/L	White wheat flour bread *(all meals 100g flour + 10g margarine + 50g cheese)*	Meal alone	3.0, 0		6.9		1.20 (p<0.05)
			Meal + 1000 IU Vit A			8.3		
Garcia-Casal et al (1998)	n= 6M+11F, SF 29µg/L	Rice (100g polished rice + 10g margarine)	Rice alone	4.0, 0	N/A	3.9	N/A	-
			Rice + 496IU Vit A			8.4		2.15 (p<0.05)
			Rice + 1376IU Vit A			7.5		1.92 (p<0.05)
RI – Hb incorp			Rice + 2526IU Vit A			11.7		3.00 (p<0.05)
	n= 6M+11F, SF 27µg/L	Corn bread (100g corn flour + ferrous fumerate + 10g margarine + 50g cheese)	Corn alone	6.0, 0		5.8		-
			Corn + 338IU Vit A			9.8		1.68 (p<0.05)
			Corn + 554IU Vit A			13.9		2.39 (p<0.05)
			Corn + 661IU Vit A			11.8		2.03 (p<0.05)
	n=3M+17F, SF 31µg/L	Wheat bread (100g white wheat flour + 10g margarine + 50g cheese)	Wheat alone	3.0, 0		4.2		-
			Wheat + 1009IU Vit A			7.4		1.76 (p<0.05)
			Wheat + 600IUVit A			7.1		1.69 (p<0.05)
			Wheat + 1372IU Vit A			7.5		1.78 (p<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%)*		*Absorption Ratio*
						Actual	*Adjusted*	
	n=3M+8F, SF 21µg/L	Rice (as above)	Rice alone	4.0, 0		2.1		-
			1376IU Vit A			4.6		2.19 (p<0.05)
			342IU β-carotene + coffee			6.4		3.04 (p<0.05)
			558IU β-carotene			8.8		4.19 (p<0.05)
	n=6M+14F, SF 24µg/L	Corn (as above)	Corn alone	6.0, 0		3.0		-
			554IU Vit A			6.6		2.20 (p<0.05)
			395IU β-carotene			8.5		2.83 (p<0.05)
			902IU β-carotene + coffee			6.3		2.10 (p<0.05)
	n= 3M+16F, SF 18µg/L	Wheat (as above)	Wheat alone	3.0, 0		3.0		-
			600IU Vit A			5.5		1.83 (p<0.05)
			500IU β-carotene			8.3		2.76 (p<0.05)
			1211IU β-carotene + coffee			8.4		2.80 (p<0.05)
Layrisse et al (1998)	n=4M+8F, SF 52µg/L	Maize flour bread + ferrous fumerate + 50g cheese + 10g margarine	Meal alone	?	N/A	2.7	N/A	-
			Meal + 1000 IU Vit A	?		5.1		1.88 (p<0.05)
RI – Hb incorp	n=4M+9F, SF 19µg/L	Maize flour bread (50g flour) + 2.5g ferrous fumerate + 50g cheese + 10g margarine	Meal alone	?		3.6		-
			Meal + 1000 IU Vit A	?		10.6		2.94 (p<0.05)
Walczyk et al (2003)	n=7M+5F, 22 (19-23)yrs, SF 28 (7-180)µg/L	Corn bread + 200ml water + ferrous sulphate	Meal alone	2.5, 0	N/A	5.0	N/A	-
			Meal + Vit A(1000µg retinol)			4.3		0.91 (ns)
	n=1M+7F, 22 (19-23)yrs, SF 13 (5-44)µg/L	Corn bread + 200ml coffee + ferrous sulphate	Meal alone	2.5, 0		4.0		-
SI – Hb incorp – NTIMS			Meal + Vit A(1000µg retinol)			3.5		0.91 (ns)
	n=6M+ 4F, 27 (19-35)yrs, SF 36 (9-132)µg/L	Corn bread + 200ml water	Meal alone	0.5, 0		3.1		-
			Meal + Vit A(1000µg retinol)			3.0		0.99 (ns)
RI – Hb incorp +	n=4M+5F, 26 (20-29)yrs,	Corn bread + 200ml coffee	Meal alone	0.5, 0		2.1		-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%)*		*Absorption Ratio*
						Actual	*Adjusted*	
WBC	SF 22 (6-188)µg/L n=4M+6F, 27 (20-36)yrs, SF 19 (5-80) µg/L	Corn bread + 200ml coffee + ferrous fumerate *(all meals 50g flour)*	Meal + Vit A(1000µg retinol) Meal alone Meal + Vit A(1000µg retinol)	5.0, 0		2.0 3.1 2.9		0.96 (ns) - 0.94 (ns)

1.21 Fermented products

Studies investigating whether fermented foods such as sauerkraut and soy sauce increase iron absorption have found mixed results (Table 1.10) (Gillooly *et al.*, 1983; Baynes *et al.*, 1990; MacFarlane *et al.*, 1990; Fidler *et al.*, 2003). Baynes et al (1990) found that soy sauce had no effect on iron absorption when added to a soy flour meal, while iron absorption increased by 90 and 225% when 12.5 and 25ml soy sauce were added to a rice meal (Baynes *et al.*, 1990). In contrast, Fidler et al (2003) found that the addition of 10g soy sauce to a rice meal decreased iron absorption by 29%. The variation in these results can be explained by the different raw ingredients used in preparing the soy sauces, the different manufacturing methods used and the differences in the meal compositions to which soy sauce was added (Fidler *et al.*, 2003).

Table 1.10 Effect of fermented foods on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; Haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Baynes et al (1990)	n=12F, SF 16.6µg/L	250-300g soy flour meal (included 100g tomatoes + 50g onions)	Soy flour alone	3.0, 0	50.0	8.7	7.0	-
			Soy flour + 25ml soy sauce	3.0, 0		7.2	5.8	0.83 (ns)
RI – Hb incorp	n=12F, SF 13.1µg/L	100g cooked rice	Rice + soy flour	3.0, 0	53.3	5.2	3.9	-
			Rice + 25ml soy sauce	3.0, 0		13.9	10.4	2.67 (p<0.01)
	n=12F, SF 7.8µg/L	*Soy sauce = traditionally fermented Japanese soy sauce, prepared from soybeans*	Rice alone	3.0, 0	40.3	3.5	3.5	-
			Rice + 25ml soy sauce	3.0, 0		11.4	11.3	3.25 (p<0.001)
	n=12F, SF 12.9µg/L		Rice alone	3.0, 0	74.1	5.4	2.9	-
			Rice + 12.5ml soy sauce	3.0, 0		10.3	5.6	1.90 (p<0.01)
Fidler et al (2003)	n=10F, SF 16µg/L	Rice (50g dry rice, fortified with ferrous sulphate)	Rice alone	5.1, 0	N/A	8.5	N/A	-
			Rice + 10g soy sauce	5.7, 0		6.0		0.71 (p<0.05)
SI – Hb incorp	*All 19-29yrs*	*Soy sauce = Chinese soy sauce, prepared from defatted soybean meal + wheat bran*						

1.22 Polyphenols

1.22.1 Studies using single meals

Polyphenols are found in tea, coffee, cocoa, red wine, some leafy vegetables, grains, nuts, herbs and spices (Zijp, 2000). Several studies have shown tea to be strong inhibitor of non haem iron absorption. The addition of tea (5g/200ml) to a bread meal reduced iron absorption by 68% and by 77% when added to a meal containing rice, vegetable soup and 100mg ascorbic acid (Disler *et al.*, 1975b). Adding 200ml black tea to a hamburger meal reduced iron absorption by 61% (Lynch *et al.*, 1985). The addition of one cup of tea (5g/150ml) to a maize porridge meal reduced iron absorption by 43% and by 78% when added to a maize porridge meal containing ascorbic acid (Derman *et al.*, 1977). The addition of 5g tea/200ml to 30g malted sorghum and 50mg ascorbic acid reduced iron absorption by 57% (Gillooly *et al.*, 1984a). Several studies have compared the effects of tea and coffee on non haem iron absorption from single meals. Derman et al (1977) found iron absorption decreased by 57% when one cup of tea (5g tea) was served with a maize porridge meal containing 100mg ascorbic acid, while one cup of coffee reduced iron absorption by 37%. Non haem iron absorption was reduced by 61 and 33% when one cup of tea (4g dry tea) and one cup of coffee (14g coffee) respectively were added to a hamburger meal (Hallberg & Rossander, 1982c). In another study the addition of one cup of tea (1.75g dry tea) to a hamburger meal decreased iron absorption by 64%, while one cup of drip coffee decreased iron absorption by 39% (Morck *et al.*, 1983). Zijp (2000) concluded that one cup of tea reduces non haem iron absorption by 60 to 70% while one cup of coffee reduces iron absorption by approximately 40%. No research has investigated the effect of timing of tea intake in humans, however between meal tea consumption has been estimated to inhibit non haem iron absorption by approximately 20% (Zijp, 2000).

Other studies have investigated the effect on non haem iron absorption when tea replaces coffee in a meal. When tea (2.5g tea) was served instead of coffee in two separate breakfast meals, iron absorption decreased by 56% and 47% in two different groups of subjects (Hallberg *et al.*, 1986). This study however, did not adjust for the iron status of

subjects. Iron absorption decreased by 75% when black tea replaced coffee in a meal containing bread (Hurrell *et al.*, 1999).

Herbal teas decrease non haem iron absorption but to a lesser extent than black tea. The addition of Chinese green tea to a pasta meal reduced non haem iron absorption by 28% (Samman *et al.*, 2001). A study by Hurrell et al (1999) found the inhibition of iron absorption by black tea was 79-94%, peppermint tea 84%, pennyroyal 73%, vervain 59%, limeflower 52% and chamomile 47%. Drinks containing a higher amount of polyphenols decreased iron absorption more significantly than those containing less polyphenols. At an identical concentration of polyphenols, black tea had a greater inhibitory effect than the other herbal teas and an equal inhibitory effect to peppermint tea (Hurrell *et al.*, 1999).

The effect of tea and coffee on iron absorption appears to be dose related. Hurrell et al (1999) found that as black tea was diluted, iron absorption increased. In another study, one cup of single strength tea (1.75g tea) caused iron absorption to decrease by 83%, and one cup of triple strength tea (5.25g tea) by 91% when added to a semi synthetic meal (Reddy & Cook, 1991). Morck et al (1983) found iron absorption from a semi purified meal was reduced by 83% with one cup of instant coffee and by 91% when the concentration of the instant coffee was doubled. Layrisse et al (2000) observed that iron absorption from a breakfast meal given with 2g instant coffee was not significantly different to the breakfast meal given alone. However, the addition of 4g instant coffee resulted in a significant 48% reduction in iron absorption. Increasing tannic acid from 12 to 833mg significantly inhibited iron absorption in a dose dependent manner from 30 to 79% when added to a white wheat bread meal (Siegenberg *et al.*, 1991).

Red wine, cocoa, vegetables, legumes and condiments containing high levels of polyphenols also inhibit non haem iron absorption (Gillooly *et al.*, 1983; Gillooly *et al.*, 1984a; Tuntawiroon *et al.*, 1991; Cook *et al.*, 1995; Hurrell *et al.*, 1999). One glass of red wine served with a bread roll meal reduced iron absorption by 75%, and was two to three times more inhibitory than white wine which contained few polyphenols (Cook *et al.*, 1995). Red wine, however had little effect on iron absorption from a more complex

composite meal (Hallberg & Rossander, 1982c). Cocoa reduces iron absorption (Gillooly *et al.*, 1984a) but to a lesser extent than that of tea (Hurrell *et al.*, 1999). An inverse correlation has been reported between vegetable foods high in polyphenols (spinach, eggplant, lentils) and iron absorption (Gillooly *et al.*, 1983). Removing the outer layers of sorghum grain reduced their polyphenol contents and increased iron absorption significantly. Iron absorption from a sorghum cultivar without polyphenols was significantly greater than from a sorghum cultivar with a high polyphenol content (Gillooly *et al.*, 1984a). Increasing the amount of Yod Kratin, a vegetable high in polyphenols caused a successive decrease in iron absorption from a composite meal up to 87% with 20g of Yod Kratin (Tuntawiroon *et al.*, 1991). Phenolic rich extracts obtained from rosemary decreased non haem iron absorption by 21% when added to a meal consisting of pasta, a meat sauce, vegetables and bread (Samman *et al.*, 2001). The addition of chilli (25mg polyphenols) to a rice and vegetable meal decreased iron absorption by 38%. However, the addition of tumeric (50mg polyphenols) had no effect on iron absorption (Tuntipopipat *et al.*, 2006). It is likely that both the quantity and quality of polyphenols determine their extent of effect on iron absorption. Nuts (MacFarlane *et al.*, 1988b) and legumes (Lynch *et al.*, 1984) which contain significant amounts of polyphenols also have a marked inhibitory effect on iron absorption. Table 1.11 summarises the single meal studies that have investigated the effects of polyphenols on non haem iron absorption.

Table 1.11 Effect of polyphenols on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Disler et al (1975b)	n=8F, SF 30.8 (2-124) µg/L, 26-60yrs	Bread	Bread + water	?	35.8	10.4	9.3	-
			Bread + 5g dry tea/200ml	?		3.3	3.0	0.32 (p<0.001)
RI – Hb incorp	n=8F, SF 26.9 (3-76)µg/L, 26-60yrs	Rice + vegetable soup + 100mg ascorbic acid	Meal + water	?	34.7	10.8	9.4	-
			Meal + 5g dry tea/200ml	?		2.5	2.2	0.23 (p<0.001)
Derman et al (1977)	n=22F, SF 17.0µg/L	40g dry maize + 0mg aa	Meal alone	2.0, 0	50.5	3.8	3.0	-
			Meal + 1 cup of tea (5g tea/150ml)	2.0, 0		2.1	1.7	0.57 (p<0.01)
RI – Hb incorp	n=8F, SF 10.0µg/L	40g dry maize + 50mg aa	Meal alone	2.0, 0	42.1	34.0	28.6	-
			Meal + 1 cup of tea (5g tea/150ml)	2.0, 0		6.7	6.4	0.22 (p<0.001)
	n=11F, SF 14.2µg/L	40g dry maize + 100mg aa	Meal alone	2.0, 0	44.2	35.4	32.0	-
			Meal + 1 cup of tea (5g tea/150ml)	2.0, 0		15.1	13.7	0.43 (p<0.0001)
	n=11F, SF 6.5µg/L	40g dry maize + 100mg aa	Meal alone	2.0, 0	51.2	38.3	29.9	-
			Meal + 1 cup of coffee (3g ground coffee)	2.0, 0		24.1	18.8	0.63 (p<0.001)
	All 21-71yrs							
Hallberg & Rossander (1982c)	n=8M, 33yrs, Hb 149g/L + 2F, 35yrs, Hb 132g/L	110g hamburger (82g meat), 60g string beans, 150g potatoes	Meal + 250ml water	3.0, 0.66	58.5	15.8	10.8	-
			Meal + 250ml tea (4g dry tea)	3.0, 0.66		6.0	4.1	0.39 (p<0.01)
RI – Hb incorp + WBC	n=7M, 34yrs Hb 145g/L + 3F, 31yrs, Hb 141g/L		Meal + 250ml water	3.0, 0.66	42.2	13.6	12.9	-
			Meal + 250ml coffee (14g coffee)	3.0, 0.66		8.9	8.4	0.67 (p<0.01)
	n=4M, 25yrs Hb 162g/L + 6F, 45yrs Hb 133g/L		Meal + 250ml water	3.0, 0.66	34.3	9.1	10.6	-
			Meal + 250ml red wine (3.9mg iron)	6.9, 0.66		10.9	12.7	1.09 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Gillooly et al (1983) RI – Hb incorp	n=8F, SF 23µg/L	100g broccoli puree	Broccoli alone Broccoli + 500mg tannic acid	3.0, 0 3.0, 0	N/A	29.7 1.5	N/A	- 0.05 (p<0.0001)
Morck et al (1983) RI – Hb incorp	n=11M, 25 (19-30)yrs, SF 60 (27-156)µg/L	Hamburger meal (113g beef patty + bun)	Meal + 200ml water Meal + 200ml tea (1.75g dry tea) Meal + 200ml drip coffee	1.4, 0.9 1.4, 0.9 1.4, 0.9	15.73	3.71 1.32 2.25	9.43 3.36 5.72	- 0.36 (p<0.001) 0.61 (p<0.05)
	n=9M, 31 (23-50)yrs, SF 87 (46-181)µg/L	SS meal (egg albumin, corn syrup solids, corn oil)	Meal + 200ml water Meal + 200ml drip coffee Meal + 200ml instant coffee (1.5g coffee) Meal + 200ml instant coffee (3g coffee)	4.1, 0 4.1, 0 4.1, 0 4.1, 0	N/A	5.88 1.64 0.97 0.53	N/A	- 0.28 (p<0.001) 0.17 (p<0.001) 0.09 (p<0.001)
Gillooly et al (1984a) RI – Hb incorp	n=16F, SF 31.7µg/L n=12F, SF 26.1µg/L n=13F, SF 11.9µg/L n=18F, Hb 139g/L n=7F, SF 9.1µg/L n=9F, SF 22.5µg/L	30g sorghum 30g malted sorghum + 50mg aa 250ml full cream milk + 3g Fe + sugar	Whole BP (high pp) Pearled BP (low pp) Whole BP (high pp) Pearled BP (low pp) Whole BP (high pp) Whole albino (low pp) Sorghum alone Sorghum + tea (5g tea leaves / 200ml) Milk alone 10g cocoa (280g lignin / kg)	3.0-4.2, 0 *for all meals*	34.4 41.1 29.3 30.5 36.3 45.9	N/A	1.7 3.5 2.4 6.3 1.9 4.3 9.4 4.0 7.5 3.5	- 2.06 (p<0.05) - 2.63 (p<0.05) - 2.26 (p<0.05) - 0.43 (p<0.05) - 0.47 (p<0.05)
Lynch et al (1985)	n=11M, 25yrs, SF 59µg/L	Quarter pound hamburger	Meal + water	?	N/A	11.13	N/A	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp		patty and white bun	Meal + tea (200ml black)	?		4.37		0.39 (p<0.01)
Reddy & Cook (1991)	n=9M + F, 28yrs, SF 47µg/l	SS meal (egg albumin, dextrimaltose, corn oil)	Meal alone	4.1, 0	N/A	7.45	N/A	-
			Meal + 1 cup single strength tea (1.75g/200ml)	4.1, 0		1.23		0.17 (p<0.01)
RI – Hb incorp			Meal + 1 cup triple strength tea (5.25g/200ml)	4.1, 0		0.64		0.09 (p<0.01)
Siegenberg et al (1991)	n=12F, SF 11.5µg/L	80g white wheat bread + 15g potato + margarine	Bread alone	3.0, 0	66.8	9.1	5.5	-
			Bread + 12mg tannic acid	3.0, 0		6.0	3.6	0.70 (p=0.05)
	n=12F, SF 10.4µg/L		Bread alone	3.0, 0	59.7	7.4	5.0	-
RI – Hb incorp			Bread + 26mg tannic acid	3.0, 0		3.3	2.2	0.48 (p<0.01)
	n=15F, SF 8.3µg/L		Bread alone	3.0, 0	62.5	18.4	11.8	-
			Bread + 55mg tannic acid	3.0, 0		5.0	3.2	0.33 (p<0.001)
	n=14F, SF 24.8µg/L		Bread alone	3.0, 0	40.8	8.5	8.3	-
			Bread + 263mg tannic acid	3.0, 0		1.5	1.5	0.22 (p<0.001)
	n=11F, SF 24.0µg/L		Bread alone	3.0, 0	50.0	13.9	11.1	-
			Bread + 833mg tannic acid	3.0, 0		2.6	2.1	0.21 (p<0.0001)
Tuntawiroon et al (1991)	*n=10-11M in each group All 20-40 yrs*	Typical South East Asian meal (50g fried fish, 100g rice, 55g curry)	0g Yod Kratin (YK)	5.36	N/A	12.8	11.7	-
			3g YK (87.6tae)	5.03		6.4	5.0	0.43 (p<0.001)
			5g YK (146tae)	5.20		3.5	2.8	0.24
RI – Hb incorp			10g YK (292tae)	6.05		2.4	2.0	0.17
			15g YK (438tae)	5.52		2.0	1.6	0.14
			20g YK (584tae)	5.80		1.70	1.3	0.11
Cook et al (1995)	n=7M + 1F, 24yrs, SF 49 (36-66)µg/L	70g dinner roll (0.54mg Fe)	Roll + water	Roll + wine 0.74-1.0mg Fe	N/A	15.89	N/A	-
			Roll + aramon (red) wine (1.95g/L pp)			5.05		0.32 (p<0.005)
RI – Hb incorp								

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Roll + pinot noir (red) wine (2.98g/L pp)			4.05		0.25 (p<0.005)
			Roll + white wine (0.19g/L pp)			11.43		0.72 (ns)
	n=2M + 6F, 26yrs, SF 29 (20-42)µg/L		Roll + water			14.12		-
			Roll + low alcohol aramon (1.83g/L pp)			3.31		0.23 (p<0.005)
			Roll + low alcohol pinot noir (2.97g/L pp)			3.08		0.22 (p<0.001)
			Roll + low alcohol white (0.22g/L pp)			11.66		0.83 (ns)
	n=7M+1F, 24yrs, SF 37 (27-53)µg/L		Roll + low alcohol white			10.38		-
			Roll + low alcohol pinot noir			2.39		0.23 (p<0.001)
			Roll + white wine			9.43		-
			Roll + pinot noir			3.32		0.35 (p<0.001)
			Each drink - 120mL					
Layrisse et al (1997)	n= 4M + 3F, SF 17µg/L	Bread (100g precooked maize flour), 50g cheese, 10g margarine	Meal alone	6.0, 0	N/A	5.1	N/A	-
			Meal + 2g American coffee	6.0, 0		7.7		1.51 (ns)
			Meal + 4g espresso coffee	6.0, 0		8.2		1.61 (ns)
			Meal + 4g cappuccino coffee	6.0, 0		7.8		1.53 (ns)
	n=1M + 9F, SF 26µg/L		Meal alone	6.0, 0		4.4		-
			Meal + 2g American coffee	6.0, 0		5.3		1.20 (ns)
			Meal + 4g espresso coffee	6.0, 0		4.6		1.05 (ns)
			Meal + 8g espresso coffee	6.0, 0		3.1		0.58 (ns)
	n=2M + 18F, SF 28µg/L	Bread (100g white wheat flour), 50g cheese, 10g margarine	Meal alone	3.0, 0		6.8		-
			Meal + 2g American coffee	3.0, 0		1.2		0.18 (p<0.05)
			Meal + 4g espresso coffee	3.0, 0		0.4		0.06 (p<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Meal + 8g espresso coffee	3.0, 0		0.7		0.10 (p<0.05)
Hurrell et al (1999)	n=2M + 7F, 25yrs, SF 34 (9-90)µg/L	50g Fe fortified bread roll + 10g butter + 275ml beverage	Water	2.1, 0	N/A	12.9	N/A	-
			Assam tea A (274mg pp)	2.1, 0		0.74		0.06 (p<0.001)
RI – Hb incorp			Peppermint tea (177mg pp)	2.1, 0		2.01		0.16 (p<0.001)
			Pennyroyal tea (121mg pp)	2.1, 0		3.53		0.27 (p<0.001)
	n=5M + 5F, 23yrs, SF 39 (18-144)µg/L		Water	2.1, 0		5.63		-
			Assam tea A (274mg pp)	2.1, 0		0.89		0.16 (p<0.001)
			Vervain tea (116mg pp)	2.1, 0		2.32		0.41 (p<0.001)
			Lime flower tea (58mg pp)	2.1, 0		2.71		0.48 (p<0.01)
	n=6M + 4F, 27yrs, SF 61 (13-193)µg/L		Water	2.1, 0		4.46		-
			Assam tea B (396mg pp)	2.1, 0		0.92		0.21 (p<0.001)
			Camomile tea (52mg pp)	2.1, 0		2.35		0.53 (p<0.001)
			Cocoa (116mg pp)	2.1, 0		1.29		0.29 (p<0.001)
	n=3M + 6F, 23yrs, SF 33 (20-59)µg/L		Water	2.1, 0		6.58		-
			Ass tea B (100%)	2.1, 0		0.59		0.09 (p<0.001)
			Ass tea B (50%)	2.1, 0		1.05		0.16 (p<0.001)
			Ass tea B (25%)	2.1, 0		1.18		0.18 (p<0.001)
	n=9F, 22yrs, SF 50 (13-104)µg/L		Water	2.1, 0		4.33		-
			Ass tea B (25%)	2.1, 0		0.66		0.15 (p<0.001)
			Ass tea B (10%)	2.1, 0		1.48		0.32 (p<0.05)
			Ass tea B (5%)	2.1, 0		1.47		0.34 (p<0.01)
	n=1M + 9F, 28yrs, SF 47 (12-76)µg/L		Coffee (120mg pp)	2.1, 0		2.88		-
			Assam tea A	2.1, 0		0.71		0.25
Layrisse et al (2000)	n=8M + 9F, 15-50yrs, SF 31µg/L	Breakfast meal (100g corn flour, 50g cheese, 10g margarine)	Meal alone	3.0, 0	N/A	7.5	N/A	-
			Meal + 2g coffee (American type); 50 mg tannin [ ]	3.0, 0		6.7		0.89 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
RI – Hb incorp			Meal + 4g coffee (espresso type); 100 mg tannin [ ]	3.0, 0		3.9		0.52 (p<0.05)
			Meal + 2g tea (1.6g tea leaves); 295 mg tannin [ ]	3.0, 0		3.9		0.52 (p<0.05)
Reddy et al (2000)	n=8M+F, SF 37µg/L	Beef enchilada	Meal alone (128mg pp)	1.9, ?	N/A	11.3	13.9*	-
	n=9M+F, SF 77µg/L		Meal + coffee (304mg pp)	1.9, ?		2.3	5.9*	0.42
RI – Hb incorp	n=10M+F, SF 28µg/L	Chicken chow mein	Meal alone (67mg pp)	0.7, ?		11.5	10.7*	-
	n=9M+F, SF 77µg/L		Meal + tea (224mg pp)	0.7, ?		3.2	8.1*	0.76
	n=10M+F, SF 28µg/L	Green pepper, beef and	Meal alone (152mg pp)	2.7, ?		19.7	18.4*	-
	n=9M+F, SF 77µg/L	rice	Meal + tea (362mg pp)	2.7, ?		4.9	12.4*	0.67
	n=8M+F, SF 37µg/L	Tuna, noodles	Meal alone (25mg pp)	0.3, ?		6.8	8.3*	-
	n=9M+F, SF 77µg/L		Meal + coffee (224mg pp)	0.3, ?		3.2	8.1*	0.98
	All 19-40yrs							
Samman et al (2001)	n=10F, 26 (19-39)yrs, SF 20.7µg/L	70g pasta, meat sauce (90g minced pork, 28g cream, 20g carrots) + 20g white bread	Meal alone	1.93, 0.27	38.6	12.1	15.6	-
			Meal + Chinese green tea extract (37.3mg pp)			8.9	11.2	0.72 (p<0.05)
RI – Hb incorp + WBC	n=14F, 25 (19-39)yrs, SF 23.8µg/L	As above	Meal alone	1.62 ,0.43	29.3	7.5	11.2	-
			Meal + rosemary extract (32.7mg pp)			6.4	8.8	0.79 (p<0.01)
Tuntipopipat et al (2006)	n=10F, 18-35yrs, SF 67 (28-119)µg/L	White rice (50g dry), vegetable soup (100g cabbage, 30g mushrooms, 20g carrots), 12mL fish sauce, 120mL water	Meal alone	4.7, 0	N/A	9.7	N/A	-
			Meal + 4.2g dried chilli (25.2mg pp)	4.84, 0		6.0		0.62 (p<0.01)
SI	n=10F, 18-35yrs, SF 49 (14-125)µg/L		Meal alone	4.7, 0		8.7		-
			Meal + 0.5g dry tumeric	4.71, 0		8.9		1.02 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron Content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual Adjusted*	*Absorption Ratio*
			(50.13mg pp)				

*Adjusted to a SF [ ] of 30ug/

1.22.2 Effect of polyphenol intake on iron status - Observational studies

The effect of tea and coffee consumption on the iron status of young females is somewhat inconclusive. Mehta et al (1992) observed that anaemic subjects in the American NHANES II study drank less tea (3.1 cups per day) than non anaemic subjects (3.5 cups per day). However, data was based on a single 24 hour diet recall and consumption ranged from 0-35 cups coffee per day to 0-20 cups of tea per day. In contrast, a negative correlation was found between serum ferritin levels and tea consumption in French female students (Galan *et al.*, 1985). However, in most developed countries no relationship has been found between tea (Preziosi *et al.*, 1994; Galan *et al.*, 1998; van de Vijver *et al.*, 1999) or coffee intake (Razagui *et al.*, 1991; van de Vijver *et al.*, 1999) and iron status. Heath et al (2001) found that tea and coffee consumption were not implicated in the risk of mild iron deficiency in New Zealand women. Daily tea drinking was however, one factor associated with an increased risk of iron deficiency anaemia in hospitalised New Zealand infants aged 9-23 months (Wilson *et al.*, 1999). Razagui et al (1991) found that in mentally handicapped menstruating women those with depleted iron stores had a significantly higher intake of tea and a lower intake of vitamin C during meals (563 ml/meal/day) compared with women who had sufficient iron stores (184 ml/meal/day). A meta-analysis found that tea consumption does not appear to affect iron status in populations where most people have adequate iron status. However, in populations with marginal iron status, there does appear to be a negative association between tea consumption and iron status (Temme & Van Hoydonck, 2002). A review of 35 studies found that there was insufficient evidence to draw conclusions on the effect tea has on iron status (Nelson & Poulter, 2004).

1.22.3 The New Zealand situation

In females aged 19–24 years in the National Nutrition Survey, 47% were regular (at least three times per week) consumers of tea, 53% of coffee and 10% of herbal tea. For females aged 25–44 years, 61% regularly consumed tea, 63% regularly consumed coffee and 13% regularly consumed herbal tea. Seventeen percent of females aged 19-24 years and 14% of

females aged 25-44 years consumed red wine at least once per week (Russell *et al.*, 1999). No data is available on whether these drinks were consumed with meals or on the polyphenol intakes of New Zealanders. Data from the United Kingdom suggests that the total intake of dietary polyphenols is ~1g/day with the main dietary sources being fruit and beverages (Scalbert & Williamson, 2000).

1.23 Oxalate / oxalic acid

The low bioavailability of iron from some vegetables such as spinach may be due to their high content of oxalic acid. Adding 1g of calcium oxalate to cabbage reduced iron absorption by 39% (Gillooly *et al.*, 1983). However, the effect of oxalic acid on iron absorption appears to be small compared to other inhibitors and enhancers of iron absorption. No relationship was observed between iron absorption and three vegetables all with a high content of oxalic acid. Iron absorption was poor from spinach and beetroot greens, but high from beetroot (Gillooly *et al.*, 1983). Rhubarb, a fruit high in oxalate caused a mild increase in iron absorption from a meal (Ballot *et al.*, 1987). This effect however, was more likely to be caused by the Vitamin C content of the rhubarb. The mean daily intake of oxalate in English diets has been calculated to be 70 to 150mg/day, with tea contributing the greatest proportion of oxalate (Zarembski & Hodgkinson, 1962). No data is available on the dietary intake of oxalate in New Zealand.

1.24 Calcium

1.24.1 Studies using single meals

Calcium inhibits both haem and non haem iron absorption. However, the effect of calcium on iron absorption from single meal studies is less clear and weaker than other inhibitors of non haem iron absorption. Monsen and Cook (1976) observed that the addition of 178-182mg calcium and 198-374mg phosphate to a semi synthetic meal decreased non haem iron absorption by approximately 50 to 70%, while the addition of calcium or phosphate alone did not significantly decrease iron absorption.

Serving 250ml milk (273mg calcium) with a hamburger meal containing 220mg calcium did not significantly reduce iron absorption (Hallberg & Rossander, 1982c). No significant effect on iron absorption was seen when 150ml milk or 125g yoghurt were added to a meal containing 320mg calcium (Galan *et al.*, 1991), or when 250ml milk (273.6mg Ca) or 150g yoghurt (181.5mg Ca) were added to a meal containing 218mg calcium (Rosado *et al.*, 2005). This study did not adjust for subject's iron status. The addition of cheese (127mg calcium) to a hamburger meal had no effect on non haem iron absorption (Roughead *et al.*, 2002). When milk (679mg calcium) or cheese (606mg calcium) replaced beef in a standard meal iron absorption reduced by 71 and 62% respectively (Cook & Monsen, 1976). It is unknown how much of this effect was due to the removal of the beef or whether the milk and cheese had an independent additional inhibitory effect. No significant effect on iron absorption was seen when the same amount of milk and cheese replaced ovalbumin in a semi synthetic meal (Cook & Monsen, 1976).

Other studies have shown clearly that calcium does inhibit non haem iron absorption (Dawson-Hughes *et al.*, 1986; Deehr *et al.*, 1990; Cook *et al.*, 1991b; Hallberg *et al.*, 1992). Calcium salts (600mg as calcium phosphate, calcium citrate and calcium carbonate) all decreased iron absorption when taken with a meal. The inhibition was less from a meal of high iron bioavailability and low calcium content (average 27%) than from a meal of low iron bioavailability and high calcium content (average 54%) (Cook *et al.*, 1991b). The addition of 500mg elemental calcium as calcium carbonate or hydroxyapatite to a breakfast meal containing 227mg calcium reduced iron absorption by 43 and 46% respectively (Dawson-Hughes *et al.*, 1986). The same amount of calcium added to a meal containing 238mg calcium as calcium citrate malate (CCM) or milk reduced iron absorption by 28 and 59% respectively (Deehr *et al.*, 1990). 165mg calcium given as calcium chloride, milk or cheese had a 46-59% inhibitory effect on non haem iron absorption from wheat rolls (Hallberg *et al.*, 1991), while the addition of calcium as milk (215mg calcium), cheese (595mg calcium), or as a milk shake (315mg calcium) to a pizza or hamburger meal all reduced non haem iron absorption by 46-64% (Hallberg *et al.*, 1992).

The effect of calcium on non haem iron absorption is thought to be dose related. In a study by Hallberg et al (1991) 40mg of calcium chloride added to wheat rolls reduced iron absorption from a meal by 40%. With increasing amounts of calcium there was a continuous decrease in iron absorption up to 75% with 300mg calcium. Increasing the dose further had little effect on iron absorption. This may explain why in some studies the addition of calcium did not inhibit iron absorption, especially those studies where the baseline meals are already high in calcium (Hallberg *et al.*, 1991). The conflicting results observed may not only be due to differences in the composition of baseline meals, but also due to the different calcium compounds used (Deehr *et al.*, 1990).

Table 1.12 Effect of calcium on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Monsen & Cook (1976)	n=21F, 25 (21-31)yrs, SF 27 (12-49)µg/L	SS meal (dextrimaltose, corn oil, egg albumin) (24mg Ca, 40mg P)	Meal alone	4.1, 0	N/A	1.7	N/A	-
			Meal + $CaHPO_4$ + K_2HPO_4 (178mg Ca, 374mg P)	4.1, 0		0.8		0.50 (p<0.001)
RI – Hb incorp	n=11F, 24 (21-29)yrs, SF 25 (12-49)µg/L	SS meal + 100g beef (20mg Ca, 216mg P)	Meal alone	?		8.8		-
			Meal + $CaCl_2$ + K_2HPO_4 (182mg Ca, 198mg P)	?		4.0		0.46 (p<0.001)
	n=13 M, 25 (17-35)yrs, SF 55 (15-229)µg/L	SS meal (dextrimaltose, corn oil, egg albumin) (24mg Ca, 40mg P)	Meal alone	4.1, 0		2.2		-
			Meal + $CaCl_2$ + K_2HPO_4 (178mg Ca, 374mg P)	4.1, 0		0.6		0.30 (p<0.001)
			Meal + $CaCl_2$ (178mg Ca)	4.1, 0		1.5		0.71 (ns)
			Meal + K_2HPO_4 (374mg P)	4.1, 0		1.5		0.70 (ns)
Cook & Monsen (1976) RI – Hb incorp	n=8F, 23 (21-27)yrs, SF 28 (6-132)µg/L	Standard meal (beef, potatoes, corn, bread, margarine, ice milk, peaches) (202mg Ca)	Std meal	4.1, ?	N/A	5.5	N/A	-
			Std meal + milk (679mg Ca) (replaces beef)	1.8, 0		1.6		0.29 (p<0.01)
		SS meal (dextrimaltose, corn oil, ovalbumin (o/a)) (202mg Ca)	SS meal	4.1, 0		0.7		-
			SS meal + milk (679mg Ca) (replaces o/a)	4.1, 0		0.9		1.29 (ns)
	n=8F, 20 (19-23)yrs, SF 33 (9-82)µg/L	Standard meal (as above)	Std meal	4.1, ?		9.4		-
			Std meal + cheese (606mg Ca) (replaces beef)	2.3, 0		3.6		0.38 (p<0.01)
		SS meal (as above)	SS meal	4.1, 0		2.0		-
		Replacements made on basis of protein content (20.2g)	SS meal + cheese (606mg Ca) (replaces o/a)	4.1, 0		2.4		1.20 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Hallberg & Rossander (1982c) RI – Hb incorp + WBC	n=7M, 23 yrs, Hb 148g/L + 2F, 20yrs, Hb 124g/L	110g hamburger (82g meat), 60g string beans, 150g potatoes (220mg Ca)	Meal + 250ml water Meal + 250ml milk (273mg calcium)	3.0, 0.66 3.2, 0.66	34.4	7.8 6.8	9.1 7.9	- 0.86 (ns)
Dawson-Hughes et al (1986) RI – WBC	n= 13F, 65 (59-70)yrs	Breakfast meal - corn chex cereal, nonfat dried milk, graham cracker, margarine, formula beverage (227mg Ca)	Meal + placebo Meal + 500mg calcium carbonate Meal + 500mg hydroxyapatite	3.6, 0 3.6, 0 3.6, 0	N/A	6.3 3.2 1.7	N/A	- 0.51 (p<0.01) 0.27 (p<0.01)
Deehr et al (1990) RI – WBC	n=19F, 63 (52-72)yrs, SF 111 (30-216)µg/L	Breakfast meal (12g corn chex cereal, 11g dried milk, 14g graham cracker, 10g margarine, 317g formula beverage (238mg Ca)	Meal + Placebo Meal + CCM (450-550mg Ca) Meal + CCM + 450ml orange juice (541mg Ca) Meal + 450ml whole milk (446mg Ca)	3.18, 0 3.18, 0 3.36, 0 3.36, 0	N/A	8.3 6.0 7.4 3.4	N/A	- 0.72 (p<0.05) 0.89 (ns) 0.41 (p<0.05)
Cook et al (1991b) RI – Hb incorp	n=9F, 21 (20-22)yrs, SF 21 (11-37)µg/L n=8F + 1M, 23 (20-28)yrs, SF 24 (6-75)µg/L n=10M, 23 (21-25)yrs, SF	37mg Fe (ferrous sulphate) + enhancing meal (94g hamburger patty + 70g bun) 18mg Fe (ferrous sulphate) + enhancing meal (94g hamburger patty + 70g bun) 18mg Fe (ferrous sulphate)	Meal alone Meal + calcium carbonate (300mg Ca) Meal alone Meal + calcium carbonate (600mg Ca) Meal alone	3.7, 1.4 3.7, 1.4 3.7, 1.4 3.7, 1.4 3.7, 1.4	N/A	2.12 1.61 13.0 7.3 3.9	N/A	- 0.76 (p<0.05) - 0.56 (p<0.001) -

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	74 (19-171)µg/L	+ enhancing meal (94g hamburger patty + 70g bun)	Meal + calcium carbonate (600mg Ca)	3.7, 1.4		3.3		0.84 (ns)
	n=2F + 5M, 23 (22-26)yrs, SF 54 (28-164)µg/L		Meal alone	3.7, 1.4		10.1		-
			Meal + calcium citrate (600mg Ca)	3.7, 1.4		6.1		0.60 (ns)
	n=6M + 1F, 24 (23-28)yrs, SF 75 (47-108)µg/L		Meal alone	3.7, 1.4		7.3		-
			Meal + calcium phosphate (600mg Ca)	3.7, 1.4		3.2		0.43 (p<0.05)
	n=5M + 5F, 23 (22-27)yrs, SF 42 (5-409)µg/L	Enhancing meal (94g hamburger patty + 70g bun)	Meal alone	3.7, 1.4		13.4		-
			Meal + calcium carbonate (600mg Ca)	3.7, 1.4		9.1		0.68 (p<0.001)
			Meal + calcium citrate (600mg Ca)	3.7, 1.4		11.9		0.89 (ns)
			Meal + calcium phosphate (600mg Ca)	3.7, 1.4		8.2		0.61 (p<0.05)
	n=7m, 3F, 25 (22-39)yrs, SF 66 (12-359)µg/L	Inhibiting meal (Egg, muffin, bran flakes, sugar, low fat milk, coffee)	Meal alone	4.7, 0		1.2		-
			Meal + calcium carbonate (600mg Ca)	4.7, 0		0.7		0.58 (p<0.05)
			Meal + calcium citrate (600mg Ca)	4.7, 0		0.5		0.43 (p<0.01)
			Meal + calcium phosphate (600mg Ca)	4.7, 0		0.4		0.37 (p<0.01)
Galan et al (1991)	n=13 M, 31.8 (22-45)yrs, SF 128 (36-220)µg/L	Typical French meal (150g steak, 30g cheese, 65g fries, 100g carrots, 150g apple, 50g bread, water) (320mg Ca)	Meal alone	4.4, 2.1	17.7	2.2	5.1	-
			Meal + 150ml skimmed milk (172mg Ca)	4.5, 2.1		2.1	4.9	0.96 (ns)
RI – Hb incorp			Meal + 125g plain yoghurt (233mg Ca)	4.6, 2.1		2.0	4.7	0.92 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Hallberg et al (1991)	n=4M(3bd) + 5F(1bd)	80g rolls made from white wheat flour ($CaCl_2$ added to dough), 20g butter + 150ml water (10mg Ca)	Rolls alone	3.8, 0	39.3	21.7	22.0	-
			Rolls + $CaCl_2$ (40mg Ca)	3.8, 0		13.1	12.8	0.61 (p<0.001)
	n=4M(3bd) + 7F		Rolls alone	3.8, 0	25.2	12.4	20.0	-
RI – Hb incorp + WBC			Rolls + $CaCl_2$ (75mg Ca)	3.8, 0		5.7	11.2	0.46 (p<0.001)
	n=3M(1bd) +7F		Rolls alone	3.8, 0	29.9	16.9	20.8	-
			Rolls + $CaCl_2$ (165mg Ca)	3.8, 0		6.8	8.8	0.41 (p<0.001)
	n=3M(2bd) +7F		Rolls alone	3.8, 0	22.5	14.6	19.2	-
			Rolls + $CaCl_2$ (300mg Ca)	3.8, 0		3.6	6.4	0.26 (p<0.001)
	n=10F(3bd)		Rolls alone	3.8, 0	25.4	10.9	18.0	-
			Rolls + $CaCl_2$ (600mg Ca)	3.8, 0		2.4	3.6	0.23 (p<0.001)
	n=2M(1bd) +8F	80g rolls made from white wheat flour ($CaCl_2$ added to baked rolls) + 20g butter + 150ml water (10mg Ca)	Rolls alone	3.8, 0	39.2	21.6	21.6	-
			Rolls + $CaCl_2$ (40mg Ca)	3.8, 0		21.3	22.4	1.0 (ns)
	n=6M(3bd) + 4F(1bd)		Rolls alone	3.8, 0	31.8	24.4	23.6	-
			Rolls + $CaCl_2$ (75mg Ca)	3.8, 0		19.9	19.6	0.84 (p<0.01)
	n=3M(2bd) + 5F(2bd)		Rolls alone	3.8, 0	44.6	29.6	23.2	-
			Rolls + $CaCl_2$ (165mg Ca)	3.8, 0		15.6	14.0	0.54 (p<0.001)
	n=7M(3bd) + 3F		Rolls alone	3.8, 0	26.5	21.3	26.0	-
			Rolls + $CaCl_2$ (300mg Ca)	3.8, 0		9.6	12.0	0.44 (p<0.001)
	n=8F(1bd)		Rolls alone	3.8, 0	29.5	17.7	26.8	-
			Rolls + $CaCl_2$ (600mg Ca)	3.8, 0		7.4	13.2	0.41 (p<0.001)
	n=3M(1bd) + 7F	80g wheat rolls + 20g butter (10mg Ca)	Rolls + 150ml water	3.8, 0	28.6	13.1	18.8	-
			Rolls + 150ml low fat milk (165mg Ca)	3.8, 0		6.0	7.2	0.43 (p<0.001)
	n=6M(2bd) +4F		Rolls alone	3.8, 0	31.7	19.4	24.0	-
			Rolls + 20g cheese (165 mg Ca)	3.8, 0		10.4	13.6	0.54 (p<0.001)
	All 19-58yrs							
Hallberg et al	n=4M(1bd) + 6F(1bd)	Wheat rolls + 25mg	Meal alone	3.8, 0	33.8	8.1	10.4	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
(1992)		phytate (10mg Ca)	Meal + 300mg Ca	3.8, 0		4.4	5.6	0.54 (p<0.0001)
	n=3M(1bd) + 6F(1bd)	Pizza meal (dough, tomato	Meal alone	4.2, 0	33.4	8.6	11.6	-
RI – Hb incorp + WBC		puree, olives, anchovies,	Meal + 80g cheese (595mg	4.3, 0		3.2	4.4	0.36 (p<0.0001)
		tomatoes) (55mg Ca)	Ca)					
	n=4M(4bd) + 5F	Hamburger + wheat rolls	Meal alone	4.3, 0.6	33.5	22.8	22.0	-
		(75g beef) (15mg Ca)	Meal + 200ml milk (215mg	4.4, 0.6		8.7	8.8	0.37 (p<0.0001)
			Ca)					
	n=6M(4bd) + 4F(1bd)	Hamburger meal (115g	Meal alone	3.6, 1.1	48.4	22.1	14.8	-
		beef, fries, mustard, onion,	Meal + milkshake (milk + ice	3.9, 1.1		12.8	8.4	0.54 (p<0.0001)
	All 19-56yrs	tomato ketchup, cucumber)	cream) (315mg Ca)					
		(80mg Ca)						
Roughead et al (2002)	n=17M+F; 22-53yrs; SF 54 (6-201)µg/L	Hamburger meal (90g beef, 53g hamburger bun,	Meal alone	4.3, 1.1	N/A	6.6	N/A	-
		68g fries, 240g apple juice,	Meal + 28g cheese (127mg	4.3, 1.1		7.4		1.12 (ns)
RI – WBC		40g tomato ketchup)	Ca)					

The effect of calcium served at different times of the day on iron absorption has been investigated. Calcium given as milk or cheese (340mg calcium) in a breakfast meal had no effect on non haem iron absorption from a hamburger meal eaten 2 or 4 hours later (Gleerup *et al.*, 1993). In another study, Gleerup et al (1995) observed that on average, females absorbed 30-50% more iron (0.4mg more iron per day) over 10 days when milk or cheese (providing 937mg calcium/day) were consumed separately from the main meals compared to when the same amount of calcium was spread across four meals.

1.24.2 Studies using complete diets

The inhibitory effect of calcium on iron absorption from complete diets appears to be less than that from single meals. In fact, only one study has found calcium to have a significant inhibitory effect on non haem iron absorption from a complete diet (Minehane & Fairweather-Tait, 1998). This study was performed over two days only. Using stable isotopes and faecal monitoring it was found that the addition of 400mg calcium (calcium carbonate) to breakfast, lunch and dinner over one day reduced iron absorption by 70% compared to a day when no calcium was added (Minehane & Fairweather-Tait, 1998). An increase in daily dietary calcium intake from 780 to 2382mg led to a slight but not statistically significant reduction in iron retention in male subjects using a chemical balance technique over 39 days (Snedeker *et al.*, 1982). In a 6 week chemical balance study, Tidehag et al (1995) found no effect of increased milk intake with daily meals on iron absorption in ileostomy patients. In contrast, iron absorption was higher (but not significantly) when milk was added to cereal based diets for one day compared to a day when milk was not added (Turnland *et al.*, 1990). No significant differences were observed in non haem iron absorption from a diet high in calcium (1281mg/d) versus a diet low in calcium (280mg/d) in iron replete subjects who selected their own diets over a five day period (Reddy & Cook, 1997) or between a basic diet (224mg Ca/day) and the basic diet supplemented with milk (826mg Ca/day), calcium lactate (802mg Ca/day), or milk mineral isolate (801mg ca/day) over a 4 day period in subjects with low iron status (SF<40μg/L) (Grinder-Pedersen *et al.*, 2004).

1.24.3 Effect of calcium on iron status – intervention studies

The effect of calcium supplementation on iron status was reviewed by Bendich (2001) who concluded that calcium supplementation as high as 1200mg per day does not affect iron status in healthy premenopausal women. No changes in iron stores were observed in pre menopausal women when 500mg calcium (as $CaCO_3$) was given with two meals per day for 12 weeks compared with untreated controls (Sokoll & Dawson-Hughes, 1992) or when 400mg calcium was given 3 times per day to iron replete subjects consuming a Western diet over a 6 month period (Minehane & Fairweather-Tait, 1998). Supplementation with 500mg of calcium carbonate twice a day with meals had no effect on serum ferritin levels in both lactating and non lactating women 6 months post partum over a 6 month period (Kalkwarf & Harrast, 1998). Further studies are needed to determine whether calcium intake affects iron status in women who are anaemic or have low iron stores. It is assumed that dietary calcium has the same effect on iron status as supplemental calcium.

1.24.4 Effect of calcium on iron status – observational studies

The relationship between calcium intake and iron status shows varying results. Studies in elderly subjects have found no association between calcium intake and serum ferritin levels (Fleming *et al.*, 1998; Liu *et al.*, 2003; Milman *et al.*, 2004). One study in pre menopausal women found no association between calcium intake and iron status (Brussard *et al.*, 1997). However, several studies in pre menopausal women in developed countries have found a negative association between calcium or dairy product intake and iron status (Takkunen & Seppanen, 1975; Galan *et al.*, 1985; Preziosi *et al.*, 1994; Rangan *et al.*, 1997; Galan *et al.*, 1998). Van de Vijver et al (1999) found that across six European countries dietary calcium intake was weakly inversely associated with blood iron status, irrespective of whether calcium was taken simultaneously with iron. In contrast, a study in China found calcium intake to be positively correlated with iron status. However, those subjects with a high dairy intake were likely to have had a high intake of haem iron (Root *et al.*, 1999). Heath et al (2001) found that calcium intake was not associated with the risk of mild iron deficiency in New Zealand women.

1.24.5 The New Zealand situation

The National Nutrition Survey found that in women aged 15-24 years the mean intake of calcium was 826mg and 743mg for those aged 25–44 years (Russell *et al.*, 1999). Twenty eight percent of females aged 19–24 years and 26% of women aged 25-44 years had an inadequate intake of calcium (Russell *et al.*, 1999). Three percent of women aged 25-44 years and 0% of those aged 19-24 years took a calcium supplement (Russell *et al.*, 1999). Calcium intake may have increased in recent years as several foods have been approved to be fortified with calcium since 1996 (Ministry of Health, 2003). The main dietary sources of calcium for women aged 19-24 years are milk (36%), cheese (11%), non alcoholic beverages (7%), dairy products (5%), bread (5%), and vegetables (4%). For females aged 25-44 years the main dietary sources of calcium are milk (38%), cheese (11%), bread and non-alcoholic beverages (6% each), and vegetables and dairy products (5% each) (Russell *et al.*, 1999).

1.25 Protein

1.25.1 Studies using single meals

Many foods high in protein including eggs, milk proteins, nuts, legumes and soy protein tend to inhibit non haem iron absorption.

Eggs and egg albumin

Both egg albumin and egg yolk appear to mildly inhibit the absorption of non haem iron. Iron absorption decreased by 78% when the equivalent of 2.9 eggs replaced beef in a standard meal. The same amount of egg albumin (on a protein basis) reduced iron absorption by 61% (Cook & Monsen, 1976). It is unknown how much of the inhibition was due to the addition of egg or the removal of beef from the meal. Iron absorption did not change significantly when egg powder replaced egg albumin in a semi synthetic meal (Cook & Monsen, 1976). Rossander et al (1979) found that non haem iron absorption

decreased by 28% when a boiled egg was added to a Western breakfast meal. However, the actual amount of iron absorbed increased slightly due to the high iron content of the egg (Rossander *et al.*, 1979). The addition of 100g egg albumin to a maize porridge meal had no significant effect on non haem iron absorption (Bjorn-Rasmussen & Hallberg, 1979). Doubling the egg albumin content in a semi synthetic meal reduced iron absorption by 39%, while the removal of egg albumin from the same meal tripled iron absorption (Monsen & Cook, 1979). The addition of 30g egg albumin to a semi synthetic meal reduced iron absorption by 55%. However, when 50, 100 and 300mg phytic acid were added to the same semi synthetic meal, the addition of 30g egg albumin had no significant effect on iron absorption (Reddy *et al.*, 1996). It is likely that these small amounts of phytate were causing maximal iron absorption (Reddy *et al.*, 1996). The inhibitory effect of egg albumin and egg yolk on iron absorption depends on the composition of the meal they are added to, and shows less of an effect when added to meals of low iron bioavailability.

Bovine serum albumin

The addition of 35g egg albumin to a semi synthetic protein free meal reduced iron absorption by 72% compared with a 47% reduction with the same amount bovine serum albumin (BSA). Doubling the amount of BSA did not lead to any further decrease in iron absorption (Hurrell *et al.*, 1988). In another groups of subjects the addition of 30g BSA to a baladi bread meal increased iron absorption by 61% and had no significant effect when added to a maize meal (Hurrell *et al.*, 1988).

Milk proteins

Cook et al (1981) observed that an equivalent amount of casein and egg albumin (based on protein content) had similar effects on non haem iron absorption from a semi synthetic meal. Hurrell et al (1989) compared the effect of egg albumin, casein and whey on non haem iron absorption when added to a semi synthetic meal. Replacing egg albumin with sodium caseinate (Alanate 110®) reduced mean iron absorption to approximately half that

of egg albumin, although the difference was not statistically significant, while replacing egg albumin with whey protein (ultrafiltered) reduced iron absorption significantly by 61%. The extent to which acid hydrolysis or enzyme digestion could overcome the inhibitory effect of the casein and whey was also investigated. Iron absorption was higher from meals prepared with enzyme hydrolysed casein (84%) compared with sodium caseinate and egg albumin. Enzyme hydrolysis of whey protein to 16% (Lactry®) and 36% (Lad®) reduced non haem iron absorption compared with egg albumin but to a lesser extent than that of intact whey protein.

Nuts and legumes

Nuts and legumes are a poor source of bioavailable iron and tend to inhibit non haem iron absorption by binding iron in the intestinal lumen (MacFarlane *et al.*, 1988a; Hurrell *et al.*, 1992). Iron absorption from bread and nuts, including walnuts, almonds, peanuts and hazelnuts was 1.8% compared with an average of 6.6% for bread alone (MacFarlane *et al.*, 1988b). Coconut did not reduce iron absorption significantly (MacFarlane *et al.*, 1988b). All nuts, excluding coconut, contain significant amounts of phytates and polyphenols (MacFarlane *et al.*, 1988b). Iron absorption ranged from 0.84 to 1.91% for a variety of legumes (black beans, split peas, lentils, soybeans, mung beans) served as soup (Lynch *et al.*, 1984). Other calculated values of iron absorption include 1.5% for black beans (Cook *et al.*, 1972) and 2.4% for lentils (Sayers *et al.*, 1974). MacFarlane et al (1988a) found that iron absorption from soy beans was 1.7% compared with 0.9% from lupines, while Morck et al (1982) found iron absorption to be 1.06 and 1.60% from boiled and baked whole soy beans served with water to male subjects.

Soy protein

The effect of soy beans on non haem iron absorption has been studied more extensively than other legumes. Soy beans are a major source of protein for many people around the world and are increasingly being used by the food industry (Allen & Ahluwalia, 1997). Results at times appear conflicting because the inhibiting effect of soy beans on non haem

iron absorption is offset in part by the high iron content of soy bean protein (Hallberg & Rossander, 1984). For example, one study found that when 33g fat free soy flour was added to a high phytate meal, iron absorption increased by 50%. When the meals were adjusted to contain similar amounts of iron, 33g fat free soy flour decreased iron absorption by 20% (Hallberg & Rossander, 1984). In addition some studies have reported that iron from soybean ferritin is well absorbed (Murray-Kolb *et al.*, 2003; Davila-Hicks *et al.*, 2004; Lonnerdal *et al.*, 2006).

Several studies have compared the effects of soy protein isolate (SPI) and egg albumin on non haem iron absorption (Morck *et al.*, 1982; Reddy & Cook, 1991; Hurrell *et al.*, 1992; Lynch *et al.*, 1994; Reddy *et al.*, 1996). The addition of 30g egg albumin to a semi synthetic meal reduced non haem iron absorption by 55%, while 30g SPI decreased iron absorption by 77%. When 300mg phytate was included in the semi synthetic meal, the addition of egg albumin and SPI decreased iron absorption, but not significantly (Reddy *et al.*, 1996).

When SPI and various forms of SPI replaced egg albumin in a meal iron absorption decreased by 69-94% (Reddy & Cook, 1991; Hurrell *et al.*, 1992; Lynch *et al.*, 1994). Cook et al (1981) found that when egg albumin was replaced with full fat soy flour (FFSF), textured soy flour (TSF) and SPI non haem iron absorption decreased by 82, 65 and 92% respectively. SPI, which had the greatest inhibitory effect on iron bioavailability contained a higher level of phytate and protein. SPI also inhibited non haem iron absorption to a greater extent than sodium caseinate when replacing egg albumin in a meal (Cook *et al.*, 1981). Iron absorption from a soy based infant formula was significantly less than that from cow's milk formula when compared in adult subjects (Gillooly *et al.*, 1984b; Derman *et al.*, 1987).

Replacing meat with soy protein lowers the amount of non haem iron absorbed. This is likely to occur through both the reduction of the MFP factor and through soy protein's additional inhibition on non haem iron absorption. When 75g hydrated TSF was added to 70g beef iron absorption decreased by 96% compared with 100g beef (Lynch *et al.*, 1985).

Adding 30g of soy flour to 70g beef in a hamburger meal decreased iron absorption by 62% compared with 100g beef (Lynch *et al.*, 1985). The addition of 30g textured soy protein and 30g TSF to a 100g beef hamburger meal reduced iron absorption by 45 (Lynch *et al.*, 1985) and 61% (Cook *et al.*, 1981) respectively. Adding 15g TSF to a hamburger meal containing 42g beef did not reduce iron absorption significantly (Hallberg & Rossander, 1982d).

Studies have investigated whether various processing methods decrease the inhibitory effects of soy bean products on iron absorption. Morck et al (1982) observed a significant 50 to 100% increase in non haem iron absorption when test meals containing either SPI or whole soybeans were baked at 200°C to destroy possible inhibitory compounds. Iron absorption was still however, less than from meals containing egg albumin. Derman et al (1987) however found no significant effect on iron availability when SPI was heated to 200°C for 2 hours prior to use.

The nature of the substances in soy bean products that inhibits iron absorption is unclear. The removal of phytate from soy flour by acid washing (Hallberg & Rossander, 1982d) had no significant effect on non haem iron absorption. Hurrell et al (1992) found that even after removal of virtually all phytic acid, iron absorption from a soy protein meal improved, but was still only half that from a egg albumin control, indicating that other factors contribute to the poor iron bioavailability from soy protein products. Lynch et al (1994) suggested that the two major inhibitors of non haem iron absorption in soy bean products are phytic acid and a protein related moiety contained in the conglycinin (7S) fraction. MacFarlane et al (1990) found an inverse relationship between iron absorption and the proportion of higher molecular weight fractions in soy bean products. However, this finding was not consistent so firm conclusions were unable to be drawn. In vitro studies suggest that high molecular weight peptides may be involved in the inhibition of non haem iron absorption (Schricker *et al.*, 1982; Kane & Miller, 1984). Both soy sauce and miso, products where the protein is broken down during through fermentation enhance iron bioavailability (Baynes *et al.*, 1990; MacFarlane *et al.*, 1990). Plant nodulation may have a role in determining the form of iron in soya beans and its level of absorption (Murray-Kolb *et al.*,

2003). Table 1.13 summarises single meal studies investigating the effect of various proteins on iron absorption.

Table 1.13 Effect of protein on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Cook & Monsen (1976)	n=10F, 23 (19-26)yrs, SF 23 (2-68)µg/L	Standard meal (beef, potatoes, corn, bread, margarine, ice milk, peaches)	Std meal	4.1, ?	N/A	8.2	N/A	-
			Std meal + 43g powdered egg (replaces beef)	5.2, 0		1.8		0.22 (p<0.01)
RI – Hb incorp			SS meal					
	n=7F, 26 (19-32)yrs, SF 22 (7-50)µg/L	SS meal (dextrimaltose, corn oil, ovalbumin (o/a))	SS meal + 43g powdered egg (replaces o/a)	4.1, 0		0.8		-
				4.1, 0		0.7		0.88 (ns)
			Std meal	4.1, ?				
			Std meal + EA (replaces beef)	4.1, 0		10.0		-
			Replacements made based on protein content (20.2g)			3.9		0.39 (p<0.01)
Bjorn-Rasmussen & Hallberg (1979)	n=6M+F, *All 29-42yrs*	50g maize flour as a porridge	Meal alone	5.0, 0	N/A	0.85	N/A	-
			Meal + 100g EA (20g protein)			1.18		1.56 (ns)
RI – Hb incorp + WBC								
Monsen & Cook (1979)	n=14M, 23 (18-32)yrs, SF 82 (9-373)µg/L	SS meal (37g egg albumin, 35g corn oil, 67g dextrimaltose) + 200ml water	SS meal	4.1, 0	N/A	2.3	N/A	-
			SS meal + 74g EA (doubled)	4.1, 0		1.4		0.61 (p<0.02)
	n=13 M, 24 (18-36)yrs, SF 47 (9-144)µg/L		SS meal	4.1, 0		3.8		-
RI – Hb incorp			SS meal - EA (0g EA)	4.1, 0		9.6		2.52 (p<0.001)
Rossander et al (1979)	n=12M, 29yrs, Hb 142g/L + 9F, 29yrs, Hb 129g/L	Western breakfast meal (2 wheat rolls + 12g margarine + 10g orange marmalade + 15g cheese) + 8g coffee in 150ml	Meal alone (7g protein)	2.8, 0	52.0	9.3	7.2	-
	n=10M, 25yrs, Hb 149g/L + 2F, 20yrs, Hb 135g/L		Meal + boiled egg (13g protein)	4.1, 0		7.6	5.8	0.82 (ns)
RI – Hb incorp + WBC								

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		water						
Cook et al (1981)	n=15M, 26 (24-30)yrs, SF 53 (12-164)µg/L	SS meal (dextrimaltose, corn oil)	Meal + EA (29.4g protein)	4.1, 0	N/A	2.49	N/A	-
			Meal + Sodium caseinate (29.4g protein)			2.74		1.10 (ns)
RI – Hb incorp			Meal + ISP (29.4g protein)			0.46		0.19 (p<0.001)
	n=10M, 28 (21-35)yrs, SF 69 (26-149)µg/L	SS meal (dextrimaltose, corn oil)	Meal + EA (14.7g protein)	4.0, 0	N/A	5.50		-
			Meal + FF soy flour (14.7g protein)			0.97		0.18 (p<0.001)
			Meal + TSF (14.7g protein)			1.91		0.35 (p<0.001)
			Meal + ISP (14.7g protein)			0.41		0.08 (p<0.001)
	n=11M, 23 (18-37)yrs, SF 48 (27-70)µg/L	Hamburger meal (bun, 55g fries, 180ml vanilla milkshake, meat patty)	Meal + 100g beef	?	19.88	3.20	6.44	-
			Meal + 100g beef + 30g TSF (3:1 Beef: soy)			1.24	2.49	0.39 (p<0.001)
			Meal + 70g beef + 30g TSF (2:1 Beef: soy)			1.51	3.04	0.47 (p<0.001)
Hallberg & Rossander (1982d)	n=9M (6bd), 24yrs, Hb 141g/L + 2F, 23yrs, Hb 134g/L	60g string beans, 150g mashed potatoes, hamburger patty	Meal + 42g beef (7g protein)	2.35, 0.25	46.9	9.9	8.4	-
RI – Hb incorp + WBC	n=9M (4bd), 26yrs, Hb 142 g/L + 1F, 22yrs, 123g/L		Meal + 42g beef + 15g textured soy patty (7g protein)	3.8, 0.25	40.9	8.2	7.2	0.86 (ns)
Morck et al (1982)	n=11M, 27 (19-50)yrs, SF 31 (2-130)µg/L	SS meal (corn syrup solids + corn oil)	Meal + 18.4g EA	4.0, 0	N/A	5.05	N/A	-
			Meal + 16.9g ISP			0.56		0.11 (p<0.0001)
RI – Hb incorp		SS meal (as above) +	Meal + 18.4g EA	4.0, 0		10.19		-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		100mg aa	Meal + 16.9g ISP			3.20		0.31 (p<0.0001)
	n=7M, 22 (44-50)yrs, SF 55 (18-94)µg/L	SS meal (as above)	Meal + 18.4g EA	5.6, 0		5.94		-
			Meal + 16.9g ISP			0.36		0.06 (p<0.0001)
		SS meal (as above) + 100g beef	Meal + 18.4g EA	5.6, 1.0		7.47		-
			Meal + 16.9g ISP			1.44		0.19 (p<0.01)
			All above meals contain 14.7g protein					
	n=9M, 24 (18-35)yrs, SF 46 (14-172)µg/L	Soy beans + water	ISP – uncooked	4.0, 0		0.64		-
			ISP – baked			1.28		1.99 (p<0.001)
			Whole soybean – boiled			1.06		-
			Whole soybean - baked			1.60		1.50 (p<0.05)
Gillooly et al (1984b)	n=12F, SF 12.1µg/L	50g infant formula + 20mg aa	SPI	3.0, 0	34.0	2.4	2.8	-
			Milk	3.0, 0		5.3	6.2	2.21 (p<0.02)
	n=7F, SF 20.5µg/L	50g infant formula + 40mg aa	SPI	3.0, 0	32.2	7.2	8.9	-
RI – Hb incorp			Milk	3.0, 0		19.5	24.2	2.71 (p<0.02)
Hallberg & Rossander (1984)	n=7M, 29yrs, Hb 151g/L + 3F, 21yrs, Hb 140g/L	Latin American meal (80g dry maize chapattis, 31g dry black beans, 50g polished rice cooked)	Meal alone + ferrous sulphate (250mg PA)	10.7, 0	31.5	5.2	6.0	-
RI – Hb incorp + WBC			Meal + 33g fat free soy flour (400mg PA)	10.7, 0		4.0	4.8	0.80
Lynch et al (1984)	n=10M, 20 (18-30)yrs, SF 52 (19-157)µg/L	Soup – 100g legumes + 50g cooked broth	Soy beans (11g protein)	4.0, 0	16.32	1.66	4.06	N/A
			Black beans (5.1g protein)	4.0, 0		0.84	2.06	
			Lentils (7.8g protein)	4.0, 0		1.20	2.94	
RI – Hb incorp	n=10M, 19 (18-20)yrs, SF 61 (20-132)µg/L		Split peas (8g protein)	4.4, 0	18.82	1.09	2.32	
			Mung beans (8g protein)	2.9, 0		1.91	4.06	
Lynch et al (1985)	n=9M, 25 (23-27)yrs, 54	Meat patty	100g beef	0.9, 1.2	N/A	24.82	N/A	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	(20-108)µg/L		70g beef + 75g hydrated TSF	4.5, 0.8		0.87		0.04 (p<0.001)
RI – Hb incorp	n=12M, 20 (18-27)yrs, SF	Hamburger bun, 68g fries,	Meal + 100g beef	3.0, 1.2		5.05		-
	44 (5-118)µg/L	145ml vanilla milkshake	Meal + 70g beef + 30g soy	4.2, 0.8		1.90		0.38 (p<0.01)
	n=10M, 24 (21-26)yrs, SF		flour					
	67 (8-208)µg/L		Meal + 100g beef	5.9, 1.2		5.94		-
			Meal + 100g beef + 30g	5.9, 1.2		3.24		0.55 (p<0.01)
			textured soy protein					
Derman et al (1987)	n=12F, SF 16.7µg/L	Formula + 0mg aa	Milk (1.5g protein)	?	36.7	8.3	9.0	-
		Formula + 0mg aa	Soybean (2.3g protein)			4.4	4.8	0.53 (p<0.025)
RI – Hb incorp	n=8F, SF 25.2µg/L	Formula + 5.8mg aa	Milk (1.5g protein)		45.3	11.9	10.5	-
		Formula + 5.8mg aa	Soybean (2.3g protein)			1.8	1.6	0.15 (p<0.05)
	n=11F, SF 17.9µg/L	Formula + 5.8mg aa	Soy bean (2.3g)		41.0	7.5	7.3	-
		Formula + 5.8mg aa	ISP (2.3g)			11.3	11.0	1.51 (p<0.05)
	n=12F, SF 21.0µg/L	10g protein + 30mg aa	ISP (10g)		N/A	4.4		-
		10g protein + 30mg aa	Soya bean flour (10g)			2.7		0.61 (p<0.05)
	n=6F, SF 11.2µg/L	Milk + 0mg aa	ISP (25g)		N/A	5.8		-
		Milk + 0mg aa	Heated ISP (25g)			6.7		1.15 (ns)
	n=16F, SF 33.2µg/L	Milk + 10mg aa	ISP (2.3g)		45.2	9.9	8.8	-
		Milk + 10mg aa	Heated ISP (2.3g)			13.5	11.9	1.36 (ns)
Hurrell et al (1988)	n=8M, 30 (19-39)yrs, SF	SS meal (67g hydrolysed	Meal alone	4.1, 0	N/A	10.64	N/A	-
	92 (13-367)µg/L	maize starch, 35g corn oil,	Meal + 35g EA	4.1, 0		2.95		0.28 (p<0.05)
RI – Hb incorp		12ml vanillan extract)	Meal + 30.2g BSA	4.1, 0		5.67		0.53 (p<0.05)
			Meal + 60.4g BSA	4.1, 0		6.14		0.58 (p<0.05)
	n=8M, 26 (22-34)yrs, SF	Maize meal (60g corn	Meal alone	4.2, 0		0.49		-
	50 (19-118)µg/L	meal) + 14g butter	Meal + 30g BSA	4.2, 0		0.57		1.15 (ns)
	n=2M+3F, 26 (23-29)yrs,	Baladi bread meal (60g	Meal alone	4.0, 0		3.04		-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	SF 30 (14-76)µg/L	82% extraction wheat flour) + 14g butter	Meal + 30g BSA	4.0, 0		4.89		1.61 (p<0.05)
MacFarlane et al (1988b)	n=12F, SF 9.8µg/L	60g white bread	Bread alone		51.1	5.9	4.6	-
			Bread + 50g Walnut			0.7	0.5	0.12 (p<0.0005)
	n=14F, SF 14.2µg/L		Bread alone		58.8	11.1	7.6	-
RI – Hb incorp			Bread + 50g Almond			1.7	1.2	0.15 (p<0.0005)
	n=8F, SF 17.9µg/L		Bread alone		25.3	8.1	12.8	-
			Bread + 50g Peanut			2.2	3.5	0.27 (p<0.01)
	n=28F, SF 16.6µg/L	60g white bread + margarine	Bread alone		52.1	4.4	3.4	-
			Bread + 50g Hazelnut			2.3	1.8	0.53 (p<0.0005)
	n=11F, SF 16.1µg/L		Bread + 50g Brazil nut		60.9	2.6	1.7	-
	n=12F, SF 17.3µg/L	60g white bread + 150ml milk	Bread + milk alone		58.2	4.6	3.2	-
			Bread + milk + 50g Coconut			4.0	2.7	0.87 (ns)
MacFarlane et al (1988a)	n=11F, SF 15.0µg/L	Soybeans or lupines	Soybeans (7g protein) (83mg phytate)	3.0, 0	39.6	1.7	1.7	-
			Lupines (7g protein) (negligible phytate)	3.0, 0		0.9	0.9	0.53 (p<0.0047)
RI – Hb incorp								
Hurrell et al (1989)	n=4M+3F, 26 (23-37)yrs, SF 40 (8-144)µg/L	SS meal (67g hydrolysed maize starch, 35g corn oil, 12 ml vanilla extract)	Meal + EA	4.1, 0	N/A	6.67	N/A	-
			Meal + Caseinate (Alenate 110)	4.1, 0		3.65		0.55 (ns)
RI – Hb incorp			Meal + Casein, 84% enzyme hydrolysed (Sheffield Products)	4.1, 0		7.67		1.15
	n=8M, 28 (21-46)yrs, SF 90 (16-140)µg/L		Meal + EA	4.1, 0		2.53		-
			Meal + Whey protein (intact, ultrafiltered)	4.1, 0		0.98		0.39 (p<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Meal + Whey protein (16% enzyme hydrolysed)	4.1, 0		1.06		0.42
			Meal + Whey protein (36% enzyme hydrolysed)	4.1, 0		1.71		0.68 (ns)
			All replacements 30g protein					
MacFarlane et al (1990)	n=13F, SF 28.7µg/L	7g protein in each meal	Soy flour	3.0-4.0mg	65.6	4.6	2.8	-
			Tofu (uf)	total iron		2.9	1.8	0.63 (p<0.001)
	n=12F, SF 22.3µg/L	Tofu, silken tofu, sufu,	Soy flour		69.9	3.7	2.1	-
RI – Hb incorp		natto, tempeh, barley miso,	Silken tofu (uf)			13.9	8.0	3.75 (p<0.0001)
	n=12F, SF 10.0µg/L	soybean miso all served as	Tofu (uf)		-	3.0		-
		a soup containing 100g	Silken tofu (uf)			6.3		2.10 (p<0.001)
	n=27F, SF 8.7µg/L	tomatoes + 50g onions	Tofu (uf)		64.5	8.4	5.2	-
			Sufu (f)			8.3	5.1	0.99 (ns)
	n=23F, SF 24.1µg/L	Soy flour served as a drink	Soy flour		45.2	4.8	4.2	-
		(with water, sugar, vanilla	Sufu (f-1 wk)			5.3	4.7	1.10 (ns)
	n=11F, SF 8.0µg/L	essence)	Sufu (f-1 wk)		-	6.4		-
			Sufu (f-3 wk)			8.5		1.33 (ns)
	n=13F, SF 17.3µg/L	Rice miso served with	Soy flour		38.8	3.8	3.9	-
		water	Tempeh (f-24 hr)			6.5	6.7	1.71 (p<0.0001)
	n=12F, SF 5.6µg/L		Tempeh (f-24 hr)		-	14.7		-
			Tempeh (f-72 hr)			9.8		0.67 (p<0.01)
	n=15F, SF 7.3µg/L		Soy flour		40.1	5.2	5.2	-
			Natto (f-20 hr)			10.8	10.8	2.07 (p<0.001)
	n=12F SF 18.9µg/L		Soy flour + rice		44.2	2.0	1.8	-
			Rice miso (f-6 mo)			17.0	15.4	8.50 (p<0.0001)
	n=14F, SF 12.7µg/L		Soy flour		49.8	2.4	1.9	-
			Barley miso (f-6 mo)			6.4	5.1	2.66 (p<0.0001)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	n=12F, SF 28.1µg/L		Soy flour		37.4	1.3	1.4	-
			Soybean miso (f-6 mo)			3.9	4.2	3.00 (p<0.0001)
	n=13F, SF 29.9µg/L		Soy flour + rice		52.8	0.8	0.6	-
			Soy flour: rice miso protein ratio (75:25)			8.8	6.7	11.0 (p<0.0001
	n=11F, SF 14.4µg/L		Soy flour + rice		40.4	0.9	0.9	-
			Soy flour: rice miso protein ratio (50:50)			8.7	8.6	9.66 (p<0.0001)
	n=7F, SF 5.0µg/L		Beef + rice		58.7	6.6	4.5	-
			Rice miso			10.4	7.1	1.58 (ns)
Reddy & Cook (1991) RI – Hb incorp	n=15M+F, 26 (24-30)yrs, SF 53 (45-62)µg/L	SS meal (egg albumin, dextrimaltose, corn oil)	Meal alone Meal + ISP (replacing EA)	4.1, 0	N/A	2.49 0.46	N/A	- 0.19 (p<0.01)
Hurrell et al (1992)	n=6M+2F, 24yrs, SF 59µg/L	SS meal (67g hydrolysed corn starch, 36g corn oil, 12g vanilla extract) + 200ml water	Meal + EA	5.7, 0	N/A	6.34	N/A	-
			Meal + SPI I (277.2mg PA)			1.50		0.24 (p<0.05)
RI – Hb incorp	n=5M+4F, 23yrs, SF 38µg/L		Meal + EA	6.4, 0		5.75		-
			Meal + SPI II (237.6mg PA)			0.92		0.16 (p<0.001)
	n=7M+1F, 23yrs, SF 68µg/L		Meal + EA	5.5, 0		5.48		-
			Meal + SPI III (214.5mg PA)			0.53		0.10 (p<0.001)
	n=3M+4F, 22yrs, SF 35µg/L		Meal + EA	5.5, 0		9.72		-
			Meal + SPI I (161.7mg PA)			1.36		0.14 (p<0.001)
			All meals – 30g protein added					
Lynch et al (1994) RI – Hb incorp	n=3M + 3F, 22yrs, SF 38 (26-57)µg/L	SS meal (67g hydrolysed maize starch, 36g corn oil, 12ml vanilla extract)	Meal + EA Meal + SPI I (1.70% PA) Meal + hydrolysed soybean	7.2, 0	N/A	3.10 0.28 1.86	N/A	- 0.09 (p<0.01) 0.59 (ns)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			proteins 1 (0.23% PA)					
			Meal + hydrolysed soybean proteins 2 (<0.05% PA)			5.33		1.68 (p<0.01)
	n=5M + 4F, 23yrs, SF 41 (30-56)µg/L		Meal + EA	6.5, 0		4.49		-
			Meal + SPI 2 (0.84% PA)			0.72		0.16 (p<0.01)
			Meal + glycinin (11S) (0.65% PA)			0.96		0.21 (p<0.01)
			Meal + glycinin (11S), low phytate (0.06% PA)			5.48		1.24 (ns)
	n=3M + 6F, 28yrs, SF 60 (41-87)µg/L		Meal + EA	6.5, 0		4.82		-
			Meal + glycinin (11S) (0.65% PA)	6.5, 0		1.10		0.23 (p<0.01)
			Meal + glycinin (11S), low phytate (0.06% PA)	6.5, 0		4.72		0.98 (ns)
			Meal + glycinin (11S), low phytate (0.06% PA)	2.6, 0		5.38		1.12 (ns)
	n=4M + 6F, 43yrs, SF 71 (57-88)µg/L		Meal + EA	6.5, 0		5.84		-
			Meal + SPI 3 (0.15% PA)			1.79		0.31 (p<0.01)
			Meal + conglycinin (7S) (2.52% PA)			1.87		0.32 (p<0.01)
			Meal + conglycinin (7S) low phytate (0.04% PA)			2.47		0.44 (p<0.01)
			All meals – 30g protein added					
Reddy et al (1996)	n=7F+5M, SF 34(28-41) µg/L	SS meal (67g maltodextrose + 36g corn oil + 12ml vanilla extract)	Meal alone	4.1, 0	N/A	21.69	N/A	-
			Meal + EA	4.1, 0		9.67		0.45 (p<0.01)
RI – Hb incorp		SS meal (as above) +	Meal alone	4.1, 0		2.15		-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		300mg phytic acid (84.6mg Phytate)	Meal + EA	4.1, 0		1.41		0.65 (ns)
	n=4F+5M, SF 49(38-64) µg/L	SS meal (as above)	Meal alone	5.2, 0		11.44		-
		SS meal (as above)	Meal + SPI	5.2, 0		2.60		0.23 (p<0.001)
		+300mg phytic acid (84.6mg Phytate)	Meal alone	5.2, 0		1.82		-
			Meal + SPI	5.2, 0		1.22		0.67 (ns)
	n=6F+2M, SF 41(31-54) µg/L	SS meal (as above) + 50mg Phytate	Meal alone	4.1, 0		1.70		-
			Meal + EA	4.1, 0		2.14		1.26 (ns)
		SS meal (as above) +100mg Phytate	Meal alone	4.1, 0		0.93		-
	All 23-25yrs		Meal + EA	4.1, 0		1.73		1.86 (ns)
			All meals - 30g protein added					
Murray-Kolb et al (2003) RI – Hb incorp	n=18F, 19-23yrs, SF 11.2 (3.1-45)µg/L	Soybeans -intrinsically labeled, hydroponically grown, nonnodulating	As a soup (46.5g soybeans) or as muffins (23.25g soybeans)	4.5, 0 3.0, 0	58.9	25.9	17.5	N/A

1.25.2 Effect of protein on iron status – intervention studies

Only three intervention studies have been undertaken which assess the effect of dietary protein on iron status. Swain et al (2002a) found that there were no significant differences in iron status between women with an average baseline serum ferritin of 27μg/L who consumed either 40g SPI or 40g whey protein per day over a 24 week period in addition to their normal diets. No difference in iron status was observed between groups of men, women and children when subjects consumed 1 of 7 beef products extended with varying amounts and types of soy protein (Bodwell *et al.*, 1987) or when men consumed patties of all beef or beef extended with soy isolate concentrate or flour in 1 to 2 meals per day (Morris *et al.*, 1987) as the main protein source over a 6 month period.

1.25.3 Effect of protein on iron status – observational studies

One study has reported serum ferritin concentrations as being significantly lower in vegetarians who replaced meat with soy bean products compared with non vegetarians (Shaw *et al.*, 1995). This effect is probably due to the lack of meat in the vegetarian diet rather than the effect of soy bean products themselves.

1.25.4 The New Zealand situation

The mean and median daily protein intakes for women aged 19-24 years is 78 and 76g and for women aged 25-44 years is 77 and 75g. The main sources of protein include beef and veal, bread, milk, poultry and fish / seafood. For women aged 19-24 and 25-44 years, nuts and seeds provided 1% and <1% of protein in their diets, while eggs and egg dishes provided 1 and 3% of the total daily protein intake. Twenty two percent of women aged 19-24 years and 17% of women aged 25-44 years ate eggs more than 4 times per week. Nuts were eaten at least once per week by 8% of women aged 19-24 years and by 19% of women aged 25-44 years. Thirty eight and 34% of women aged 19-24 and 25-44 years respectively ate peanut butter at least once per week (Russell *et al.*, 1999). As much of the

protein in the New Zealand women's diet is from meat products (Russell *et al.*, 1999), iron bioavailability is not likely to be compromised due to the intake of other protein foods.

1.26 Phytates / dietary fibre

1.26.1 Studies using single meals

Phytates or phytic acid (myo-inositol-hexa-phosphate, IP_6) acts as a storage compound of phosphates and minerals. They are found in grains, cereals, legumes, seeds, nuts, vegetables and fruit. Approximately 90% of phytates come from cereals in Western diets (Allen & Ahluwalia, 1997).

Meals or food containing high levels of phytate tend to inhibit iron absorption (Lynch *et al.*, 1984; MacFarlane *et al.*, 1988b; Tuntawiroon *et al.*, 1990; Cook *et al.*, 1997). Tuntawiroon et al (1990) found that differences in iron absorption between meals could be explained by their phytate content, while a strong inverse correlation was found between iron absorption and the phytate content of different cereal grains (Cook *et al.*, 1997). Iron in nuts and legumes is poorly absorbed which can be attributed to their high contents of both phytates and polyphenols (Lynch *et al.*, 1984; MacFarlane *et al.*, 1988b). The addition of phytate to a meal also inhibits iron absorption. Adding 2g sodium phytate to a broccoli meal reduced iron absorption by 80 and 77% in two separate studies (Gillooly *et al.*, 1983; Gillooly *et al.*, 1984a). The addition of 238-372mg phytic acid to a hamburger meal reduced iron absorption by 50-66% (Hallberg *et al.*, 1987), while the addition of 300mg phytic acid to semisynthetic meals containing either no protein, egg albumin, beef or SPI reduced iron absorption by 53-90% (Reddy *et al.*, 1996)

Phytate appears to have a dose dependent effect on non haem iron absorption (Hallberg *et al.*, 1989; Siegenberg *et al.*, 1991; Brune *et al.*, 1992; Reddy *et al.*, 1996). For example, 2mg sodium phytate added to wheat rolls reduced iron absorption by 22%, while adding 250mg sodium phytate reduced iron absorption by 85% (Hallberg *et al.*, 1989). Siegenberg et al (1991) found there was no difference between bread meals containing 10 and 14 mg

phytate. However, when 34 and 58mg of phytate were added in bran to bread, iron absorption reduced by 47 and 53% respectively.

Studies have investigated whether the degradation or removal of phytic acid by various methods increases iron absorption. Hallberg and Rossander (1982d) replaced normal soy flour with dephytinised soy flour in a hamburger patty and observed no significant effect on iron absorption. Iron absorption was inhibited to the same extent when either dephytinised bran or whole bran was added to plain muffins (Simpson *et al.*, 1981). This lack of effect was likely to have been caused by an incomplete dephytinisation process (Simpson *et al.*, 1981). In contrast, when the phytate content of sorghum was reduced by pearling iron absorption increased significantly (Gillooly *et al.*, 1984a). The removal of phytate from bran using endogenous phytase or dilute hydrochloric acid significantly improved iron absorption (Hallberg *et al.*, 1987), while the addition of phytase to a breakfast meal significantly increased iron absorption (Layrisse *et al.*, 2000). Hurrell et al (1992) found that iron absorption increased 4 to 5 times when the phytic acid in soy protein isolate was reduced from 4.9-8.4 to <0.01mg/g. Even small amounts of phytate were strongly inhibitory and phytic acid had to be reduced to <10mg phytic acid per meal before a meaningful increase in iron absorption was observed. In another study only cooking procedures which extensively degraded phytic acid, or amylase pre-treatment, which substantially liquefies cereal porridges, improved iron absorption from cereal products (Hurrell *et al.*, 2002). Hurrell et al (2003) found that phytic acid degradation improved iron absorption from a range of cereal based meals. The change in iron absorption was related to the type of cereal and initial levels of iron absorption. More recently, genetic modification has been used to reduce the phytic acid content of maize. One of these studies found that iron absorption improved with a low phytate maize (Mendoza *et al.*, 1998), however no improvement in iron absorption was seen when the low phytate maize porridge was fortified with additional iron (Mendoza *et al.*, 2001). Phytic acid (IP_6) can be dephosphorylated to yield lower inositol phosphates such as IP_5, IP_4, IP_3, and IP_2. Brune et al (1992) observed that only IP_5 and IP_6 inhibited iron absorption whereas inositol esters with fewer phosphate groups had limited effects. Sandberg et al (1999) concluded that to

improve iron absorption from cereals and legumes, degradation needs to be to less phosphorylated inositol phosphates than IP_3.

Bran has a high content of phytate and strongly inhibits non haem iron absorption. An early study found that more iron was absorbed from white bread compared with whole meal bread, despite the whole meal bread containing a higher content of iron (Dobbs & Baird, 1977). Bjorn-Rassmussen (1974) found that non haem iron absorption decreased in relation to the increasing bran content in a meal. The addition of 12g wheat bran to muffins reduced non haem iron absorption by 74% when added to a meal of low iron bioavailability and by 51-58% when added to meals of high iron bioavailability (Simpson *et al.*, 1981). Adding 10% bran (by weight) to a meal reduced iron absorption by 90% (Reddy & Cook, 1991), while the addition of 25g whole bran (236mg phytic acid) to a wheat bun containing no phytic acid reduced iron absorption by 78% (Hallberg *et al.*, 1987). Adding 25% bran to a hamburger bun reduced iron absorption by 39% (Lynch *et al.*, 1985). The addition of 25g bran to wheat rolls decreased iron absorption by 90-91% in both vegetarians and non vegetarians (Brune *et al.*, 1989). Replacing 30g white flour with 24g white flour and 6g bran iron absorption decreased by 63% (Gillooly *et al.*, 1984a). The phytate content in bran may not be solely responsible for bran's inhibiting effect on non haem iron absorption (Simpson *et al.*, 1981; Hallberg *et al.*, 1987). Hallberg et al (1987) suggested that the tannins in bran or other unknown factors may contribute to bran's inhibitory effect on non haem iron absorption. However, most research suggests that it is the phytate in bran rather than the fibre that is the main inhibitory factor of iron absorption (Gillooly *et al.*, 1984a; Brune *et al.*, 1992). For example, Brune et al (1992) found that fermenting whole meal rye bread to a low level of phytate had a similar effect on iron absorption as control rolls containing lower fibre levels and the same low phytate levels.

Dietary fibre refers to the non starch polysaccharides that are not digested by secretions of the gastrointestinal tract. Fibres include cellulose, hemicelluloses, pectins, gums and mucilages and non polysaccharides fibres such as lignins. Single meal studies using radioiron isotopes to investigate the effects of fibre on non haem iron absorption have shown conflicting results. Cook et al (1983) compared a high fibre meal with a low fibre

meal in the same group of subjects. Both meals contained similar levels of nutrients including iron. Mean iron absorption from the low fibre meal was 2.4 times higher than that of the high fibre diet. When bran, pectin or cellulose were added to muffins, only bran reduced non haem iron absorption significantly (Cook, 1983). Rossander (1987) found that bran strongly inhibited non haem iron absorption, isphagula (a hemicellulose preparation) slightly inhibited iron absorption, while pectins and guar gum had no effect on iron absorption. Gillooly et al (1984a) found that lignin had an inhibitory effect on iron absorption while pectin and guar gum had no effect. A balance study in ileostomy patients found an inhibitory effect of pectins on iron absorption (Sandberg *et al.*, 1982). Table 1.14 summarises the effects of phytates and dietary fibre on non haem iron absorption in single meal studies.

Table 1.14 Effects of phytate on iron absorption (single meal studies)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Dobbs & Baird (1977)	n=4M+9F, 35-71yrs, Hb >120g/L	100g bread + 250ml distilled water	White bread	6.0, 0	N/A	8.51	N/A	-
			Whole meal bread	8.8-8.9, 0		2.81		0.33 (p<0.01)
RI - WBC								
Simpson et al (1981)	n =10M+F, 23 (22-25)yrs, SF 66(35-143)µg/L	150g milkshake + 2 x 60g muffins	Plain muffins	?	N/A	2.39	N/A	-
			Bran muffins (12g wheat bran)			0.62		0.26 (p<0.0001)
RI – Hb incorp	n=13M+F, 25 (19-42)yrs, SF 51 (8-323)µg/L	150g milkshake + 100g beef + 2 x 60g muffins	Plain muffins			3.46		-
			Bran muffins (12g wheat bran)			1.69		0.49 (p<0.001)
		150g milkshake + 100mg aa	Plain muffins			9.29		-
			Bran muffins (12g wheat bran)			3.89		0.42 (p<0.0001)
	n=6M+4F, 26 (20-33)yrs, SF 79 (24-187)µg/L	150g milkshake + 100mg aa + 2 x 60g muffins	Plain muffins			2.43		-
			Bran muffins			0.99		0.41 (p<0.0001)
			Lyphilised whole bran (LWB) muffins			1.29		0.53 (p<0.005)
			Lyphilised dephytinised bran (LDB) muffins			1.37		0.56 (p<0.01)
	n=9M+9F, 23 (19-28)yrs, SF 57 (10-200)µg/L	150g milkshake + 100mg aa + 2 x 60g muffins	Plain muffins			3.02		-
			LDB muffins			2.23		0.74 (ns)
			LDB muffins - insoluble			3.22		1.06 (ns)
			LDB muffins - soluble			2.43		0.81 (ns)
Hallberg &	n=2M(2bd), 37yrs, Hb	60g string beans, 150g	15g fat free soy flour (74mg	3.9, 0.25	35.8	4.9	5.6	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Rossander (1982d)	144g/L + 7F, 32yrs, Hb132g/L	mashed potatoes + 42 g beef patty	phytic)					
			15g fat free dephytinised soy flour (0mg phytic)	3.5, 0.25		4.4	5.2	0.93 (ns)
RI – Hb incorp + WBC								
Cook et al (1983)	n=7M, 27 (22-37)yrs, SF 86 (27-978)µg/L	140-180g muffins (made with milk) + margarine	Plain	4.4, 0	N/A	1.27	N/A	-
			12g red hard wheat bran			0.35		0.27 (p<0.005)
RI – Hb incorp			8g citrus pectin			0.93		0.72 (ns)
			8g a-cellulose			1.27		0.99 (ns)
	n= 9M, 26 (18-48)yrs, SF 49 (7-135)µg/L	140-180g muffins (made with water) + margarine + 180ml apple juice (50mg aa)	Plain			3.52		-
			5g bran			1.52		0.43 (p<0.005)
			5g pectin			3.26		0.93 (ns)
			5g cellulose			3.53		1.00 (ns)
	n=10F + 5M, 26yrs, SF 57 (17-252)µg/L		Low fibre (0.4g) meal	7.4, 0		6.07		-
			High fibre (5.1g fibre) meal	7.4, 0		2.96		0.48 (p<0.001)
Gillooly et al (1983)	n=19F, 39 (21-76)yrs, SF 23.3µg/L	100g broccoli puree	Broccoli alone	3.0, 0	N/A	15.2	N/A	-
			Broccoli + 2g sodium phytate	3.0, 0		3.5		0.23 (p<0.0005)
RI – Hb incorp								
Gillooly et al (1984a)	n=16F, SF 31.7µg/L	30g sorghum	Whole BP (high Phytate)	3.0-4.2, 0	34.4	-	1.7	-
			Pearled BP (low Phytate)				3.5	2.06 (p<0.05)
	n=12F, SF 26.1µg/L		Whole BP (high Phytate)		41.1		2.4	-
RI – Hb incorp			Pearled BP (low Phytate)				6.3	2.63 (p<0.01)
	n=10F, SF 18.1µg/L		Albino pearlings (high Phytate)		34.0		1.5	-
			Pearled albino (low P)				3.5	2.33 (p<0.0005)
	n=16F, SF 25.0 µg/L	50g broccoli	Broccoli alone		34.9		18.5	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Broccoli + 2g sodium phytate				3.7	0.20 (p<0.0005)
	n=8F, SF 16.7µg/L	Flour served a porridge	30g white flour		34.1		11.6	-
			24g white flour +6g bran				4.3	0.37 (p<0.005)
Lynch et al (1985)	n=13M, 23yrs, SF 66µg/L	113g beef + bun	White flour bun	?	N/A	9.68	N/A	-
			Bran bun (25% bran)			5.90		0.61 (p<0.01)
RI – Hb incorp								
Hallberg et al (1987)	n=7M (6bd) + 4F (2bd)	Wheat buns (W) (80g flour) + 20g margarine + 150ml water	W alone (0mg PA)	4.0, 0	44.3	35.1	39.6	-
			W + 25g bran (236mg PA)	3.5, 0		8.0	8.8	0.22 (p<0.001)
	n=5M (4bd) + 4F		W + 25g bran (237mg PA)	3.8, 0	34.3	5.5	5.2	-
RI – Hb incorp + WBC			W + 25g bran (water) (226mg PA)	4.5, 0		5.8	6.4	1.23 (ns)
	n=5M (4bd) + 5F		W + 25g bran (256mg PA)	3.5, 0	31.4	4.2	4.8	-
			W + 25g bran (HCl) (0mg PA)	3.9, 0		23.9	30.8	6.41 (p<0.01)
	n=6M (5bd) + 3F (1bd)		W alone (0mg PA)	3.8, 0	38.8	25.2	30.0	-
			W + 25g bran (HCl) (0mg PA)	3.9, 0		31.5	38.0	1.27 (p<0.0001)
	n=4M (4bd) + 5F (1bd)		W + 25g bran (232mg PA)	3.4, 0	39.4	5.8	5.2	-
			W + 25g bran (HCl) – restituted with NaPhy (227mg PA)	3.7, 0		12.0	10.8	2.08 (p<0.005)
	n=4M (1bd) + 6F (3bd)		W + 25g bran (172mg PA)	3.2, 0	31.2	2.1	2.6	-
			W + 25g bran, enym-dephytinised (20mg PA)	3.4, 0		4.6	6.2	2.38 (p<0.005)
	n=4M (2bd) + 5F (1bd)		W (15mg PA)	4.3, 0	41.2	11.6	10.0	-
			W + 25g bran, enzym-	3.4, 0		6.7	5.6	0.56

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			dephytinised (25mg PA)					
	n=2M (1bd) + 8F (2bd)		W (0mg PA)	3.6, 0	44.2	25.8	24.0	-
			W + 25g bran, enzym-dephytinised + water (0mg PA)	3.7, 0		19.6	18.8	0.78 (p<0.02)
	n=5M (3bd) + 4F (1bd)	60g string beans, 150g mashed potatoes, 82g meat, wheat buns	Meal alone (0mg PA)	3.8, ?	51.6	13.4	9.6	-
			Meal + NaPhy (372mg PA)	3.8, ?		6.4	4.8	0.5 (p<0.001)
	n=5M (5bd) + 4F	60g string beans, 150g mashed potatoes, wheat buns	Meal alone (0mg PA)	4.1, 0	35.5	11.6	12.8	-
			Meal + NaPhy (372mg PA)	4.2, 0		4.4	4.4	0.34 (p<0.001)
	n=6M (3bd) + 6F	60g string beans, 150g mashed potatoes, 82g meat, wheat buns	Meal alone (0mg PA)	7.2, ?	27.4	8.3	12.8	-
			Meal + mFePhy + MgPhy + KPhy (245mg PA)	6.5, ?		3.3	5.2	0.41 (p<0.001)
	n=5M (1bd) + 6F (2bd)		Meal + bran (257mg PA)	6.7, ?	35.2	5.0	4.8	-
			Meal + mFePhy + mgPhy +					
	All 19-58 yrs		KPhy (238mg PA)	6.2, ?		4.7	4.0	0.83 (ns)
Brune et al (1989)	n=4M + 9F; 61 (35-76)yrs, SF 52.5 (25-197)µg/L *(all*	Wheat rolls (80g flour) + 20g margarine + 150ml water	Wheat roll (80g wheat flour; 4mg PA)	3.8, 0	N/A	15.9	N/A	-
RI – Hb incorp + WBC	*vegetarians)*		Wheat roll + bran (55g wheat flour + 25g bran; 214mg PA)	3.8, 0		1.4		0.09 (p<0.001)
	n=3M(2bd)+ 3F(1bd), 54 (24-70)yrs, SF 85.7 (9-263) µg/L		Wheat roll (80g wheat flour; 4mg PA)	3.8, 0		22.3		-
			Wheat roll + bran (55g wheat flour + 25g bran; 214mg PA)	3.8, 0		2.2		0.10 (p<0.002)
Hallberg et al	n=3M+6F(3bd)	Wheat roll (WR) (80g	Meal alone	4.1, 0	28.2	17.6	21.6	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
(1989)		white wheat flour, 60% extraction) + 20g margarine +150ml water	Meal + 2mg Phytate	4.1, 0		13.6	16.8	0.82 (p<0.001)
	n=9F(3bd)		Meal alone	4.1, 0	37.9	22.2	22.7	-
RI – Hb incorp + WBC			Meal + 5mg Phytate	4.1, 0		13.7	14.0	0.61
	n=6F		Meal alone	4.1, 0	39.0	17.4	18.8	-
			Meal + 10mg Phytate	4.1, 0		7.7	8.4	0.41
	n=4M(1bd)+5F (1bd)		Meal alone	4.1, 0	35.2	16.8	19.6	-
			Meal + 25mg Phytate	4.1, 0		6.1	6.4	0.36 (p<0.001)
	n=5M(1bd)+5F (1bd)		Meal alone	4.1, 0	42.6	17.9	17.2	-
			Meal + 50mg Phytate	4.1, 0		5.1	5.2	0.31
	n=4M(1bd)+6F (1bd)		Meal alone	4.1, 0	31.2	16.7	21.2	-
			Meal +100mg Phytate	4.1, 0		5.4	6.8	0.29
	n=2M(2bd)+8F		Meal alone	4.1, 0	30.0	14.0	18.8	-
			Meal + 250mg Phytate	4.1, 0		2.3	2.8	0.18 (p<0.001)
	All 19-47 yrs		*(Phytate added as sodium phytate)*					
Tuntawiroon et al (1990)	n=8	60g Rolls	Rice (17mg Phytate)	1.6, 0	N/A	N/A	12.8	-
			Wheat (0mg Phytate)	0.96, 0			34.9	2.73
	n=6	60g Rolls	Rice (38mg Phytate)	1.74, 0			4.5	-
RI – Hb incorp			Wheat (2mg Phytate)	1.74, 0			16.3	3.56
	n=14	60g Gruel	Rice (26mg Phytate)	1.01, 0			2.6	-
			Wheat (0mg Phytate)	1.00, 0			35.0	13.46
	n=8	60g Gruel	Rice (0mg Phytate)	3.00, 0			17.6	-
			Wheat (0mg Phytate)	3.00, 0			16.8	0.95
	n=10	Starch + rice	Rice starch gruel (26mg Phytate)	1.01, 0			4.8	-
			Boiled rice (34mg Phytate)	1.04, 0			5.6	1.17

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Reddy & Cook (1991) RI – Hb incorp	n=9M+F, 28yrs, SF 47µg/L	SS meal (egg albumin, dextrimaltose, corn oil)	Meal alone Meal + 10% bran (by weight)	4.1, 0 4.1, 0	N/A	7.45 0.75	N/A	- 0.10 (p<0.01)
Siegenberg et al (1991) RI – Hb incorp	n=16F, SF 6.7µg/L	80g wheat bread + margarine + 15g potato (10mg Phytate)	Bread alone Bread (14mg Phytate)	5.0-6.0, 0	78.3	14.3 12.6		- 0.88 (ns)
	n=14F, SF 11.6µg/L		Bread alone Bread (22mg Phytate)		60.2	14.5 11.1		- 0.77 (p<0.01)
	n=16F, SF 10.8µg/L	Additions to bread - varying amounts of phytate	Bread alone Bread (34mg Phytate)		57.1	14.4 7.7		- 0.53 (p<0.01)
	n=11F, SF 6.9µg/L	free + phytate containing maize bran	Bread alone Bread (58mg Phytate)		57.3	9.5 4.5		- 0.47 (p<0.01)
Brune et al (1992) RI – Hb incorp + WBC	n=1M+9F (2bd)	Control roll (80g -55% extraction wheat flour) + 20g butter + 150ml water (5.5-6.5mg Phytate)	Control roll Whole rye + wheat roll-55% extract (5.7mg Phytate)	4.0, 0 3.7, 0	33.2	18.8 18.2	24.4 23.6	- 0.94 (ns)
	n=7M(2bd)+3F		Control roll Whole wheat + white wheat roll-55% extract (8.2mg Phytate)	4.0, 0 3.7, 0	27.8	19.8 15.6	27.2 23.2	- 0.79 (p<0.05)
	n=1M+8F(3bd)		Control roll Wheat roll- 85% extract (15mg Phytate)	4.0, 0 3.4, 0	37.4	22.2 8.9	22.8 9.2	- 0.39 (p<0.01)
	n=8M(2bd)+2F(1bd)		Control roll Whole rye + wheat roll-85% extract (34.1mg P)	4.0, 0 3.4, 0	25.0	16.2 6.1	25.6 9.2	- 0.32 (p<0.01)
	n=3M(2bd)+7F(2bd)		Control roll	4.0, 0	42.0	28.0	22.0	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
			Wheat bran + white wheat roll-55% extract (227mg P)	3.0, 0		3.8	3.2	0.13 (p<0.01)
Hurrell et al (1992)	n=6M+2F, 24yrs, SF 59µg/L	SS meal (67g hydrolysed corn starch, 36g corn oil, 12g vanilla extract) + 200ml water + SPI (30g protein)	I (native P) (277.2mgPA)		N/A	1.50	N/A	-
RI – Hb incorp			V (A-S reduced P) (6.6mg PA)			3.15		2.10 (p<0.05)
	n=5M+4F, 23yrs, SF 38µg/L		II (native P) (237.6mg PA)			0.92		-
			VI (A-S reduced P) (33mg PA)			1.91		2.07 (p<0.05)
			X (restored P) (326.7mg PA)			1.08		1.17 (ns)
	n=7M+1F, 23yrs, SF 68µg/L		III (native P) (214.5mg PA)			0.53		-
			VIII (E- reduced P) (0.33mg PA)			2.50		4.75 (p<0.001)
			XI (restored P) (122.1mg PA)			0.78		1.45 (ns)
	n=3M+4F, 22yrs, SF 35µg/L		IV (native P) (161.7mg PA)			1.36		-
			VII (A-S reduced P) (9.9mg PA)			4.17		3.06 (p<0.001)
			IX (E-reduced P) (0.33mg PA)			5.48		4.02 (<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
Reddy et al (1996)	n=7F+5M, SF 34µg/L	SS meal (67g hydrolyzed	Meal alone	4.1, 0	N/A	21.69	N/A	-
		corn starch, 36g corn oil,	Meal + 300mg PA	4.1, 0		2.15		0.10 (p<0.0001)
RI – Hb incorp		12ml vanilla extract)	Meal + EA (30g protein)	4.1, 0		9.67		-
			Meal + EA + 300mg PA	4.1, 0		1.41		0.15 (p<0.0001)
	n=4F+6M, SF 30 µg/L		Meal alone	6.2, ?		10.77		-
			Meal + 300mg PA	6.2, ?		1.83		0.17 (p<0.0001)
		(beef = 39.4g cooked	Meal + beef (30g protein)	6.2, ?		26.73		-
		freeze-dried)	Meal + beef + 300mg PA	6.2, ?		5.76		0.22 (p<0.001)
	n=4F+5M, SF 49µg/L		Meal alone	5.2, 0		11.44		-
			Meal + 300mg PA	5.2, 0		1.82		0.16 (p<0.0001)
		(SPI = phytate free	Meal + SPI (30g protein)	5.2, 0		2.60		-
		(0.01mg/g)	Meal + SPI + 300mg PA	5.2, 0		1.22		0.47 (ns)
	n=6F+2M, SF 41µg/L	SS meal (as above)	50mg PA	4.1, 0		1.70		-
		SS meal (as above)	100mg PA	4.1, 0		0.93		0.55 (p<0.05)
	All 23-25yrs	SS meal + 30g EA	50mg PA	4.1, 0		2.14		-
		SS meal + 30g EA	100mg PA	4.1, 0		1.73		0.81 (ns)
Cook et al (1997)	n=7M+4F, 27yrs, SF 47.0	50g cereal with 2.5mg	Bitter quinona (0.885% PA)	?	N/A	0.23	0.24*	No significant
	(13-108)µg/L	$FeSO_4$ added	Millet (0.340% PA)			0.44	0.44	difference between
RI – Hb incorp			Rice (0.075% PA)			0.72	0.72	cereals
			Maize (0.360% PA)			0.40	0.40	
	n=4M+8F, 23yrs, SF 47.0		Sweet quinona (0.770% PA)			0.58	0.56	
	(5-197)µg/L		Oat (0.310% PA)			0.52	0.50	
			Wheat (0.079% PA)			0.58	0.56	
Mendoza et al	n=14M, 19-35yrs, SF 67.0	Tortillas (43g)	Low phytate maize (LPM)	?	14.1	2.88	8.15	-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
(1998) RI – Hb incorp	(20.0-187.0) µg/L		(368mg PA) Wild type maize (WTM) (847mg PA) LPM + WTM			1.93 1.65	5.48 4.68	0.67 (p<0.001) 0.57 (p<0.001)
Sandberg et al (1999) RI – Hb incorp + WBC	n=11M+F, SF 73µg/L	White wheat rolls	White wheat rolls	4.0, 0	31.3	21.3	24.3	-
		Test = white wheat roll with added inositol phosphates	Roll + 10mg P as IP_5			12.3	15.2	0.61 (p<0.005)
	n=10 M+F, SF 81µg/L		White wheat rolls	4.1, 0	35.9	16.9	20.0	-
			Roll + 10mg P as IP_4			15.8	18.0	0.90 (ns)
	n= 9 M+F, SF 110µg/L		White wheat rolls	3.8, 0	17.8	7.7	17.6	-
			Roll + 10mg P as IP_3			6.8	16.8	0.98 (ns)
	n=10 M+F, SF 66µg/L		White wheat rolls	4.4, 0	38.2	20.4	20.8	-
			Roll + 1.6mg P as IP_6, 0.6mg P as IP_5, 2.9mgP as IP_4, 6.9mg P as IP_3			9.8	10.0	0.46 (p<0.001)
	n=8 M+F, SF 67µg/L		White wheat rolls	4.0, 0	35.1	29.0	29.1	-
			Roll + 2.2mg P as IP_6, 0.9mg P as IP_5, 6.2mg P as IP_4, 15.1mg P as IP_3			9.3	9.8	0.36 (p<0.001)
	n=10 M+F, SF 92µg/L		Rye rolls	4.0, 0	25.1	16.3	25.6	-
			Rye roll + 2.3mg P as IP_6, 2.6mg P as IP_5, 14.9mg P as IP_4, 10.9mg P as IP_3	3.4, 0		6.1	9.2	0.32 (p<0.001)
	All 18-54 yrs							
Layrisse et al (2000) RI – Hb incorp	n=5M + 9F, 15-50yrs, SF 29µg/L	Basal breakfast (100g corn flour, 50g cheese, 10g margarine) + 3mg Fe (ferrous sulphate)	Meal alone Meal + 304U phytase	3.0, 0 3.0, 0	N/A	5.1 10.1	N/A	- 1.96 (p<0.05)

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		Basal breakfast (100g corn flour, 50g cheese, 10g margarine) + 3mg Fe (ferrochel)	Meal alone Meal + 304U phytase	3.0, 0 3.0, 0		7.9 13.2		- 1.67 (p<0.05)
Davidsson et al (2001a) SI – Hb incorp - NTIMS	n=10F, 22 (20-28)yrs, SF 13 (5-28)µg/L	217g pea-protein isolate infant formula	Native PA (513umol/L) Dephytinised PA (<1umol/L)	0.78, 0 0.65, 0	N/A	20.7 33.1	N/A	- 1.60 (p<0.0001)
Mendoza et al (2001) RI – Hb incorp	n=14F, 19-42yrs, SF 21.9 (14.1-34.3)µg/L	Maize porridge meal (NaEDTA)	Wild type maize (817mg P/100g) Low phytate maize (361mg P/100g)	4.4, 0 4.4, 0	N/A	5.73 5.40	N/A	- 0.94 (ns)
		Maize porridge meal ($FeSO_4$)	Wild type maize (817mg P/100g) Low phytate maize (361mg P/100g)	4.4, 0 4.4, 0		1.69 1.91		- 1.13 (ns)
Hurrell et al (2003) RI – Hb incorp	n=6M+3F, 25 yrs, SF 53 (10-326)µg/L n=3M+7F, 25 yrs, SF 27 (8-119)µg/L n=5M+6F, 25 yrs, SF 28	All meals 50g cereal + 300ml water or whole milk (as stated) *Dephytinised porridges (0.002% to <0.001% PA), with the exception of wheat-soy blend (0.02% PA)*	Rice (0.16% PA) Rice, dephytinised Oat (0.67% PA) Oat, dephytinised Maize (0.26% PA) Maize, dephytinised Wheat (0.12% PA)	?	N/A	1.73 5.34 0.33 2.79 1.80 8.92 0.99	N/A	- 3.09 (p<0.001) - 8.36 (p<0.0001) - 4.96 (p<0.0001) -

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
	(7-125)µg/L		Wheat, dephytinised			11.54		11.60 (p<0.0001)
			Wheat-milk (0.12% PA)			1.30		-
			Wheat-milk, dephytinised			1.63		1.26 (ns)
	n=1M+7F, 27 yrs, SF 15 (3-79)µg/L		Sorghum A (0.87% PA)			0.94		-
			Sorghum A, dephytinised			1.26		1.34 (ns)
			Sorghum B (0.89% PA)			1.39		-
			Sorghum B, dephytinised			2.49		1.79 (p<0.01)
	n=2M+7F, 24 yrs, SF 26 (4-193)µg/L		Sorghum C (0.43% PA)			1.52		-
			Sorghum C, dephytinised			3.10		2.04 (p<0.001)
			Sorghum D (0.71% PA)			1.37		-
			Sorghum D, dephytinised			2.56		1.87 (p<0.01)
	n=3M + 4F, 23 yrs SF 48 (30-77)µg/L	Wheat porridge + 25mg aa	Native P			2.91		-
			Dephytinised			10.10		3.48 (p<0.05)
		Wheat porridge-milk + 25mg aa	Native P			2.32		-
			Dephytinised			2.58		1.11 (ns)
	n=6M, 25yrs, SF 88 (54-175)µg/L	Wheat porridge-milk	Native P			0.58		-
			Dephytinised			1.47		2.53 (p<0.05)
		Wheat porridge-milk + 25mg aa	Native P			0.89		-
			Dephytinised			2.25		2.53 (p<0.05)
	n=2M+7F, 24yrs, SF 21 (4-69)µg/L	Wheat porridge-soy	Native P (0.30% PA)			1.15		-
			Dephytinised (0.02% PA)			3.75		3.26 (p<0.005)
		Wheat porridge-soy +	Native P			2.40		-

Author / Method	*Subjects*	*Test Meal*	*Intervention*	*Iron content (mg) Non haem; haem*	*Reference Dose Absorption (%)*	*Non haem iron absorption (%) Actual*	*Adjusted*	*Absorption Ratio*
		25mg aa	Dephytinised			8.46		3.52 ($p<0.005$)
Davidsson et al (2004) SI – Hb incorp – TIMS + faecal monitoring	n=9M+F (69-191 days)	300g soya infant formula (SPI)	IF alone (90mg PA) Dephytinised IF (<1.8mg PA)	2.3-2.5, 0 2.3, 0	N/A	N/A	6.3 8.3	- 1.32 (ns)

*Mean iron absorption adjusted for iron status using ANCOVA with log SF as a covariant

1.26.2 Studies using complete diets

High fibre diets did not affect iron balance when compared with low fibre diets over periods ranging from 3 to 6 weeks (Kelsay *et al.*, 1979; Andersson *et al.*, 1983; Behall *et al.*, 1987; Kelsay *et al.*, 1988; Coudray *et al.*, 1997). Adding 16g wheat bran per day to the diet did not affect iron absorption when measured using a metabolic balance technique in patients with ileostomies over a period of one week (Sandberg *et al.*, 1982). The addition of nondigestible oligosaccharides (15g/day inulin, fructooligosaccharide or galactooligosaccharide) had no effect on non haem iron absorption in young males over a 21 day period when added to a controlled basal diet (van den Heuvel *et al.*, 1998b). Over an 8 week period, Hunt and Roughead (1999) found that premenopausal women absorbed 6 times more iron from a non vegetarian diet than from a lacto-ovo-vegetarian diet that provided similar amounts of iron, but no meat, 20% more ascorbic acid and three times more fibre and phytic acid from whole grains and legumes. This effect is likely to be due to meat consumption rather than the fibre and phytate intake.

1.26.3 Effects of phytates / fibre on iron status – intervention studies

Hunt and Roughead (1999) observed no differences in iron status when premenopausal women ate either a lacto-ovo-vegetarian diet or a non vegetarian diet (as described above) for 8 weeks. The lacto-ovo-vegetarian diet contained three times as much fibre and phytic acid as the non vegetarian diet. In another study, 40g of soy protein isolate was provided to 55 post menopausal women over 6 weeks in a randomised double blind study. Soy protein containing native phytate significantly reduced iron status, whereas soy protein with native isoflavones had no effect on serum ferritin or transferrin saturation levels (Hanson *et al.*, 2006).

1.26.4 Effects of phytates / fibre on iron status – observational studies

Most cross sectional studies have not reported any effect of phytate or fibre on iron status in young women (Galan *et al.*, 1985; Preziosi *et al.*, 1994; Rangan *et al.*, 1997). However,

serum ferritin was negatively associated with fibre intake in French women (Galan *et al.*, 1998) and Dutch adults (Brussard *et al.*, 1997) and negatively associated with phytate intake in postmenopausal women from the United States (Liu *et al.*, 2003). One study showed that iron deficient subjects consumed more cereal than subjects who were not iron deficient (Takkunen & Seppanen, 1975). Phytate consumption was not associated with mild iron deficiency in a New Zealand study (Heath *et al.*, 2001).

1.26.5 The New Zealand situation

The median intake for fibre of New Zealand females aged 19-24 years and 25-44 years was 17 and 18g/day respectively. Of this 9 and 10mg were insoluble non–starch polysaccharides (NSPs). The remaining fibre came from soluble NSPs (Russell *et al.*, 1999). This is less than the Adequate Intake of 25g/day (Commonwealth Department of Health and Ageing Australia, 2006). The major sources of fibre for females includes bread, vegetables, fruits, potatoes and kumara and breakfast cereals, providing approximately three-quarters of the dietary fibre intake. Bran or fibre based dietary supplements (including oat bran, wheat bran, wheat germ, fibre powders and fibre tablets) were used by 4% of females aged 19-24 years and by 5% of women aged 25–44 years (Russell *et al.*, 1999). No data is available on the phytate intake of New Zealanders or the phytate content of New Zealand foods.

1.27 Haem iron

Iron status appears to have little influence on the absorption of haem iron in the amounts found in meals. However, with high doses of haem iron (5-10mg) there is a strong inverse relationship with iron status and haem iron absorption (Hallberg *et al.*, 1979). Dietary factors have less impact on the absorption of haem iron compared with non haem iron. Meat and soy protein may enhance the absorption of haem iron, while calcium inhibits haem iron absorption. Ascorbic acid, phytates and polyphenols have no effect on haem iron absorption (Lynch *et al.*, 1985).

The enhancing effect of meat on haem iron absorption is similar to meats enhancing effect on non haem iron absorption from meals (Hallberg *et al.*, 1979), with two to three times more haem iron absorbed from meals containing meat than from meals without meat (Martinez-Torres & Layrisse, 1971; Hallberg *et al.*, 1979).

A study by Lynch et al (1985) found that the addition of 30g soy protein to a 100g beef patty and replacing beef with soy flour in hamburger meals increased haem iron absorption significantly. In another study haem iron absorption from a beef-soy flour patty was 22.5%. However, no comparison was made with haem iron absorption from a beef patty alone (Hallberg & Rossander, 1982d). No other studies have investigated the effect of soy protein on haem iron absorption. Although replacing beef with soy flour may increase haem iron absorption, the amount of haem iron available for absorption will decrease.

Calcium is the only dietary factor known to inhibit the absorption of haem iron from a meal. Hallberg et al (1991) found that 165mg calcium inhibited the absorption of haem iron from a hamburger meal by 25%. A similar degree of inhibition was observed for non haem iron. Because haem and non haem iron are absorbed by different pathways into the enterocyte, inhibition by calcium is likely to occur within the enterocyte in a transport step common to both non haem and haem iron (Hallberg *et al.*, 1991; Hallberg *et al.*, 1998). The inhibition of haem iron by calcium was similar in meals served with and without meat, indicating that calcium's effect on haem iron absorption is a direct inhibitory effect and not simply counteracting meat's enhancing effect on haem iron absorption (Hallberg *et al.*, 1993). Roughead et al (2002), however found that the addition of 127mg calcium as cheese to a meal had no effect on haem iron absorption.

1.28 Summary of dietary factors affecting iron absorption

While there is a large amount of research indicating the individual effects of dietary factors on non haem iron absorption, there is less information looking at their combined effects in a single meal. One study found that meat, ascorbic acid and phytic acid had the most influence on non haem iron absorption accounting for 16.4% of the variation in iron

absorption (Reddy *et al.*, 2000). Table 1.15 summarises the factors that influence non haem and haem iron absorption.

Table 1.15 Factors influencing dietary iron absorption

	Factors influencing iron absorption	***Dietary enhancers of iron absorption***	***Dietary inhibitors of iron absorption***
Non haem iron	Iron status of subject Amount of non haem iron available 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 subject Amount of dietary haem iron Food preparation (time, temperature)	Meat Soy protein	Calcium

Adapted from Hallberg (2002)

1.29 Justification of assessment methods used

1.29.1 Assessment of Iron Status

Several indices should be used to measure iron status (Gibson, 2005b). The current study determined iron status using serum ferritin and haemoglobin.

Serum ferritin reflects the amount of storage iron in the body (Cook *et al.*, 1974). It is the only measure of iron status that can distinguish between deficient, excess and normal iron stores. However, serum ferritin levels are falsely elevated by inflammation and infection (Hulthen *et al.*, 1998). Pilon (1981) reported the average intra subject day to day coefficient of variation for serum ferritin to be 14.5%. Diurnal variation for serum ferritin appears to be minimal (Gibson, 2005b).

Iron is an essential component of haemoglobin. Haemoglobin is a relatively insensitive measurement of iron deficiency with concentration only falling in iron deficiency anaemia (Gibson, 2005b). The use of haemoglobin to measure iron deficiency has several limitations. A low haemoglobin level not only occurs in iron deficiency anaemia, but in conditions of inflammation, vitamin B12 or folate deficiency, over hydration or acute plasma volume expansion (Gibson, 2005b). Haemoglobin values are affected by diurnal variation. Concentrations of haemoglobin may be up to 10g/L higher in the evening than in the morning (Gibson, 2005b).

1.29.2 Assessment of Dietary Intake

All methods of dietary assessment have their advantages and limitations. A 24 hour recall and a food frequency questionnaire were used to assess subject's dietary intake in the current study. A description of these methods including their advantages and disadvantages are discussed.

During a 24 hour recall the subject recalls their exact food and beverage intake to an interviewer for the previous day or 24 hour period. Detailed descriptions of all food and drinks are obtained including cooking methods and brand names. Quantities of food are estimated using household measures. The interview protocol must be standardised and it is important that the interviewer avoids asking leading questions and making judgmental comments (Gibson, 2005c). A 24 hour recall is not suitable to use in individuals, but is useful for assessing the average usual intake of large groups of people. The size of the group needed to determine the average usual nutrient intake depends on the degree of precision required and the day to day variation in nutrient intakes. In this situation the group must be representative of the population being studies and all days of the week should be adequately represented (Gibson, 2005c).

The 24 hour recall has a low respondent burden and therefore high compliance. It is quick and relatively inexpensive. Subjects may be less likely to alter food intakes due to the

element of surprise a 24 hour recall brings. The 24 hour recall is reliant on memory and the subject's ability to estimate portion sizes correctly. Respondents may overestimate consumption of 'good' foods such as fruit and vegetables and underestimate consumption of 'bad' foods such as takeaways (Gibson, 2005c).

A food frequency questionnaire uses a list of foods or food items to record frequency of intake over a given time period (Gibson, 2005c). The questionnaire can be self or interview administered. It is designed to obtain qualitative, descriptive data on usual intakes of food. The method is relatively quick with little respondent burden and a high response rate. The accuracy of the food frequency questionnaire is lower and it is often used in combination with other methods of dietary assessment (Gibson, 2005c). A food frequency questionnaire can be a useful method of assessing the intake of a particular food group or nutrient.

1.29.3 Anthropometric Assessment

Height and weight were used to assess anthropometric status in the current study. Measurements of height and weight are easy, quick and non invasive. It is essential that standardised techniques are used for measuring height and weight to ensure accurate and precise measurements (Gibson, 1990).

Body Mass Index (BMI) can be determined from height and weight measurements. Quetelet's Index is considered by many investigators to be the best indicator of BMI for adult populations. It is easily calculated, the least biased index by height and correlates with many health related indices such as mortality risk (Gibson, 1990). BMI however, does not differentiate between excess weight caused by muscularity or adiposity (Gibson, 1990).

1.30 Study justification and aims

1.30.1 Study justification

Iron deficiency and iron deficiency anaemia are serious global issues affecting people in both developed and developing countries. Young females are especially vulnerable to iron deficiency as seen in the 1997 National Nutrition Survey (Russell *et al.*, 1999). It is important to know the extent to which iron deficiency exists and which factors contribute to iron deficiency. Only two other studies in New Zealand have investigated the prevalence of iron deficiency in young New Zealand females (Fawcett *et al.*, 1998; Heath *et al.*, 2001). Both of these studies were undertaken in the South Island of New Zealand.

A possible solution for increasing an individual or populations iron status is to increase the iron bioavailability of their diet. Several groups throughout the world are working to identify the factor in meat that enhances non haem iron absorption (commonly known as the MFP factor). Scientists in the IFNHH at Massey University have developed and tested a number of meat extracts in the hope of identifying the MFP factor. Various extracts have been tested using iron binding characteristic procedures, Ussing Chambers, haemoglobin repletion in piglets and mice and Caco-2 cell methodology. The <0.5kDa sarcoplasmic fraction has been shown to be effective in increasing iron absorption in Caco 2 cells, Ussing chambers and mice. As humans will be the potential end users of a meat extract, it is essential that this meat extract's effectiveness is tested in a human population.

1.30.2 Study aims

The main aims of this study were:

- To determine the iron status of subjects (non vegetarian women aged 18-40 years living in the Manawatu region).
- To investigate the dietary iron intakes including haem and non haem iron of subjects.

- To compare the nutrient intakes of subjects with the Nutrient Reference Values for Australia and New Zealand.
- To investigate which factors affected iron status in these subjects.
- To assess the effectiveness of a meat extract (<0.5kDa sarcoplasmic fraction) in increasing non haem iron absorption from a single meal given to human subjects.

Chapter 2

Dietary intakes and iron status of non vegetarian women aged 18-40 years living in the Manawatu region

2.1 Introduction

Iron deficiency is the most common nutritional deficiency worldwide and is associated with a number of consequences including anaemia. The 1997 National Nutrition Survey found that iron deficiency mainly affected women aged 15 to 44 years (Russell *et al.*, 1999). Only two other studies in New Zealand have investigated the iron status of pre menopausal women. Both of these studies were undertaken in the South Island (Fawcett *et al.*, 1998; Heath *et al.*, 2001). In May 2006, the Nutrient Reference Values for Australia and New Zealand were published. The RDI for iron for women aged 19-50 years increased to 18mg/day (Commonwealth Department of Health and Ageing Australia, 2006). The RDI for calcium and folate also increased for young women (Commonwealth Department of Health and Ageing Australia, 2006). Cross sectional studies undertaken in young women have found iron status to be negatively associated with calcium (Takkunen & Seppanen, 1975; Galan *et al.*, 1985; Preziosi *et al.*, 1994; Rangan *et al.*, 1997; Galan *et al.*, 1998), dairy products (Galan *et al.*, 1985; Galan *et al.*, 1998), tea (Galan *et al.*, 1985), fibre (Brussard *et al.*, 1997; Galan *et al.*, 1998) and phosphorus (Preziosi *et al.*, 1994) intake and positively associated with haem iron (Preziosi *et al.*, 1994; Brussard *et al.*, 1997; Rangan *et al.*, 1997; Galan *et al.*, 1998), meat (Takkunen & Seppanen, 1975; Brussard *et al.*, 1997; Galan *et al.*, 1998; Heath *et al.*, 2001) and total iron (Preziosi *et al.*, 1994; Galan *et al.*, 1998) intake. The aim of this study was to assess the dietary intakes, iron status and factors affecting iron status of non vegetarian women aged 18-40 years living in the Manawatu region. The information gathered in this study was also used to select women with low iron stores to take part in the Meat Study (Chapter 3).

2.2 Methods

2.2.1 Participant Recruitment

The research protocol was reviewed and approved by the Manawatu - Whanganui Human Ethics Committee in 2004 (No. 04/02/002) and the Massey University Human Ethics Committee in 2003 (PN Protocol No. 03/145). Extensions on the timeframes permitted were applied for where necessary.

Subjects were recruited from the Manawatu region between August 2004 and September 2006 to take part in a larger study known as the Meat Study. Nine advertisements were placed in the public notices section of The Tribune, a community newspaper, delivered freely to all households in the Manawatu region on a Sunday. These advertisements were placed on 19th September 2004, 3rd April 2005, 5th November 2005, 20th November 2005, 22nd January 2006, 29th January 2006, 19th February 2006, 5th March 2006, and the 7th May 2006. One advertisement was placed in the Manawatu Standard on Wednesday 15th September, 2004. The Guardian newspaper (a local newspaper distributed freely on a Thursday to all houses in the Manawatu area) ran an article about the Meat Study (September 30th 2004) asking women who were interested in taking part to contact the researcher. Copies of the advertisement and article can be seen in Appendix 5.1. Posters were placed on notice boards at Massey University, in local shops, gyms, swimming pools and the Palmerston North Public Library. Posters were also distributed using email and local contacts. An interview took place on Classic Hits Radio Station in Palmerston North on October 6th, 2004 which explained the study and the participants we were looking for. Criteria included women aged between 18 and 40 years, who were not vegetarian or who were not pregnant or breastfeeding. This age group was chosen as most women were likely to be menstruating and therefore were more likely to have lower iron stores than postmenopausal women (Galan *et al.*, 1998; Cade *et al.*, 2005). They were also likely to have stopped growing, an important factor as growth increases the absorption of non haem iron (Fairweather-Tait, 1995a). Exclusion criteria included being vegetarian or vegan (due to the possibility of having to eat meat or the meat extract during the Meat Study), allergies

to iron containing products (due to the very small risk of adverse reaction with infusions of intravenous iron) (Abrams, 2003), donation of blood in the past 12 weeks (for ethical reasons due to the quantity of blood to be taken during the Meat Study), smoking (to ensure subjects included in the Meat Study were a homogenous group), a high alcohol intake (>2 glasses of wine or 1 pint of beer/day) (due to alcohol's effect on serum ferritin levels) (Gibson, 2005b), pregnancy or breastfeeding in the past year (these women were deemed at higher risk of iron deficiency and were excluded for ethical reasons), or planning to become pregnant in the next 6 months (for ethical reasons and due to the increased absorption of non haem iron during pregnancy and the impact this would have on the results of the Meat Study). Subjects who were taking iron or multivitamin supplements were excluded if they were not willing to stop using these during the trial period as well as for a month before the trial period began in order to avoid excessively high intakes of iron during the Meat Study.

Once a volunteer expressed interest in the study she was sent an information sheet (Appendix 5.2) explaining the study and asked to contact the researcher if she wished to participate in the study or if she had any further questions. An initial interview was then organised.

A total of 127 women contacted the researcher regarding the study. Women were excluded from the study due to currently breastfeeding or breastfeeding in the past year (n=2), being over the age of 40 (n=1), not being prepared to stop using multivitamin supplements during the Meat Study (n=2), not consuming red meat (n=1) and having a perceived aversion to ingesting the meat extract (n=1). Five women were unable to take part in the study due to other commitments. Twenty women decided not to take part for reasons unknown or were unable to be contacted again following the sending out of information sheets. Ninety five women took part in the initial dietary interview.

2.2.2 Dietary interview

Written informed consent was obtained at the initial dietary interview held in the Institute of Food, Nutrition and Human Health (IFNHH) at Massey University (see consent form,

appendix 5.2). At this interview each subject was given a code letter(s). Questions were asked to confirm details on the exclusion criteria for the Meat Study as outlined in the information sheet. Details were asked about lifestyle factors including activity levels and use of dietary supplements. A copy of the interview schedule can be found in Appendix 5.3.

Standing height was measured in metres using a portable stadiometer (Surgical and Medical Products, Australia) with measurements made to the nearest centimeter (cm). Body weight was measured in kilograms (kg) using conventional weighing scales (Seca, Vogel and Halke, Germany). Measurements were made to the nearest kg using standardised procedures by a trained assessor. Shoes and extra clothing (jackets) were removed. Quetelet's Body Mass Index (BMI) was calculated from height and weight.

Dietary intake was assessed using a 24 hour recall, with subjects recalling their food intake from the previous day. The multiple pass procedure was used when carrying out the 24 hour recall. The booklet "Food Portion Sizes: A Photographic Atlas" (Nelson *et al.*, 2002) was used to help quantify portion sizes. Following this, a non validated food frequency questionnaire (FFQ) was used to determine subject's intake of foods containing iron and foods known to enhance or inhibit iron absorption over the past three months (see Appendix 5.3). The main purpose of this questionnaire was to obtain information to help in prescribing a diet low in iron and enhancers of iron absorption for the 5 days prior to the Meat Study taking place.

2.2.3 Dietary analysis

Dietary intakes from the 24 hour recall were analysed using FoodWorks Professional Edition Version 4.00 (Xyris Software, 2005). All entries into FoodWorks were manually checked for accuracy by the researcher. Data from the FFQ was converted into average serving sizes prior to dietary analysis as determined by the researcher, for example 120g for meat, fish and poultry; and 200mL for drinks of coffee and tea.

The iron content in meat was assumed to be 40% haem and 60% non haem iron (Monsen *et al.*, 1978). The haem and non haem iron content of each meat, fish, poultry or seafood item consumed in 24 hour recall was calculated manually by the researcher from the total iron data obtained using FoodWorks Professional Edition Version 4.00 (Xyris Software, 2005). For mixed meals, the total iron content of the meat portion of the meal was idenitified using FoodWorks Professional Edition Version 4.00 (Xyris Software, 2005). From this the non haem and haem iron contents were calculated using the 40% haem to 60% non haem iron ratio. Data was entered into Microsoft Excel and all figures were checked to ensure no mistakes were made when transferring the data.

2.2.4 Measurement of iron status

All subjects had a 10mL blood sample taken at MedLab Central in Palmerston North. Biochemical iron status was determined on a non-fasting venipuncture blood sample using serum ferritin and haemoglobin (Hb). Serum ferritin is a reliable and sensitive parameter for assessment of iron stores in healthy subjects (Cook *et al.*, 1974). It is the only measure of iron status that can reflect normal, deficient or excess iron stores (Gibson, 2005b) and a low concentration of serum ferritin is characteristic only of iron deficiency (Dallman *et al.*, 1980). Diurnal variation for serum ferritin is minimal and the day to day coefficient of variation for serum ferritin in healthy subjects over a period of weeks is 15% (Gibson, 2005b). Haemoglobin is an insensitive measure of iron deficiency falling only in the later stages of iron deficiency when iron stores are exhausted and the supply of iron to the tissues is compromised (Gibson, 2005b). However, it is able to provide a measure of the severity of iron deficiency once iron deficiency anaemia has developed. Subjects (n=7) with an abnormal C - reactive protein (CRP) level (>5mg/L) were asked to have their bloods retested at least three weeks later. This cut off level was based on criteria provided by MedLab Central. A high CRP is associated with infection which may falsely elevate serum ferritin concentrations (Hulthen *et al.*, 1998). Serum ferritin was measured on an automated analyser by an immunoturbidimetric assay based on the immunological agglutination principle with enhancement of the reaction by latex (Roche Diagnostics, Indianapolis, IN). This method has been standardised against NIBSC Reagents for ferritin

(human spleen – 80/578). The assay has a coefficient of variation of 6.0% (intra runs) and 5.6% (inter runs) at a level of 32μg/L. Haemoglobin was measured using an automated hematology analyser XE-2100 using the SLS-Hb (sodium lauryl sulfate-Hb) method (Sysmex Corporation, Auckland, NZ). CRP was measured on an automated analyser by an immunoturbidimetric assay based on the principle of particle-enhanced immunological agglutination (Roche Diagnostics, Indianapolis, IN). The CRP had a coefficient of variation of 1.63% (within runs) and 2.17% (between runs).

All subjects were sent a letter thanking them and informing them of their blood test results with an explanation of what their result meant and whether they met the criteria for the Meat Study. Subjects who showed signs of iron deficiency anaemia (SF<12μg/L and Hb<120g/L) or who had excessively high iron stores (SF>300μg/L) were sent a copy of their results and it was recommended that they visit their GP or contact the medical practitioner in the IFNHH at Massey University. Examples of these letters can be seen in Appendix 5.4. For the purpose of this study, depleted iron stores have been defined as the existence of low iron stores (SF<20μg/L) and iron deficient erythropoiesis as a SF<12μg/L and Hb>120g/L.

2.2.5 Statistical analysis

Statistical analysis was performed by the researcher using SPSS for Windows, release 14.0 (SPSS Inc, 2005). Differences with $p<.05$ were considered significant unless indicated otherwise. Mean and median intakes of macro and micronutrients were reported and compared with Nutrient Reference Values for Australia and New Zealand (Commonwealth Department of Health and Ageing Australia, 2006).

Dietary intakes from the 24 hour recall were compared with data from the FFQ using Spearman's correlation coefficient as the data were not normally distributed. Intakes of iron, energy, protein, fat, carbohydrate, fibre, vitamin C, total vitamin A and calcium were compared.

Evidence for a difference between subjects with a serum ferritin of <20μg/L and subjects with a serum ferritin >20μg/L was tested using the independent groups t-test for continuous variables that were normally distributed, the Mann-Whitney U test for those that were not normally distributed, and the Chi-square test for categorical variables.

The relationship between dietary factors and serum ferritin levels were evaluated after preliminary study of Pearson's correlation matrix. Multiple regression analysis was conducted with serum ferritin as the dependent variable. The following variables were entered into the model using a backward stepwise method: age, BMI, and daily intakes of energy, protein, total iron, haem iron, red meat, total meat, vitamin C, vitamin A, total tea (including black tea and herbal tea), coffee, alcohol, fibre and calcium, and number of hours spent exercising or running per week. These were selected as they had previously been shown to influence serum ferritin concentrations or iron absorption from single meals. The variables at the point where the change in R^2 became significant were analysed using forced entry multiple regression analysis. These variables included age, BMI, energy intake, total iron intake, fibre, Vitamin C, Vitamin A, total meat, total tea, total coffee and exercise ($R^2 = 0.230$). The significant values (age, total iron intake, and tea intake) were analysed using forced entry multiple regression analysis. Forward stepwise analysis was used to determine the individual contribution of each predictor. Collinearity was investigated and Durbin-Watson was used to check errors in the regression were independent. Cases that might influence the model were checked using standardised residuals, Cook's distance, the average leverage, Mahalanobis distance, DFBeta values and the upper and lower limit of acceptance values for the covariance ratios. Assumptions of the regression were checked to ensure the model generalised beyond our population group using graphs of ZRESID* against ZPRED*, histograms and P-P plots.

2.3 Results

2.3.1 Study participants

A total of 95 women were recruited over a two year period (August 2004-September 2006) and took part in the initial dietary interview. Five women withdrew from the study before having their bloods taken at Medlab Central. Seven women had a high CRP and were asked to have their bloods retested. Of these, two women were excluded from the study due to having a high CRP on the second occasion and two women were excluded as second blood samples were unable to be obtained. The three remaining women were included in the study and data from their second blood test was used in the data analysis. The final population group comprised 86 women. One woman had a serum ferritin level of 386μg/L so was excluded from statistical analyses, as this number was outside the physiological range for normal iron status.

2.3.2 Iron status of study participants

The mean and median age of these women was 27.0±7.2 and 24.0 (21.0, 34.5) years, respectively. The mean and median BMI was 23.5±3.7 and 22.9 (21.5, 24.7) kg/m^2. Table 2.1 shows the serum ferritin and haemoglobin levels of the women included in this study.

Table 2.1 Serum ferritin and haemoglobin levels of the included women (n=85)

	Mean (standard deviation)	***Median (interquartile range)***
Serum ferritin (μg/L)	39.7 (19.6)	36.0 (26.0, 48.5)
Haemoglobin (g/L)	128.8 (8.6)	128.0 (123.0, 133.5)

Two women (2.4%) had iron deficiency anaemia (SF<12μg/L and Hb<120g/L), no woman had iron deficient erythropoiesis and 9 women (10.6%) had depleted iron stores (SF<20μg/L). Five of these women had a haemoglobin level of less than 120g/L. In addition, five women (5.9%) had haemoglobin levels below 120g/L with a normal serum ferritin level. All other women had normal iron stores (SF>20μg/L) (Table 2.2).

Table 2.2 Iron status of women aged 18-40 years living in the Manawatu region

Iron status	*Number of subjects*
Iron deficiency anaemia (SF<12μg/L, Hb<120g/L)	n=2 (2.4%)
Iron deficient erythropoiesis (SF<12μg/L, Hb>120g/L)	n=0 (0%)
Depleted iron stores (SF<20μg/L, Hb>120g/L)	n=4 (4.7%)
Depleted iron stores (SF<20μg/L, Hb<120g/L)	n=5 (5.9%)
Normal iron stores (SF>20μg/L, Hb>120g/L)	n= 75 (87.2%)

2.3.3 Dietary iron intakes of study participants

Seventy one women (83.5%) consumed less than the recommended dietary intake (RDI) of 18mg iron per day (Commonwealth Department of Health and Ageing Australia, 2006). Eighteen women (21.2%) did not meet the Estimated Average Requirement for iron of 8mg/day, while 45 women (53%) consumed less than 2/3 of the RDI (Table 2.3). The daily mean and median iron intakes were 12.72±6.15mg and 10.8mg, both which are below the RDI of 18mg iron/day (Commonwealth Department of Health and Ageing Australia, 2006).

Table 2.3 Dietary iron intakes of women aged 18-40 years living in the Manawatu area

Daily iron intake	*Number of subjects*
<8.00mg (EAR)	18 (21.2%)
8.00-11.99mg	27 (31.8%)
12.00mg-15.99mg	22 (25.9%)
16.00mg-17.99mg	4 (4.7%)
>18.00mg (RDI)	15 (17.6%)

The estimated mean haem and non haem iron intakes were 0.99±0.91mg/day and 11.74±5.91mg/day (Table 2.6). This equates to non haem iron contributing on average 92.2% and haem iron contributing 7.8% of total dietary iron.

2.3.4 Nutrient intakes of study participants

The nutrient intake of study participants as determined from the 24 hour recalls are displayed in Tables 2.4, 2.5 and 2.6, alongside comparisons with the EAR and RDI or AI.

Table 2.4 Mean and median intakes of macronutrients

	Mean (standard deviation)	***Median (interquartile range)***	***Estimated Average Requirement (per day)***	***Recommended Dietary Intake (per day)***
Energy (kJ)	7554.22 (2199.83)	7455.76 (5676.3, 9000.9)	9200-12500[#] (19-30yrs) 9400-11200[#] (31-50yrs)	-
Protein (g)	76.77 (24.55)	74.88 (56.75, 90.66)	37g (0.60g/kg)	46g (0.75g/kg)
Fat (g)	60.49 (28.75)	53.68 (39.94, 73.95)	-	-
Carbohydrate (g)	233.13 (82.1)	226.34 (171.15, 279.65)	-	-
Alcohol (g)	2.18 (6.66)	n/a	-	-
Protein (%)	17.74 (4.74)	17.37 (13.55, 21.23)	-	15-25%TE[**]
Fat (%)	29.23 (9.04)	29.37 (22.31, 36.24)	-	20-35%TE[**]
Carbohydrate (%)	52.34 (8.69)	51.88 (46.13, 58.27)	-	45-65%TE[**]
Alcohol (%)	0.70 (2.04)	n/a	-	-

[#] Physical Activity Level of 1.8

[**] Acceptable Macronutrient Distribution range

TE = Total energy

Table 2.5 Mean and median intakes of carbohydrates and lipids

	Mean (standard deviation)	*Median (interquartile range)*	*Estimated Average Requirement (per day)*	*Recommended Dietary Intake (per day)*
Fibre (g)	20.85 (7.91)	19.85 (14.78, 26.12)	-	25*
Total sugars (g)	116.72 (59.05)	103.58 (82.75, 142.82)	-	-
Total sugars (%)	25.29 (8.92)	24.96 (19.55, 31.11)	-	-
Saturated fat (g)	24.57 (14.11)	20.04 (15.17, 33.03)	-	-
Polyunsaturated fat (g)	9.17 (6.58)	7.18 (4.99, 11.10)	-	-
Monounsaturated fat (g)	19.77 (11.15)	16.46 (12.01, 24.78)	-	-
Cholesterol (mg)	208.59 (131.97)	172.26 (126.08, 263.76)	-	-
Saturated fat (%)	11.99 (5.09)	11.47 (8.17)	-	<10%TE (saturated and trans fat)
Polyunsaturated fat (%)	4.52 (2.55)	3.65 (2.77, 5.63)	-	-
Monounsaturated fat (%)	9.75 (3.86)	9.52 (6.91, 11.73)	-	-

*Adequate intake

TE = Total energy

Table 2.6 Mean and median intakes of iron and other micronutrients

	Mean (standard deviation)	*Median (interquartile range)*	*Estimated Average Requirement (per day)*	*Recommended Dietary Intake (per day)*
Iron (mg)	12.72 (6.15)	10.8 (8.5, 14.98)	8	18
Haem iron (mg)	0.99 (0.91)	0.76 (0.25, 1.54)	-	-
Non haem iron (mg)	11.74 (5.91)	10.24 (7.95, 14.15)	-	-
Vitamin C (mg)	145.61 (125.41)	116.77 (46.18, 184.56)	30	45
Total Vitamin A (µg)	892.4 (982.19)	662.2 (407.03, 1064.32)	500	700
Calcium (mg)	889.43 (403.08)	819.04 (593.8, 1144.46)	840	1000
Thiamin (mg)	1.35 (0.84)	1.11 (0.73, 1.69)	0.9	1.1
Riboflavin (mg)	1.76 (0.77)	1.66 (1.23, 2.18)	0.9	1.1
Niacin Equivalents (mg)	30.42 (10.56)	29.17 (21.77, 39.13)	11	14
Vitamin E (mg)	8.31 (4.87)	6.87 (5.50, 10.97)	-	7*
Vitamin B6 (mg)	1.69 (0.86)	1.6 (0.95, 2.20)	1.1	1.3
Vitamin B12 (µg)	3.74 (2.31)	3.28 (2.10, 4.54)	2.0	2.4
Folate (µg)	328.74 (183.87)	282.51 (191.12, 463.75)	320	400
Sodium (mg)	2259.79 (918.44)	2064.75 (1688.17, 2736.49)	-	460-920*
Potassium (mg)	3262.77 (1197.73)	3026.67 (2447.27, 3970.70)	-	2800*
Magnesium (mg)	314.98 (127.84)	287.42 (218.69, 382.42)	255 (19-30 yrs) 265 (31-50 yrs)	310 (19-30 yrs) 320 (31-50 yrs)
Phosphorus (mg)	1381.27 (466.66)	1282.83 (1035.73, 1711.14)	580	1000
Zinc (mg)	10.53 (4.38)	9.35 (7.73, 13.23)	6.5	8
Manganese (µg)	4174.85 (2658.79)	3453.48 (2474.86, 4945.05)	-	5000*
Copper (mg)	1.54 (0.71)	1.38 (1.06, 1.83)	-	1.2
Selenium (µg)	43.34 (37.80)	36.31 (25.06, 49.90)	50	60

*Adequate Intake

2.3.5 24 hour recall versus Food Frequency Questionnaire

A significant positive relationship was observed for iron (r=.21, p(one-tailed) <.05), energy (r=.20, p(one-tailed) <.05), protein (r=.30, p(one-tailed) <.01), carbohydrate (r=.25, p(one-tailed) <.05), fibre (r=.37, p(one-tailed) <.001), Vitamin C (r=.32, p(one-tailed) <.01), total vitamin A (r=.26, p(one-tailed) <.01) and calcium (r=.45, p(one-tailed) <.001) when the 24 hour recall was compared with the FFQ data.

2.3.6 Factors affecting serum ferritin

Table 2.7 compares the characteristics of subjects who had depleted iron stores or iron deficiency anaemia (SF<20μg/L) and those with normal iron stores (SF>20μg/L). There was no significant differences between age, body mass index, and intakes of energy, protein, fat, carbohydrate, fibre, total iron, haem iron, non haem iron, vitamin C, vitamin A or calcium intakes between those subjects who had a serum ferritin <20μg/L and those with a serum ferritin >20μg/L. Subjects with a serum ferritin <20μg/L had significantly lower haemoglobin levels (median = 118g/L) compared with those subjects who had serum ferritin levels >20μg/L (median = 129g/L), U = 140, p<.001, r = -.38. There were no significant differences between intakes of red meat, chicken, fish or seafood or total meat intakes between those subjects with a serum ferritin < 20μg/L and those with a serum ferritin >20μg/L as determined by the food frequency questionnaire.

Table 2.7 Characteristics of study participants with and without a Serum Ferritin of < 20μg/L

Characteristic	*Participants with SF < 20 (n=11)*	*Participants with SF > 20 (n=74)*	*p-value for difference*
Age (years)	30 (23, 37)	23.5 (20.8, 33.3)	0.07
Body mass Index	22.5 (19.5, 26.6)	23.0 (21.5, 24.5)	0.46
Serum ferritin (μg/L)	15 (12, 17)	38.5 (29, 52)	0.00
Haemoglobin (g/L)	118 (114, 127)	129 (125, 135.25)	0.00
Energy (kJ)	8186.58 (7366.1, 9399.02)	7234.79 (5567.03, 8740.16)	0.09
Protein (g/day)	62.17 (56.0, 85.05)	75.87 (57.07, 92.12)	0.49
Fat (g/day)	72.24 (40.99, 89.15)	52.65 (39.61, 71.04)	0.11
Carbohydrate (g/day)	226.09 (187.76, 329.37)	226.79 (167.02, 274.40)	0.35
Fibre (g)	19.78 (14.87, 28.25)	19.89 (14.62, 25.97)	0.48
Total iron (mg)	11.26 (8.52, 14.91)	10.78 (8.48, 15.52)	0.27
Haem iron (mg)	0.51 (0.0, 1.05)	0.78 (0.26, 1.57)	0.10
Non haem iron (mg)	10.24 (8.12, 14.06)	10.12 (7.77, 14.97)	0.32
Vitamin C (mg)	156.91 (35.12, 177.67)	115.43 (46.76, 194.03)	0.38
Vitamin A (μg)	745.17 (395.08, 887.31)	660.25 (413.21, 1077.71)	0.59
Calcium (mg)	927.87 (583.45, 1053.46)	798.01 (595.53, 1183.43)	0.41
Red meat intake (g/day)[#]	42.9 (8.6, 51.4)	51.4 (34.3, 87.9)	0.07
Chicken (g/day)[#]	42.9 (8.6, 51.4)	51.4 (25.7, 51.4)	0.16
Fish or seafood (g/day)[#]	8.6 (8.6, 51.4)	17.1 (8.6, 38.6)	0.40
Total meat intake (g/day)[#]	111.4 (86.7, 120)	124.8 (85.7, 154.3)	0.09
Eats red meat > 3x/week (%)[#]	45.5	64.9	*
Eats chicken > 3x/week (%)[#]	45.5	51.4	0.72
Eats fish or seafood > 3x/week (%)[#]	36.4	24.3	*
Eats any kind of meat > 3x/week (%)[#]	90.9	95.9	*
Eats any kind of meat >1x/day (%)[#]	27.3	51.4	0.07

Characteristic	*Participants with SF < 20 (n=11)*	*Participants with SF > 20 (n=74)*	*p-value for difference*
Drinks black tea (%)#	81.8	63.5	*
Drinks herbal tea (%)#	72.7	41.9	0.06
Drinks coffee (%)#	54.5	67.6	*
Use of iron supplement (%)	0	8.1	*
Use of multi vitamin (%)	27.3	27.0	*
Use of other supplements (%)	27.3	29.7	*

Values are expressed as medians (interquartile range) or percentages

*Statistical analysis was unable to be performed because assumptions for the Chi-Square Test were not met

As estimated from Food Frequency Questionnaire

Multiple regression analysis showed that serum ferritin was positively associated with age and total dietary iron intake. Age and total dietary iron intake explained 12.6% of the variation in serum ferritin ($p<.05$). Total iron intake explained 6.4% ($p<.05$) of the variation in serum ferritin within this model. Total iron intake and age were not positively correlated ($p>.05$). No statistically significant relationship was found between serum ferritin and Body Mass Index, total exercise or hours spent running per week or daily intakes of energy, protein, haem iron, red meat, total meat, vitamin C, vitamin A, total tea, coffee, alcohol, fibre or calcium ($p>.05$).

Table 2.8 Multiple regression to determine factors which influence serum ferritin

	Coefficient (B)	*Standard error (B)*	*p value*
Constant (SF)	16.64	9.05	
Age of subject	0.62	0.31	<.05
Total iron intake	0.81	0.33	<.05

Note, $R^2 = 0.126$

2.4 Discussion

2.4.1 Study participants

The subjects included in this study all volunteered to take part in a larger study known as the Meat Study. All subjects were non smokers who avoided consuming large amounts of alcohol. They may therefore have been more health conscious than the normal population. Several subjects stated that they were interested in taking part in the 'Meat Study' due to previous diagnosis of iron deficiency. Data was not collected on previous history of iron deficiency, although previous iron deficiency is known to be a risk factor for iron deficiency (Galan *et al.*, 1998). The mean and median BMI of subjects in this group was slightly lower than that of the National Nutrition Survey where the mean BMI of females aged 19-24 years was 25.0kg/m^2 and 25.7 kg/m^2 for women aged 25-44 years (Russell *et al.*, 1999), but slightly higher than the median BMI (22.7 kg/m^2) in a Dunedin study investigating factors determining iron status in women aged 18-40 years (Heath *et al.*, 2001).

2.4.2 Iron status of study participants

The incidence of iron deficiency anaemia in this population group (2.4%) was similar to that seen in other New Zealand studies (Fawcett *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001). In other Western countries the incidence of iron deficiency anaemia in women of similar age groups has been 4-5% (Brussard *et al.*, 1997; Looker *et al.*, 1997; Rangan *et al.*, 1997; Galan *et al.*, 1998). In a study of French women aged 17-42 years 1.3% of women were found to have iron deficiency anaemia (Galan *et al.*, 1985).

The prevalence of iron deficient erythropoiesis and depleted iron stores was lower in our population compared with similar groups both in New Zealand and overseas. No subjects had iron deficient erythropoiesis (SF<12 µg/L) and 10.6% had depleted iron stores (SF<20 µg/L). In contrast, the National Nutrition Survey (Russell *et al.*, 1999) found that 6 and 10% of women aged 19-24 and 25-44 years respectively had a serum ferritin level of

<12μg/L. Heath et al (2001) found that 4% of women aged 18-40 years had iron deficient erythropoiesis (SF<12 μg/L, Hb>120g/L) and 19% had depleted iron stores (SF<20μg/L, Hb>120g/L) while Fawcett et al (1998) found 6.7% of women aged 21 years to have iron deficiency (SF<12μg/L, Hb>120g/L) and 18.7% to have low iron stores (SF<20μg/L). Studies undertaken in other Western countries also show that females appear to have a higher incidence of iron deficiency and depleted iron stores compared with our population group (Leggett *et al.*, 1990; Brussard *et al.*, 1997; Looker *et al.*, 1997; Rangan *et al.*, 1997; Galan *et al.*, 1998). Rangan et al (1997) found that 19.8% of Australian women aged 15–30 years had a SF<20μg/L and 7.2% had iron deficiency (SF<12μg/L and TS<16%), while Leggett et al (1990) found 8.9% of Australian women under 65 years of age to have a SF<10μg/L. In the third National Health and Nutrition Survey (1988-1994) in the United States, 11% of women aged 20-49 years had iron deficiency (defined as an abnormal value for two or more of the following: SF<12 μg/L, transferrin saturation (TS)<15%, erythrocyte protoporphyrin>1.24μmol/L or a haemoglobin <120g/L) (Looker *et al.*, 1997). In various European studies, 22.7% of women aged 35-39 years have been shown to have depleted iron stores (SF<15μg/L) (Galan *et al.*, 1998), 16% of women aged 20-49 years had a SF<10μg/L (Brussard *et al.*, 1997) and 16% of women aged 17-42 years have had a SF<12 μg/L (Galan *et al.*, 1985). It is difficult to compare these studies directly due to the use of different cut-off values for determining iron deficiency and depleted iron stores. In a review, Hallberg (1995) found that the prevalence of iron deficiency ranged from 3.9% in Iceland to 45% in Ireland with cut-off values ranging from 10-17μg/L. Iron status in the United Kingdom appears to be particularly low, with approximately 30% of women aged 19 to 24 years, 18% of women aged 25-34 years and 24% of women aged 35-49 years having a SF<20μg/L (Ruston *et al.*, 2003). In a smaller study in the United Kingdom involving 90 women aged 18 to 45 years, the median serum ferritin level was 13.0μg/L (Harvey *et al.*, 2005).

The lower prevalence of iron deficient erythropoiesis and depleted iron stores in this population group may have been due to the exclusion of subjects who had donated blood in the past 12 weeks, the exclusion of women who did not eat meat or due to a higher dietary intake of total iron. Recent blood donation is a risk factor for mild iron deficiency (Heath

et al., 2001) and pre menopausal blood donors tend to have a lower serum ferritin concentration than non blood donors (Leggett *et al.*, 1990; Brussard *et al.*, 1997; Rangan *et al.*, 1997). Vegetarian females tend to have lower serum ferritin concentrations than non-vegetarian females, but do not appear to have a higher incidence of iron deficiency anaemia (Hunt, 2003). This finding is supported by two studies undertaken in New Zealand in females (Alexander *et al.*, 1994) and in Seventh Day Adventists (Harman & Parnell, 1998). It is has been suggested that iron homeostasis is maintained through the up regulation of iron absorption (Hunt, 2003). However, iron absorption did not appear to be improved in strict long term vegetarians consuming a high phytate intake compared with non vegetarians (Brune *et al.*, 1989). In New Zealand women are exposed to advertising about the importance of receiving adequate amounts of iron in the diet. However this does not explain the difference in the incidence of iron deficiency seen in this and other New Zealand studies (Fawcett *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001). Additionally, the researcher may have been contacted predominantly by women who were interested in iron, and therefore more conscious of receiving adequate amounts of iron in their diet.

2.4.3 Dietary intakes of iron

The mean daily intake of iron was 12.7±6.2mg/day which was higher than that of the National Nutrition Survey (10.8 and 10.5mg for females aged 19-24 and 25-44 years respectively) (Russell *et al.*, 1999) and higher than in New Zealand women of a similar age group (10.7mg/day) (Heath *et al.*, 2001). Total iron intake was also higher than that of pre-menopausal women in studies undertaken in other Western countries. Reported mean dietary iron intakes of women in Australia were 11.7mg/day (1.07mg haem iron (9.1%)) (Rangan *et al.*, 1997). In France, mean iron intakes in pre menopausal women have been reported as 11.5mg/day (9.5% haem iron) in women aged 35-39 years (Galan *et al.*, 1998), 9.4mg (13% haem iron) in women aged 18-30 years, 9.9mg (13% haem iron) in women aged 30-40 years (Preziosi *et al.*, 1994), and 10.9mg (13.7% haem iron) (Galan *et al.*, 1985). In the Netherlands, mean iron intake was 11.6mg/day (15.5% haem iron) in women aged 18-45 years (Brussard *et al.*, 1997). A median intake of 11.9mg/day was reported in a

population of women in the United Kingdom (Harvey *et al.*, 2005). In the United Kingdom's National Diet and Nutrition Survey, mean iron intakes were 10.0mg and 9.8mg for women aged 19-24 and 25-34 years, and 12.9mg for women aged 35-49 years (Henderson *et al.*, 2003). Intakes of haem iron were particularly low, at 0.4-0.5mg/day for women aged 19-49 years (Henderson *et al.*, 2003). All of these groups had a higher incidence of iron deficiency and depleted iron stores compared with our population group.

Haem iron contributed 1.0mg (7.8%) of total dietary iron intake in our study. This is similar to other research undertaken in New Zealand (Heath *et al.*, 2001) where women with mild iron deficiency consumed 0.7mg (6.5%) haem iron and women without mild iron deficiency consumed 1.0 mg (9.3%) haem iron and Australia (1.07mg haem iron (9.1%)) (Rangan *et al.*, 1997) but slightly lower than the intake seen in European countries (Galan *et al.*, 1985; Preziosi *et al.*, 1994; Brussard *et al.*, 1997; Galan *et al.*, 1998). Unlike our study, these groups all included vegetarian women, so it is surprising that their intake of haem iron was higher than that of the present study. Using the food frequency questionnaire the estimated total mean and median intake of meat in the diet of women was 126.2g/day and 119.9g/day respectively. In a study by Heath et al (2001) these values were 85.9g/day for women with mild iron deficiency (SF<20μg/L) and 110.6g/day for women with normal iron stores (SF>20μg/L).

Despite the higher mean intake of total iron, 83.5% of women consumed less than the RDI for iron of 18mg per day, 53% consumed less than 2/3 of the RDI (12mg iron per day) and 21.2% consumed less than the estimated average requirement (Commonwealth Department of Health and Ageing Australia, 2006) or the United Kingdom's lower reference nutrient intake of 8mg/day (Department of Health, 1991). Similar results were observed in French populations (Galan *et al.*, 1991; Preziosi *et al.*, 1994; Galan *et al.*, 1998). In the United Kingdom 42% of women aged 19-24, 41% of women aged 25-34 years and 27% of women aged 35-49 years had an iron intake below 8mg/day (Henderson *et al.*, 2003). The results in our study population are similar to that of the National Nutrition Survey (Russell *et al.*, 1999) which found the estimated prevalence of iron deficiency to be lower than the number of women estimated to have an inadequate intake of dietary iron.

2.4.4 Intake of other nutrients

The mean and median intakes of carbohydrate, protein and fat were all within the Acceptable Macronutrient Distribution Range (AMDR) to reduce the risk of chronic disease (Commonwealth Department of Health and Ageing Australia, 2006). Alcohol contributed on average 0.7% of total energy. Saturated fats contributed to 11.99% of total energy intake, higher than that recommended by the Nutrient Reference Values (Commonwealth Department of Health and Ageing Australia, 2006). The mean intake of protein was 76.8g, higher than the RDI for protein of 46g per day (Commonwealth Department of Health and Ageing Australia, 2006). Total sugars contributed 25.3% of total energy. The mean intake of fibre was 20.85g, lower than both the AI for fibre (25g) and the suggested dietary target for fibre of 28g/day. Energy intakes were below the recommended range of estimated energy requirements (9,200–12,500kJ/day for a woman 19-30 years and 9,400-11,200kJ for a woman aged 31-50 years with a physical activity level (PAL) of 1.8). The PAL of 1.8 was chosen as PAL's of 1.75 and above are consistent with good health (Commonwealth Department of Health and Ageing Australia, 2006).

Mean and median intakes of Vitamin C, thiamin, riboflavin, niacin equivalents, vitamin B6, vitamin B12, phosphorus, zinc, copper, potassium and sodium were all above the RDI and AIs. However, none of these nutrient intakes were above the upper limit set, and neither vitamin C nor potassium met the suggested dietary target (SDT) to reduce chronic disease risk. Sodium intakes were above the SDT of 1600mg/day (Commonwealth Department of Health and Ageing Australia, 2006). Intakes of calcium, folate, selenium and manganese were below their respective RDIs and AIs (Commonwealth Department of Health and Ageing Australia, 2006). Mean intakes of vitamin A and vitamin E were above the RDI and AI respectively, while median intakes were below the RDI and AI. The median intake of magnesium was below the RDI, while the mean intake of magnesium was above the RDI for women aged 19-30 years, but below the RDI for women aged 31-50 years (Commonwealth Department of Health and Ageing Australia, 2006). Comparing dietary intakes with the RDI can be misleading because the RDI is set at the amount of nutrient that is adequate or more than adequate for the majority of people (Department of Health, 1991).

Intakes of selenium were below the Estimated Average Requirement (EAR). Over 50% of women consumed less than the EAR for folate (51.8%), calcium (52.9%) and selenium (76.5%) (Commonwealth Department of Health and Ageing Australia, 2006).

The mean energy intake of study participants (7554.2kJ) was lower than women aged 19-24 years and women aged 25–44 years in the National Nutrition Survey (9102kJ and 8417kJ respectively), but similar to New Zealand and Australian studies investigating factors that affect iron status (Rangan *et al.*, 1997; Heath *et al.*, 2001). Intakes of protein and carbohydrate were similar between this study and women in the National Nutrition Survey (Russell *et al.*, 1999). Fat and alcohol intakes were much lower in our group of women. The average fat intake was 60.5g per day (29.2% of total energy) compared with 84g (34% of total energy) and 80g (35% of total energy) for women aged 19-24 years and women aged 25-44 years respectively (Russell *et al.*, 1999). Intakes of saturated fat, polyunsaturated fat and monounsaturated fat were lower than those observed in the National Nutrition Survey both in actual amounts and as a percentage of total energy (Russell *et al.*, 1999). Alcohol contributed on average 0.7% of total energy or 2.2g compared with 13g (4% of total energy) and 11g (3% of total energy) for women aged 19-24 years and 25-44 years in the National Nutrition Survey (Russell *et al.*, 1999). The lower intake of alcohol is likely to have been due to the exclusion of heavy drinkers from our study population. The lower intake of fat may have been due to using subjects who were non smokers and light consumers of alcohol, and therefore perhaps more health conscious. In addition several of our subjects were nutrition students so may have been aware of the health risks associated with a high fat diet.

Mean intakes of fibre, vitamin C, vitamin A, calcium, thiamin, riboflavin, vitamin B6, folate, manganese, potassium, magnesium, phosphorus and copper were all higher in our study population than those women in the National Nutrition Survey (Russell *et al.*, 1999). Mean intakes of niacin equivalents, vitamin E, vitamin B12, zinc and selenium were only slightly lower than those observed in the National Nutrition Survey (Russell *et al.*, 1999). Overall, it appears that our subjects had a more nutrient dense diet than those in the National Nutrition Survey.

2.4.5 Dietary factors affecting iron status

There were no significant differences in the dietary intakes of women who had a SF<20μg/L and those with a SF>20μg/L. Total dietary iron was the only dietary factor to show a significant positive relationship with serum ferritin concentration. This finding was supported by two large European studies involving men and women covering a wide range of ages (Preziosi *et al.*, 1994; Galan *et al.*, 1998) and a study in adolescent females (Bairati *et al.*, 1989). However, most studies in population groups involving pre menopausal women have not found this association (Galan *et al.*, 1985; Razagui *et al.*, 1991; Brussard *et al.*, 1997; Heath *et al.*, 2001).

It is likely that it is the type of iron rather than the amount of iron consumed that is most important in determining iron status. Unlike other studies, this study did not find haem iron intake (Brussard *et al.*, 1997; Rangan *et al.*, 1997; Galan *et al.*, 1998) or meat intake (Takkunen & Seppanen, 1975; Helman & Darnton-Hall, 1987; Worthington-Roberts *et al.*, 1988; Reddy & Sanders, 1990; Alexander *et al.*, 1994; Brussard *et al.*, 1997; Galan *et al.*, 1998; Ball & Bartlett, 1999; Heath *et al.*, 2001; Hua *et al.*, 2001) to be associated with higher levels of serum ferritin. This finding may have been more significant had we included non meat eaters in our study population. While not a significant result, women with a serum ferritin <20μg/L appeared to have a lower intake of all types of meat compared with those who had a serum ferritin >20μg/L as determined by the food frequency questionnaire.

Vitamin C did not affect iron status in this study, which is in agreement of most other studies in premenopausal women (Galan *et al.*, 1985; Bairati *et al.*, 1989; Preziosi *et al.*, 1994; Rangan *et al.*, 1997; Galan *et al.*, 1998; Heath *et al.*, 2001). This may be because the bioavailability of dietary iron eaten depends on the amount of Vitamin C consumed with that iron (ie. at mealtimes) rather than the total dietary intake as demonstrated by Razagui et al (1991).

This study found no association between tea or coffee intake and serum ferritin levels. However, from the food frequency questionnaire it appeared that more black and herbal tea was consumed by women who had a serum ferritin <20μg/L compared with those who had a serum ferritin >20μg/L. This finding was not significant. Galan et al (1985) found a negative association between tea consumption and serum ferritin levels, and Razagui et al (1991) found tea intake at mealtimes to be negatively correlated with serum ferritin levels. Other studies have found no relationship between tea (Preziosi *et al.*, 1994; Galan *et al.*, 1998) or coffee intake (Razagui *et al.*, 1991) and iron status. A meta-analysis found that tea consumption does not appear to affect iron status in populations who have an adequate iron status (Temme & Van Hoydonck, 2002). Polyphenol intakes were not calculated due to the limited data available on their composition in databases of New Zealand foods and beverages. It can also be assumed that weak tea would have a less of an effect on iron status than strong tea. However, this was not investigated.

There was no association between calcium and iron status in our population group, as found in two other cross sectional studies (Brussard *et al.*, 1997; Heath *et al.*, 2001). Many cross sectional studies have found a negative association between calcium or dairy product intake on serum ferritin levels (Takkunen & Seppanen, 1975; Galan *et al.*, 1985; Preziosi *et al.*, 1994; Rangan *et al.*, 1997; Galan *et al.*, 1998). However, this finding has not been seen in long term intervention studies (Sokoll & Dawson-Hughes, 1992; Reddy & Cook, 1997; Kalkwarf & Harrast, 1998; Minehane & Fairweather-Tait, 1998). In the cross sectional studies where the effects of dairy products or calcium intake on iron status have been observed, it may be due to high calcium foods replacing foods containing high amounts of bioavailable iron rather than the inhibitory effect of calcium itself (Heath *et al.*, 2001).

There was no association seen between iron status and fibre intakes in this study. Only two cross sectional studies have found a negative association between iron status and fibre intake (Brussard *et al.*, 1997; Galan *et al.*, 1998), while one study showed that iron deficient subjects consumed more cereal than subjects who were not iron deficient (Takkunen & Seppanen, 1975). Phytate levels were not calculated due to the limited food composition data available.

Alcohol was not found to have any effect on iron status in this population group. This finding should be interpreted with caution as the inclusion criteria for our study excluded heavy drinkers and only 15.3% of women had consumed any alcohol on the day their 24 hour recall was undertaken. Most studies in young women have not investigated the effect of alcohol on iron status. Studies have ranged from finding a positive association between alcohol intake and serum ferritin levels in women (Brussard *et al.*, 1997) to finding a positive effect in men only (Leggett *et al.*, 1990) to finding no association between alcohol intake and iron deficiency (Rangan *et al.*, 1997),

There was no association found between vitamin A intakes and iron status in this group. No other studies in young women have considered the effect of vitamin A intake on iron status.

Energy and protein intake was not shown to influence iron status in this study population. These findings agree with Rangan et al (1997). Brussard et al (1997) however found serum ferritin levels to be positively associated with energy and vegetable protein intakes. Other studies have not reported any association.

2.4.6 Other factors affecting iron status

Age, BMI, exercise, supplement use and their relationship with iron status were also investigated in this study. Increasing age was found to be associated with a higher concentration of serum ferritin. Hallberg (2002) suggested that one reason serum ferritin increases with age is the fact that serum ferritin is a very strong acute phase reactant. However, there appears to be no other reasons for a positive relationship between age and iron status, excluding menstrual blood loss and parity (Heath *et al.*, 2001). Parity was not investigated in this study and all women were assumed to still be menstruating. Brussard et al (1997) and Leggett et al (1990) also found increasing age to be associated with a higher iron status. These findings were likely to be due to menopause and less loss of blood in older women. Post menopausal women have been found to have significantly higher serum

ferritin levels than pre menopausal women (Galan *et al.*, 1998; Cade *et al.*, 2005). Other studies in pre menopausal women have not found age to be associated with iron deficiency (Rangan *et al.*, 1997).

Body mass index was not associated with serum ferritin levels. Whitfield et al (2003) found serum ferritin increased with an increasing BMI. Heath et al (2001) found that women with mild iron deficiency (SF<20μg/L in the absence of iron deficiency anaemia) had a slightly but significantly lower BMI than participants without mild iron deficiency.

The total number of hours spent exercising or running per week were not found to significantly influence iron status. This finding is in agreement with other studies which have found no significant relationship between frequency of active exercise and serum ferritin concentration (Leggett *et al.*, 1990) or between iron deficiency and exercise based on frequency, duration and intensity of the exercise taken (Rangan *et al.*, 1997). Comparisons of serum ferritin between athletes and control groups have shown mixed results. Some studies have reported lower serum ferritin levels in athletes, especially female and endurance athletes (Fogelholm, 1995), while other have reported higher serum ferritin values in athletes than in control subjects (Malczewska *et al.*, 2000b).

7.1% of women in this study reported using iron supplements, 27.1% of women used multivitamin/mineral supplements and 29.4% of women reported the use of other nutritional supplements. Data was not collected on whether the multivitamin/mineral supplements contained iron. These findings are similar to that of the National Nutrition Survey which found that 8% of women aged 19-24 years and 10% of women aged 25-44 years had used iron supplements and 32% of women aged 19-24 years and 27% of women aged 25-44 years had used a multivitamin and / or mineral supplement in that past year (Russell *et al.*, 1999). Insufficient data was available to determine whether the use of iron, multivitamin / mineral or other supplements had an effect on subjects iron status. Complete data was not available on the type and amount of iron in supplements subjects used and therefore was not included in the dietary analysis. This may have led to an underestimation of iron intakes (Gibson, 2005a). Interestingly, none of the women who had a SF<20μg/L

used an iron supplement compared with 8.1% of women who had a SF>20μg/L. These findings were not significant. The level of use of multivitamins and other supplements was similar between the two groups. A study in pre menopausal Australian women found vitamin and mineral supplement use were not associated with iron deficiency (Rangan *et al.*, 1997). Supplemental iron intake was found to be associated with higher iron stores in pre menopausal women in the United States (Blanck *et al.*, 2005) and the use of a vitamin and mineral supplement in the past year was found to reduce the risk of mild iron deficiency in New Zealand women aged 18-40 years (Heath *et al.*, 2001).

Finally genetic factors may have an important influence on iron status in females. For example, Whitfield et al (2003) found that genetic differences between women had a greater influence on iron stores than the effects of self reported menstruation and number of pregnancies.

2.4.7 Dietary assessment and analysis

One of the limitations of this study was the use of a 24 hour recall to assess dietary intakes from these study participants. To characterise the usual intake of a large group a 24 hour recall over a 1 day period is the method of choice, provided the sample is representative of the population and all days of the week are represented (Gibson, 2005c). This group was unlikely to be representative of the population as subjects volunteered to take part in the study. A 24 hour recall does not take into account day to day variation of an individual's food consumption and may omit foods that are consumed infrequently (Gibson, 2005c). The success of a 24 hour recall relies on the subjects memory and ability to accurately estimate portion sizes (Acheson *et al.*, 1980). A diet record would have provided better dietary information. In individuals, it has been suggested that 12 days of recording is necessary to accurately estimate an individuals usual iron intake due to the large variations of intake across days (Heath & Fairweather-Tait, 2002). However, conducting a diet record was not practical due to time and budgetary constraints. In addition, the longer the diet record the greater the respondent burden which may result in a decrease in compliance (Gibson, 2005c).

A non–validated food frequency questionnaire (FFQ) was used to assist in determining the intake of foods containing iron and the intake of foods likely to enhance or inhibit iron absorption over the past three months. Food frequencies are relatively quick to administer with a low respondent burden. They are able to evaluate dietary intakes over a longer period of time which may be more relevant as iron status is the balance between iron absorption and loss over several months (Rangan *et al.*, 1997). However, their accuracy is lower than in other methods (Gibson, 2005c). The FFQ used in this study was designed to provide guidelines for participants to keep their diets low in iron (as part of the Meat Study) rather than to determine accurately the levels of iron consumed. It was non-validated and therefore used only to give an indication of the amounts of meat, chicken, fish and seafood eaten, and the amounts of tea and coffee consumed rather than the amounts of actual nutrients consumed. Nutrient intakes from the 24 hour recall and FFQ were not highly correlated with one another. A validated FFQ would have provided more accurate and reliable information, such as the FFQ developed by Heath et al (2000) which was validated against 11 days of weighed diet records in 49 women aged 19-31 years (Heath *et al.*, 2000).

The use of food composition databases such as Foodworks to assess nutrients has several limitations. Foods in the database may differ in growing, processing, sorting and/or cooking methods, or there may be seasonal or regional differences compared with the actual foods consumed. A study in the United Kingdom found that using food composition database data rather than brand specific values to calculate the iron content of breakfast cereals could over or underestimate as individual's iron intake by as much as 22 or 28% respectively (O'Hara *et al.*, 2004). In addition, food composition databases may include errors in sampling protocols and analytical methods (Gibson, 1990). In the present study if a food was not contained in the database, a suitable substitution was made using a combination of an appropriate similar food in the database and the information contained on nutrition labels.

Energy intakes of participants were low when compared with data from the National Nutrition Survey (Russell *et al.*, 1999). Under reporting is common in dietary assessment methods and reported intakes do not always reflect actual intakes. Energy intake could be

validated by dividing reported energy intake by basal metabolic rate. This ratio determinines whether reported energy intakes are consistent with energy intakes required to live a normal lifestyle (Goldberg *et al.*, 1991). This method however, is crude and depends on estimates of basal metabolic rate, which have their own inaccuracies. Limitiations associated with the 24 hour recall and assessment of energy expenditure (a crude measure of reported hours per week of exercise) meant it was not appropriate to relate estimated energy expenditure to energy intake using the 24 hour recall to investigate the incidence of under reporting.

2.4.8 Estimation of the haem iron content of foods

Estimating the iron content of diets has difficulties associated with the direct measurement of haem iron content of foods. Foodworks only provides data on the total iron content of foods. The haem iron in this study was therefore estimated at 40% based on models used to investigate the bioavailability of dietary iron in a given meal (Monsen *et al.*, 1978). However, studies have given varying estimates of the haem iron content of meats. For example, analysis of a variety of meats found that 50-60% of iron in beef, lamb and chicken and 30-40% of the iron in pork, liver and fish was in the form of haem iron (Cook & Monsen, 1976). In New Zealand, the total haem iron content of uncooked beef *(longissimus lumborum muscle)* was approximately 82% and 76% for lamb with the proportion of soluble haem iron higher for beef than for lamb (Purchas *et al.*, 2003). An increase in the solubility of iron is associated with increased iron bioavailability (Conrad & Umbreit, 2000). The haem iron content of beef was reported to be 70-80% following cooking (Purchas *et al.*, 2003; Purchas *et al.*, 2004). In Australia, the haem content of beef was 64-70%, lamb 59-62%, pork 66%, chicken 54-70%, liver 33%, a beef sausage 36%, tuna 18% and snapper 63%. These were all cooked samples of meat (Rangan *et al.*, 1996). A study in the United States found the haem iron content of cooked chicken to range from 29-40% (Clark *et al.*, 1997). Haem iron in meat converts to non haem iron with cooking (Garcia *et al.*, 1996b; Baech *et al.*, 2003b; Purchas *et al.*, 2003). This extent to which this occurs depends on the temperature and duration of heating (Chen *et al.*, 1984; Baech *et al.*, 2003b), making it difficult to accurately estimate the haem iron content of meat. In

addition, cooking leads to decreases in soluble iron and increases in insoluble iron (Purchas *et al.*, 2003). In the present study, further calculations were made which assumed that white meat (fish, chicken and pork) contained 35% haem iron, beef contained 75% haem iron and lamb 60% haem iron. Using these figures haem iron intake was estimated to be 1.5±1.4mg/d (11.8% of total iron intake) and non haem iron 11.3±5.9mg/day (88.9%). These figures were slightly higher than the figures we obtained using haem iron estimates of 40% in all meats. However, the use of these haem iron figures in multiple regression analysis did not have any significant effect on iron status.

2.4.9 Limitations of this study

One of the major limitations of this study was that we did not investigate oral contraceptive use, blood loss through menstruation or nose bleeds, parity, intervals between pregnancies or previous diagnosis of iron deficiency anaemia. Oral contraceptive use tends to determine the extent of menstrual bleeding (Rangan *et al.*, 1997). Several studies have shown that serum ferritin is inversely related to the duration of the menstrual period (Galan *et al.*, 1985; Razagui *et al.*, 1991; Rangan *et al.*, 1997), to the duration and extent of menstrual bleeding assessed by a menstrual recall method (Heath *et al.*, 2001) or to menstrual loss as determined by direct measurement (Harvey *et al.*, 2005). Only one study has investigated the effect of nose bleeds and found them to be an important predictor of mild iron deficiency (Heath *et al.*, 2001). Parity (Looker *et al.*, 1997; Galan *et al.*, 1998) and previous diagnosis of iron deficiency anaemia (Galan *et al.*, 1998) are also known to impact on iron status. Investigating these aspects would have helped determine more clearly factors affecting iron status in young women.

2.5 Conclusions

The prevalence of iron deficiency anaemia in this group was similar to that seen in other New Zealand studies but lower than that seen in other Western countries. The incidence of iron deficient erythropoiesis and depleted iron stores was lower compared with similar groups in New Zealand and overseas. This may have been due to the exclusion of blood

donors and vegetarian women, or due to a higher dietary intake of total iron. Despite a large number of women appearing to have an inadequate intake of dietary iron, only 13% of women had depleted iron stores or iron deficiency anaemia. Only total dietary iron intake and age were positively associated with iron status. Dietary intakes of folate, calcium and selenium were low in this population with over 50% of women consuming less than the estimated average requirement for folate and calcium and over 75% of women consuming less than the estimated average requirement for selenium.

Chapter 3

The effect of a meat extract on iron absorption in young women

3.1 Introduction

Several studies have shown that iron deficiency and iron deficiency anaemia is a problem in both developed and developing countries. Solutions to this may be addressed through several means including increasing the bioavailability of dietary iron consumed. The unidentified factor in meat that increases the absorption of non haem iron from single meals is commonly referred to as the meat fish poultry (MFP) factor (Whitney & Rolfes, 2005). Research has been ongoing to determine what exactly the MFP comprises (Martinez-Torres & Layrisse, 1970; Cook & Monsen, 1976; Bjorn-Rasmussen & Hallberg, 1979; Martinez-Torres *et al.*, 1981; Layrisse *et al.*, 1984; Taylor *et al.*, 1986; Mulvihill *et al.*, 1998; Mulvihill & Morrissey, 1998a; Mulvihill & Morrissey, 1998b). Over the past several years the Institute of Food, Nutrition and Human Health (IFNHH) at Massey University has been undertaking research to identify and determine the MFP factor. An extract containing peptides and carbohydrates <0.5kDa of the sarcoplasmic fraction (Wilkinson *et al.*, 2006) has shown significant up-regulation of iron uptake and transport in Caco-2 cells (Wolber *et al.*, 2006). The aim of this exploratory study was to assess the extent to which this meat extract (<0.5kDa sarcoplasmic fraction) was able to increase the absorption of non haem iron from a single meal given to human subjects. Iron absorption was measured using the incorporation of stable isotopes ^{57}Fe (given with the meal) and ^{58}Fe (given as an intravenous infusion) into red blood cells.

3.2 Methods

3.2.1 Subject Recruitment

Subjects were recruited using the methods described in Chapter 2. This code letter allocated to subjects in study one was also used in study two. Eighteen subjects were selected to take part in this exploratory study. Previous studies investigating non haem iron absorption in women have used similar numbers of subjects (Whittaker *et al.*, 2001). Three steps were involved in selecting suitable participants to take part in the Meat Study. These included a dietary interview, a measurement of iron status and a medical screening.

Dietary interview

The process of the dietary interview is explained in Chapter 2. In addition, the Meat Study was explained in more detail, including how subjects would be selected and participants were asked about their willingness to eat a pasta meal with a tomato based sauce as part of the Meat Study. Subjects who met the dietary criteria for the Meat Study had their iron status measured at MedLab Central in Palmerston North.

Measurement of iron status

The methodology for determining subject's iron status is outlined in Chapter 2. Subjects were selected for the Meat Study based on their iron status and their estimated blood volume. Subjects who had a serum ferritin level above the 50th percentile (>30μg/L) for their age group (Gibson, 1990) were excluded from the study as these women had adequate iron stores and were therefore less likely to absorb dietary iron efficiently, while subjects with a serum ferritin level below the 10th percentile (<12μg/L) (Gibson, 1990) and a haemoglobin level of less than 120g/L were excluded from the study as these values indicated iron deficiency anaemia (Gibson, 2005b). Kristensen (2005) also used this criteria to select subjects for a study investigating pork's effect on non haem iron absorption. Other studies have used subjects with a range of serum ferrritn levels and

adjusted iron absorption results accordingly, based on a serum ferritin of 40 μg/L (Cook *et al.*, 1991a). Subjects with laboratory results outside the normal range (SF<12μg/L and Hb<120g/L or SF>300μg/L) were sent a copy of their results and it was recommended that they visit their general practitioner or contact the medical practitioner in the IFNHH at Massey University.

Blood volume was calculated using Allen's formula (Allen *et al.*, 1956) shown below.

Blood volume for women (in Litres)
$= (0.414 \times height\ (in\ metres)^3) + (0.0328 \times weight\ (in\ kilograms)) - 0.030$

Those with an estimated blood volume of greater than 4.3 Litres (L) were excluded from the study, as a higher blood volume may cause a dilutional effect, affecting the uptake of iron (and stable isotopes of iron) into the red blood cells.

Subjects with low iron stores (SF 12-30μg/L, Hb levels >110g/L) and a plasma volume of less than 4.3 L were invited to continue in the study. Thirty one women had a serum ferritin between 12 and 30μg/L and Hb levels >110g/L. One women volunteered to have her iron levels retested several months later, and met the criteria for participating in the study the second time around. Five women were excluded due to having a plasma volume greater than 4.3 L. Seven women withdrew from the study – five of these women moved away and 2 withdrew due to busy schedules. As the time between the initial recruitment and start of the Meat Study was over an 18 month period, those participants who were recruited initially and eligible for the study were asked to have a second blood test taken to check their serum ferritin levels. Two women were excluded as their serum ferritin had increased to levels greater than 30μg/L. Eighteen subjects took part in the Meat Study.

Medical interview

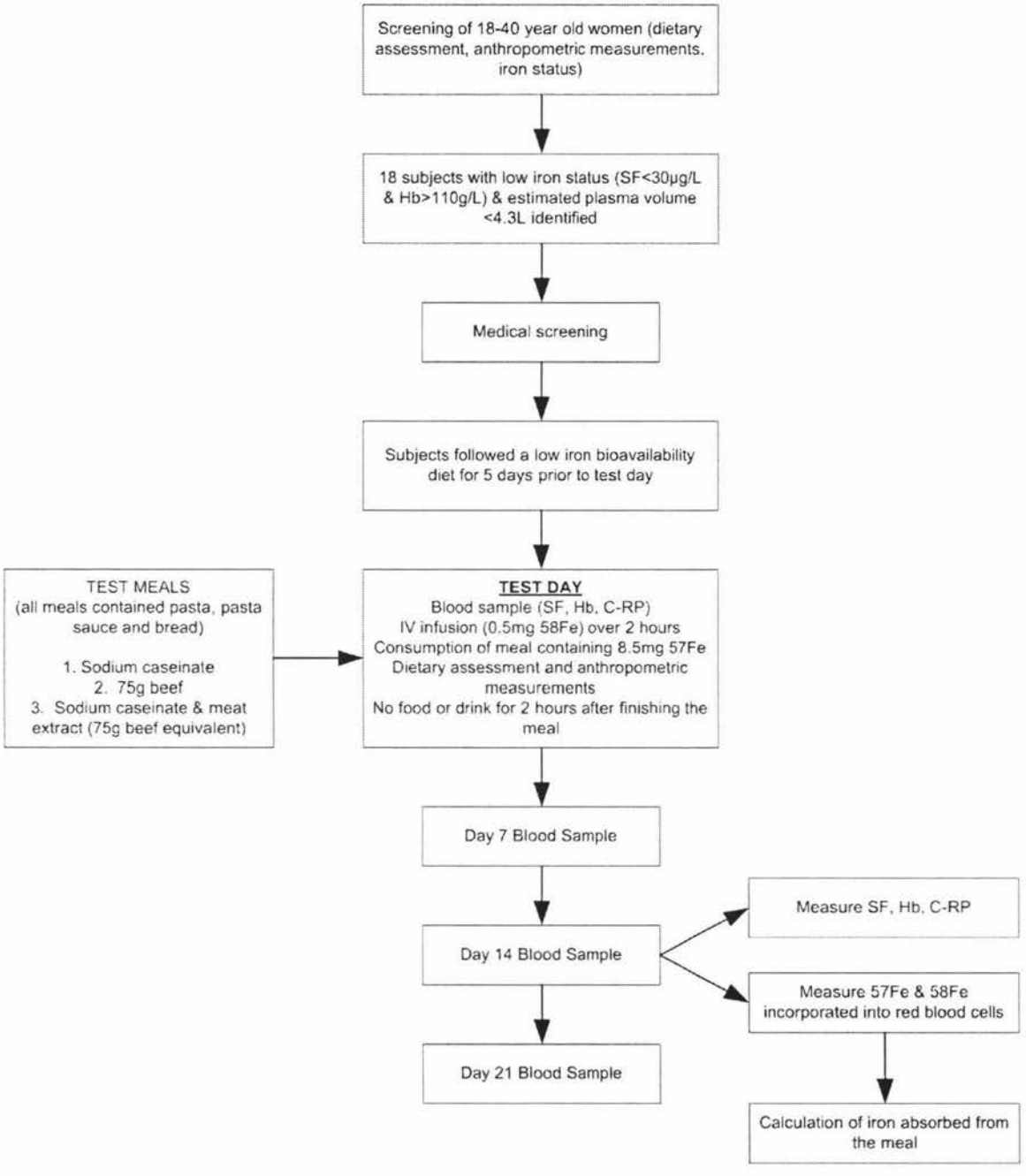
All suitable subjects for the study were interviewed by a medical practitioner, Dr Roger Lentle, who obtained a medical history from each subject and ensured that subjects were

medically suitable to continue with the study. Subjects were excluded from the study if they had any condition that may have affected their iron status including menstrual dysfunction and conditions affecting iron metabolism and red cell turnover, as these may affect the incorporation of iron into red blood cells. Subjects were excluded if they had a history of allergy or anaphylaxis due to previous reports on adverse reactions observed with the administration of intravenous iron (Fishbane & Kowalski, 2000). The risk of this occurring however is extremely unlikely due to the form and small doses of iron used (Woodhouse & Abrams, 2001). Other criteria for exclusion included conditions affecting blood coagulation due the number of blood samples that were to be taken or blood borne diseases including hepatitis to avoid any potential risks to the researchers. No subjects were excluded from the study on the basis of medical grounds.

3.2.2 Overview of the study

Subjects followed a low iron diet for the 5 days prior to the study and came into the Human Nutrition Unit at the IFNHH at 7.30am having fasted 12 hours prior. A cannula was inserted into the subjects arm, a blood sample was taken and each subject was given 0.5mg ^{58}Fe as an IV infusion over a 2 hour period. The cannula was removed and a further blood sample was taken. The subject then ate one of three meals – a meat meal, a sodium caseinate meal or a sodium caseinate meal containing the meat extract. 8.5mg ^{57}Fe was added to these meals. The subject waited for 2 hours in the Human Nutrition Unit following the consumption of the meal. During this time, the subject's weight, height and a 24 hour recall was taken. The subject then went back to their normal diets and returned 7, 14 and 21 days later for further blood samples. The blood samples taken at day 14 were analysed for the incorporation of ^{57}Fe and ^{58}Fe into the red blood cells.

Figure 3.1 Overview of the study protocol

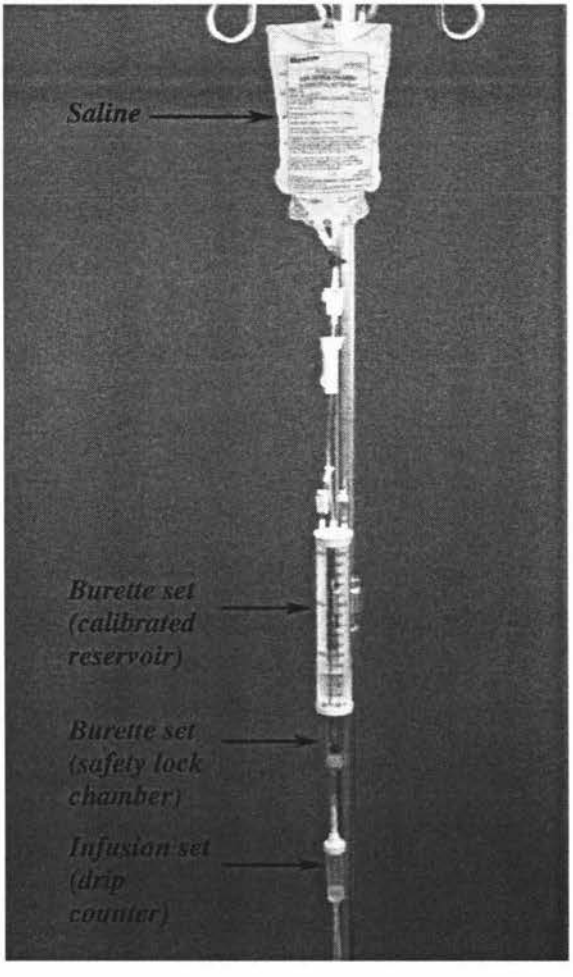


3.2.3 Calibration of equipment

The use of stable isotopes to measure non haem iron absorption has not been previously undertaken at Massey University. Investigations were made to see how many saline flushes would be necessary to remove the iron isotope (^{58}Fe) from the intravenous infusion set. Indigo carmine dye was used to represent the iron isotope.

A baseline sample of saline was collected by running the saline through the IV infusion set. The IV infusion set can be seen in Figure 3.2.

Figure 3.2 Intravenous infusion set up



0.2mg indigo carmine dye was mixed with 40mL water to produce a solution of blue coloured dye (5µg/1ml concentration). 45mL saline and 5mL of the indigo carmine dye solution were added to the calibrated reservoir. This 50mL solution was run through the IV infusion set and a sample was collected in a small plastic test tube. The first flush was given by adding 25mL saline to the calibrated reservoir. The 25mL flush was run through the IV infusion set and a sample was collected. This procedure was repeated five times. Samples were analysed using a U-2001 spectrophotometer (Alphatech Systems Ltd & Co, Parnell, Auckland) set at a wavelength of 608nm. The test sample and 1st flush sample were diluted with the addition of 2 parts saline using a pipette to 1 part test solution. The final readings were adjusted accordingly. All samples were measured in duplicate. The procedure was performed eleven times. Graphs were plotted and it was determined that 4 x 25mL saline flushes would be adequate to ensure all of the ^{58}Fe was removed from the IV infusion set.

Pipettes (LabServ, BioLab, Auckland, New Zealand) used for measuring ^{57}Fe (100-1000 µL) and the meat extract (100-1000 µL, 20-200 µL) were calibrated before use through the use of standard re-calibration techniques.

3.2.4 Instructions for subjects prior to taking part in the study

An appointment was made with subjects prior to the study to provide instructions on keeping their diet low in iron and foods that enhance iron absorption for the 5 days prior to the study. The use of the low iron diet was to avoid saturation of the mucosal cells with iron (Fairweather-Tait & Minski, 1986) prior to the study beginning. Final instructions regarding the study were also provided at this time. The appointment was made at a time suitable to both the researcher and the subject and was made at least two weeks prior to the study to ensure the subject had ample time to prepare themselves to undertake the low iron diet. Subjects were emailed prior to beginning the low iron diet and during the 5 day diet to provide encouragement and give subjects an opportunity to ask questions regarding the low iron diet or the study.

Dietary instructions were provided based on the individual's diet and through the use of general guidelines. The low iron diet was a vegetarian diet with a restriction on the intake of foods high in Vitamin C. Subjects were asked to restrict their intake of food that was fortified with iron and avoid the use of cast iron cookware. A comprehensive list of foods fortified with iron was developed based on food labels. Subjects were asked to avoid fruit juices and cordials and to limit their intake of fruit and vegetables to five servings per day. They were also asked to avoid eating fruits and vegetables deemed to have a high Vitamin C content (>15mg/100g). Where only small amounts of these vegetables were likely to be eaten (for example garlic) it was explained to subjects that this would be acceptable if eaten in small quantities. The diet aimed to meet the Food and Nutrition Guidelines for Healthy Adults (Ministry of Health, 2003) and meal ideas and recipes were provided to all subjects. A list of these dietary guidelines can be found in 5.5. All subjects kept a diet record for the 5 days prior to the study. Verbal and written instructions were provided on how to complete a food record (see Appendix 5.6). Food scales were provided to all participants to assist them in keeping their food record.

Subjects took part in the study (ate the test meal) within 1 week of their last menstrual period to minimise any loss of iron through menstruation and to ensure maximal iron absorption. Throughout the study period subjects were contacted weekly as a reminder to return for their follow up blood tests.

For the 12 hours prior to eating the test meal subjects were asked to avoid the consumption of food and beverages. They were permitted to drink water. Subjects arrived at the Institute of Food, Nutrition and Human Health at 7.30am. An explanation of the morning's procedures was given to all subjects prior to beginning the study, by a registered medical doctor who was responsible for taking the blood samples, inserting and removing the intravenous (IV) cannula, and administering the intravenous iron. This doctor was available throughout the IV infusion and for the 2 hours following the administration of IV iron. Comprehensive resuscitation equipment was available in the case of an emergency (Abrams, 2003).

3.2.5 Set up of the IV infusion set

Prior to the subject arriving on the morning of the study, the equipment for administrating the IV infusion was set up. The set up can be seen in Figure 3.2. A 500ml Viaflex bag of saline (at room temperature (25°C)) for intravenous infusion (0.9% Sodium Chloride), (Baxter Healthcare Pty Ltd, NSW, Australia) was hung on a drip stand. A latex-free add-on burette set (Alaris Medical Systems, CA, USA) was attached to the bag of saline using the burette spike. A Tutoplus infusion set without an air vent (Plasti Medical S.p.A., Villamarzana, Italy) was attached to the other end of the burette set. Saline was run through the IV infusion set by opening the roller clamps on the burette and drip set so that the calibrated reservoir had ~120ml saline in it, the safety lock chamber was full with saline and the drip counter was half full with saline with no bubbles throughout. A sample of saline was colleted in a 10mL sterile conical tube (Geiner Bio-One International, NC, USA) and frozen at -20°C for later analysis. The roller clamp on the burette and infusion set were closed and the end of the infusion set was then capped.

3.2.6 Procedure for the IV infusion of ^{58}Fe and blood sampling

A BD Insyte IV cannula (1.1 x 30mm or 1.7 x 30mm), (Becton Dickson Infusion Therapy, Utah, USA) was inserted into a vein in the subject's arm. A 25ml blood sample was taken using a Soft-Ject 20ml syringe (Henke Sass Wolf GMBH, Tuttlingen, Germany). This blood was immediately transferred into 4 tubes using a BD Precision Glide Needle (1.2mm x 38mm), (Becton Dickson, Singapore) attached to the end of the syringe. 15mL of blood was collected into two 7.5mL S-Monovette (19 I.U. Heparin/ml blood) tubes designed for trace metal analysis (Sarstedt, Numbrecht, Germany). These tubes were stored in two separate freezers at -20°C for baseline measures of the ^{57}Fe and ^{58}Fe in red blood cells. These were not analysed as it was assumed that the ^{57}Fe and ^{58}Fe in red blood cells would not differ between subjects at baseline. 4.5mL blood was collected in a K3E (15% 0.054ml) vacutainer (BD Vacutainer Systems Preanalytical Solutions, Plymouth, UK) and 5mL of blood into a Lithium Heparin (72 U.S.P units) vacutainer (BD Vacutainer, Preanalytical Solutions, NJ, USA). These tubes were stored at 4°C and sent to MedLab

Central in Palmerston North during the morning for analysis of serum ferritin, haemoglobin and C - reactive protein. A description of the assays used is described in Chapter 2.

Following the collection of the first blood sample, the end of the drip set was attached to the cannula and saline was run through by opening the roller clamp on the drip set. Once the saline was running freely, the drip set was taped to the subject's arm using a Tegaderm IV Transparent Dressing (3M Health Care, Neuss, Germany) and surgical tape (3M Healthcare, MN, USA).

At this stage, the saline was running freely into the subject. 0.5mg iron citrate (^{58}Fe) was drawn up into a 10mL syringe (BD Medical, Singapore) using a 1.2mm x 38mm Precision Glide Needle (BD, Singapore) from sterilised ampoules. For the first 12 subjects, 9mL of ^{58}Fe (54.4μg/mL) was added to the calibrated reservoir when the saline reached the 91mL mark. For the final 6 subjects, 6.3mL of ^{58}Fe (79μg/mL) was added to the calibrated reservoir when the saline reached the 94mL mark. When adding the ^{58}Fe the outer part of the calibrated reservoir was swabbed using an alcohol swab, and the iron solution was then syringed into the calibrated reservoir. The calibrated reservoir was 'swirled' to mix the saline and ^{58}Fe solution evenly. A drip rate of 18 drips was established so that the solution could be infused slowly over 120 minutes (similar to the estimated rate of iron absorption from the gastrointestinal tract of 2-5μg/minute and at a rate which avoids iron coagulating when introduced to the plasma) (Fairweather-Tait & Dainty, 2002). The infusion set was monitored continuously to ensure the speed of 18 drips per minute was maintained. The subject sat in a comfortable chair and was able to watch television, read or work over this 2 hour period.

When 20mL of solution was left remaining in the calibrated reservoir (80mL of solution infused), the drip counter was squeezed so that both the drip counter and safety lock chamber were full. The infusion continued at the same rate until the infusion ran to the bottom of the safety lock chamber. The entire IV infusion set was then flushed with 25mL saline to ensure all the iron solution was removed from the drip set. The procedure for these flushes involved opening the burette roller clamp and filling the calibrated reservoir

to 25mL. The burette roller clamp was closed and the calibrated reservoir oscillated to collect any iron solution. The safety lock chamber was squeezed to move the ball to the top, and then oscillated, to again collect extra iron solution. The flush was run to the bottom of the safety lock chamber by opening the drip set roller clamp. This procedure was repeated three times. Following the administration of the flushes the cannula was removed, with care taken to avoid the back flushing of blood.

Following the removal of the cannula, a 5mL sample of blood was taken by venipuncture in the anticubital vein of the opposite arm to which the cannula had been inserted using a Precision Glide Needle (0.9mm x 25mm), (BD, Singapore) and a 5mL Soft-Ject syringe (Henke Sass Wolf GMBH, Tuttlingen, Germany). The blood was transferred into a 7.5mL S-Monovette (19 I.U. Heparin/ml blood) tube designed for trace metal analysis (Sarstedt, Numbrecht, Germany). This tube was centrifuged in a Heraeus megafuge 1.0R for 15 minutes at 1600 Relative Centrifugal Force (RCF) at 4°C. 3mL of plasma was removed with a transfer pipette to a 4mL Nunc tube (InVitro Technologies, Auckland NZ) and stored in the freezer at -20°C. The intention was to determine the concentration of ^{58}Fe in the plasma following the infusion and thus calculate actual blood volume. However, these measurements were not made as the cost of analysis was prohibitive. Estimated blood volumes were used instead (Allen *et al.*, 1956).

3.2.7 Serving of the meal

The subject then ate one of the three test meals. These test meals are described in detail below. The first 12 subjects were randomly allocated to one of the three meals – a meat meal, a sodium caseinate meal or a sodium caseinate and meat extract meal (which will be referred to as the meat extract meal). The final 6 subjects were allocated to a meal based on their serum ferritin levels to ensure the groups were as evenly matched as possible.

The meals were stored at -20°C prior to serving. They were thawed in a refrigerator for 12-15 hours the day before consumption and reheated individually in a microwave oven on high for 2 minutes. The entire meal was transferred to a serving bowl. 8.5mg (5mL) of

^{57}Fe as iron citrate solution (1.7mg/ml) was added to each meal prior to serving using a calibrated 100-1000µl pipette. The meals were served with 1 slice of white sandwich bread and 180ml de-ionised water. The subjects were asked to eat the entire meal within a 30 minute time period. Subjects used the bread to wipe up any sauce left and at the end of the meal subjects washed the serving bowl with the deionised water and consumed this water to ensure all of the meal and ^{57}Fe was consumed. The meal was served and eaten using plastic cutlery and china bowls to ensure there was no contamination with iron from stainless steel utensils.

3.2.8 Other measurements taken

The subject remained in the lab for 2 hours after eating the meal. No food or drink (other than deionised water) was permitted during this time. During this time a 24 hour recall was taken to help measure compliance to the low iron diet. The booklet "Food Portion Sizes: A Photographic Atlas" (Nelson *et al.*, 2002) was used to help estimate portion sizes. The food diary was checked to ensure it contained the necessary detail. If not, the subject was asked to clarify the information provided. Dietary intakes from the 5 day food record and 24 hour recall were analysed using FoodWorks Professional Edition Version 4.00 (Xyris Software, 2005). The dietary data from the 5th day of the food record was compared with that from the 24 hour recall as a check on the accuracy of the food record. Height and weight were measured using the procedures described in Chapter 2.

The drip set was disconnected and recapped. Following the subject's departure, the infusion set was cut immediately above the calibrated reservoir and a small piece of Parafilm "M" (American National Can, IL, USA) was placed across the top to cover it. This was placed in a plastic bag and weighed on calibrated Denver Instrument Scales to 3 decimal places. A sample was taken from the bottom of the infusion set and collected in a 10mL sterile conical tube (Geiner Bio-One International, NC, USA). This was frozen at -20°C for later analysis to ensure that all of the ^{58}Fe had been infused.

3.2.9 Blood samples - days 7, 14 and 21

Subjects returned 7, 14 and 21 days following the meal to have further blood samples taken to measure the levels of ^{57}Fe and ^{58}Fe incorporated into red blood cells.

On days 7 and 21, a 15mL blood sample was taken by venipuncture in the anticubital vein of the arm using a Precision Glide needle (0.9mm x 25mm), (BD, Singapore) and a 20mL Soft-Ject syringe (Henke Sass Wolf GMBH, Tuttlingen, Germany). This blood was transferred into two 7.5mL S-Monovette (19 I.U. Heparin / ml blood) tubes designed for trace metal analysis (Sarstedt, Numbrecht, Germany) using a BD Precision Glide Needle (1.2mm x 38mm), (Becton Dickson, Singapore) attached to the end of the syringe. These tubes were stored in two separate freezers at -20°C. Analysis has not yet been undertaken on the levels of ^{57}Fe and ^{58}Fe incorporated into red blood cells from these samples. The 7 day sample was taken in case it was not possible to obtain results from the 14 day sample (van den Heuvel *et al.*, 1998a). It is intended that this will be measured at a later date. The 21 day sample was taken as a back up sample in the case of the 14 day sample being lost.

On day 14, 25mL blood was collected, with 15mL blood being transferred to two S-Monovette tubes using the same procedure as described above. One of these tubes was sent for analysis of the levels of ^{57}Fe and ^{58}Fe incorporated into red blood cells. In addition, 4.5mL blood was transferred to a K3E (15% 0.054ml) vacutainer (BD Vacutainer Systems Preanalytical Solutions, Plymouth, UK) and 5mL of blood was transferred into a Lithium Heparin (72 U.S.P units) vacutainer (BD Vacutainer, Preanalytical Solutions, NJ, USA). These tubes were stored in a refrigerator at 4°C and then sent to MedLab Central in Palmerston North for analysis of serum ferritin, haemoglobin and C-reactive protein (C-RP).

3.2.10 Subject's iron status

All subjects received a copy of their laboratory results (SF, Hb and C-RP taken at baseline and day 14) on the final day of the study. Subjects who had a serum ferritin of <20μg/L

and/or a haemoglobin of <120g/L were asked to speak to our medical doctor regarding their results who explained their results and referred them to their own General Practitioner where necessary. Subjects were also invited to receive dietary advice on increasing their intake and bioavailability of dietary iron by a New Zealand Registered Dietitian.

3.2.11 Preparation of stable isotope labels

Elemental stable isotopes (96.44 atom %, Certificate No. 20051886, Impurities (ppm): Al<1, Cu<100, Si<1, C<100, Zn<100, Ni<1, Mg<1) were obtained from Chemgas, France. The initial preparation of both batches of ^{58}Fe was completed in the Institute of Food, Nutrition and Human Health (IFNHH) by a trained chemist.

Stable isotopes were ordered in two batches. The first batch of isotopes came in a powder form (as iron oxide) and contained less iron (7mg) than had been ordered (17.22mg). However, this was not possible to determine until the end stage of processing and packaging into sterile vials. The initial weight was measured as iron oxide rather than pure iron. The second batch of iron was ordered as a foil. This iron had a smaller surface area than the iron as a powder so more hydrochloric acid was added to dissolve the iron and convert the isotope into a soluble form. The second batch of iron was further diluted so that the resulting ferric chloride had the same concentration as Batch 1. All acids used were of ultra high purity to avoid any trace contamination. Details of the preparation methods for the two batches of ^{58}Fe can be seen below.

Preparation of ^{58}Fe-citrate solution, IFNHH – Batch 1

17.22mg of elemental iron stable isotope was weighed on a 5-figure balance into an acid-washed Erlenmenger flask. Iron powder was dissolved in 17.5mL of 2.29M HCl with continuous stirring and gentle heating. When dissolved the solution was evaporated to dryness.

Preparation of ^{58}Fe-citrate solution, IFNHH – Batch 2

20.3mg of elemental iron stable isotope was weighed on a 5-figure balance into an acid-washed Erlenmenger flask. The iron foil was dissolved in 0.5mL of 10M HCl, and then diluted with a further 5.0mL of 1M HCl with continuous stirring and gentle heating to reach the desired concentration. When dissolved the solution was evaporated to dryness.

The dry ferric chloride (48.79mg from batch 1 and 57.52mg from batch 2) was delivered to the Ipswich Hospital NHS Trust Pharmacy in the United Kingdom where it was prepared as ferric citrate in ampoules for intravenous injection.

Preparation of ^{58}Fe-citrate solution, Ipswich Hospital NHS Trust Pharmacy – Batches 1 and 2

Sodium-tri-citrate was dissolved in ultra pure water and added to the iron chloride at a molar ratio of 25:1. The pH was checked and adjusted to 7.0 with sodium carbonate or HCl as necessary and the near volume was made up with ultra pure water. The pH was checked again and the final adjustment was made with ultra pure water. The iron concentration of the citrate solution was 54.4µg/mL (Batch 1) and 79µg/mL (Batch 2). The precise concentration of ^{58}Fe in solution was determined by thermal ionisation mass spectrometry. For each batch 2.5mL (+0.2mL for overage) of ^{58}Fe labeled citrate solution was sealed in ampoules, heat sterilised and sterility and end product tested.

Preparation of ^{57}Fe-citrate solution - IFNHH

The preparation of both batches of ^{57}Fe was completed in the IFNHH by a trained chemist. The solution was prepared in the IFNHH at Massey University using 246mg of elemental iron stable isotope (atom %, Chemgas, France, Certificate No., Impurities (ppm). Elemental stable isotopes (96.44 atom %, Certificate No. 20051886, Impurities (ppm): Al<1, Cu<100, Si<1, C<100, Zn<100, Ni<1, Mg<1) were obtained from Chemgas, France. As the ^{57}Fe citrate solution was to be added to the meals, the final solution was tasted by

the researchers to ensure its taste would not affect the consumption of the meals. The taste of the ^{57}Fe citrate solution was salty, but the salty taste was not detectable once added to the meal.

The first batch of ^{58}Fe was used for the first 12 subjects and the second batch was used on the final 6 subjects. Each subject received 0.5mg ^{58}Fe enriched iron as ferric citrate solution by intravenous infusion and 8.5mg ^{57}Fe as ferric citrate solution added to their meal. The same batch of ^{57}Fe was used for all subjects.

3.2.12 Test meals

Designing the meals

Three different types of test meals were prepared. All meals consisted of an equivalent amount of pasta, bread, and a tomato based pasta sauce. The sauce of the first meal contained lean beef (minced), and that of the second and third meals contained sodium caseinate (Tatua 100, Tatua Nutritionals, Morrinsville, New Zealand). In addition to this, the third meal contained a meat extract (<0.5kDa sarcoplasmic fraction). The meat and sodium caseinate were provided in protein equivalent quantities. The meals were designed to have a similar macro and micronutrient composition, with factors that enhanced and inhibited iron absorption kept to a minimum (for example, vitamin C and calcium). Meals were designed using the New Zealand Food Composition Tables (Athar *et al.*, 2003).

Various meals were made up using different brands of pasta, pasta sauce and casein powders to obtain a suitable meal in terms of taste, appearance and consistency. Pasta sauce was chosen as the vehicle to administer the meat extract due to its liquid consistency (similar to that of the meat extract) and taste (a savoury taste not unlike that of the meat extract). The sodium caseinate was chosen as it mixed well with the pasta sauce, had a similar consistency to mince when mixed with the pasta sauce and its taste was acceptable. Different quantities of pasta, pasta sauce, meat and sodium caseinate were trialed for acceptability. A tasting panel provided feed back throughout the process, as well as once

the final meal composition was decided upon. All subjects were invited to taste both the meat meal and the sodium caseinate meal to ensure that they would be able to eat the entire meal during the study. The majority of subjects took part and all subjects were able to consume the entire meal on the day of the study.

The final meals consisted of 120g cooked pasta (Zafarelli 100% durum wheat), 150g pasta sauce (Bertolli Five Brothers "Summer Tomato Basil" (tomatoes (95%), sugar, onions, salt, olive oil, parsley, basil, garlic, spices), a slice of white sandwich Sunny Crust bread and 180mL of deionised water. In addition, protein equivalent quantities of meat and sodium caseinate were added to the meals. The iron content of the selected pasta sauce was checked by a technician in an atomic absorption (AA) analyser. The iron content was 0.859mg/100g, less than that determined by the Food Composition Tables (1mg/100g). The estimated composition of these meals can be seen in Table 3.2.

Table 3.1 Final composition of the test meals

Meal 1	***Meal 2***	***Meal 3***
16.98g sodium caseinate (Tatua Nutritionals)	16.98g sodium caseinate + meat extract (3.073g)	75g lean beef
All meals contained 120g cooked pasta, 150g pasta sauce, 1 slice of white sandwich bread, 180mL deionised water.		

Table 3.2 Estimated composition of test meals by as determined by New Zealand Food Composition Tables (Athar et al., 2003)

Nutrient	*Pasta, bread, sauce and meat*	*Pasta, bread, sauce and sodium caseinate*
Weight (g)	371	313
Energy (kJ)	1468.4	1407.6
Protein (g)	26.8	26.8
Fat (g)	3.45	1.99
Carbohydrate (g)	54.1	54.1
Water (g)	270.54	215.17
Total iron (mg)	3.82	2.13
Calcium (mg)	55.76	58.98
Phosphorus (mg)	320.35	288.85
Vitamin C (mg)	1.50	0.17
Fibre (g)	4.33	4.33
Zinc(mg)	4	2.05
Magnesium (mg)	54.97	39.49

Preparation of meals

All meals were prepared in the food technology labs at Massey University. Plastic utensils and cooking equipment were used where possible to avoid iron contamination through cooking equipment that might contain iron (Brittin & Nossaman, 1986; Park & Brittin, 1997). 1500g dry pasta was cooked in a large pot in de-ionised water and was drained using a plastic sieve and deionised water. 120g servings of cooked pasta were weighed and served into plastic containers.

Sodium caseinate meal

Sodium caseinate (Tatua 100) was provided free of charge by Tatua Nutritionals (Tatua Co-operative Dairy Company Ltd). A copy of the product analysis certificate can be seen in Appendix 5.7. 2,400g pasta sauce and 271.68g sodium caseinate were mixed together in a large plastic bowl using plastic spoons. From this, 166.98g of this mixture was placed into each container of pasta, providing 150g pasta sauce and 16.98g sodium caseinate per meal. These meals were frozen at -20°C.

Meat meal

75g of beef was chosen as the amount of beef to be included in the meat meal based on scientific literature (Cook & Monsen, 1975; Layrisse *et al.*, 1984; Baech *et al.*, 2003a) and following recommendations from the tasting panel. Lean topside beef was purchased from Preston AE & Co Ltd in Palmerston North. It was minced before the food preparation began. 637.5g (75g x 8.5 serves) raw beef was cooked in a large non stick electric frying pan. Following cooking, the beef weighed 518.76g, meaning 75g raw meat was reduced to 61.03g cooked beef. The cooked beef was mixed with 1275g (150g x 8.5serves) pasta sauce. From this, 8 x 211.03g servings of beef and pasta sauce (61.03g beef + 150g sauce) were measured and added to the pasta. These meals were frozen at -20°C.

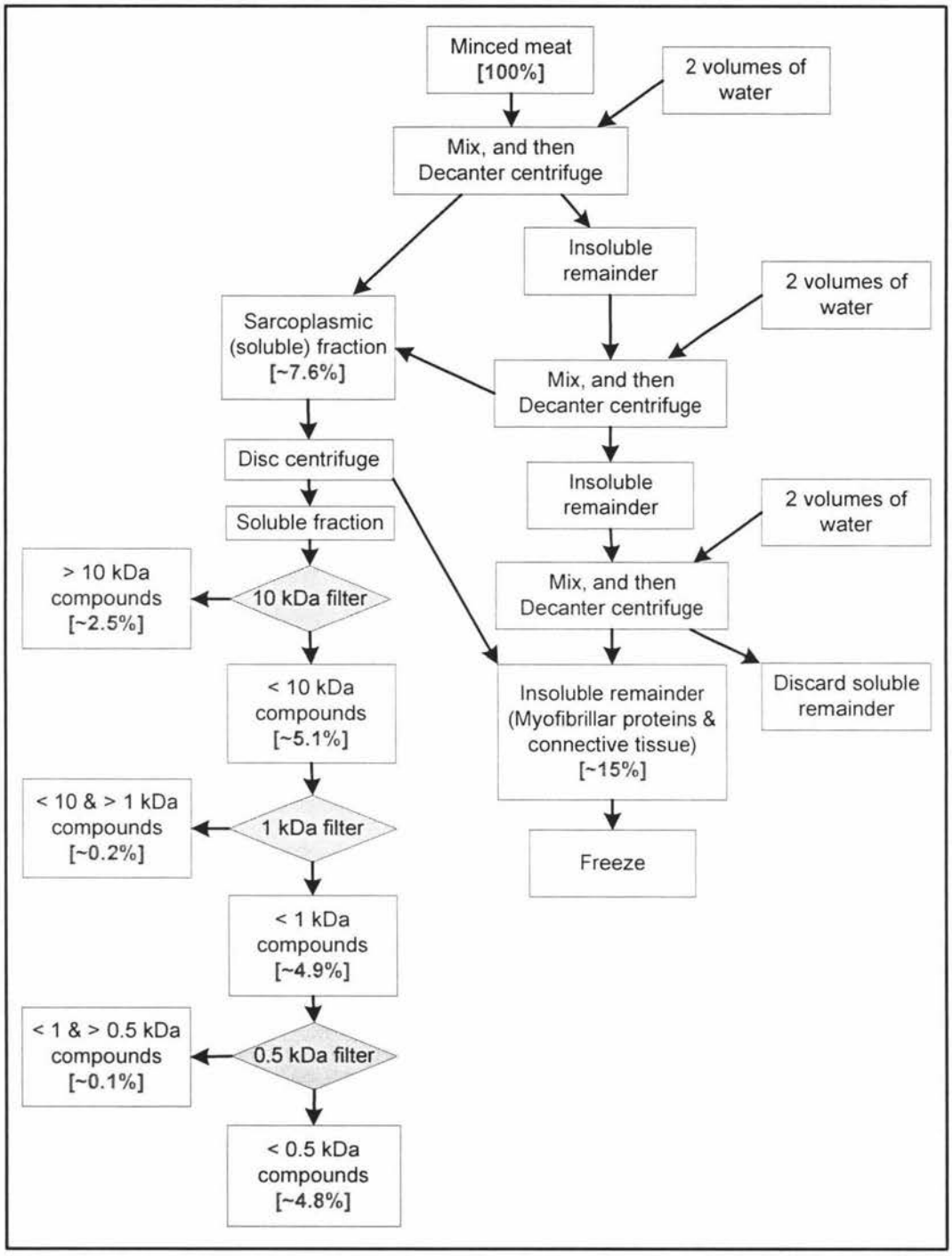
Meat extract

Studies have been ongoing in the IFNHH at Massey University to determine the factor in meat that enhances the absorption of non haem iron. Several meat extracts have been prepared and evaluated by testing the extent to which preparations bind iron (Wilkinson *et al.*, 2006), the uptake of iron into mouse duodenal mucosal cells using Ussing Chambers (Reynolds *et al.*, 2006a), the uptake of iron and its incorporation into red blood cells over a 4-week period in semi-anaemic piglets (Morel & Purchas, 2006), and over 1-2 days in iron depleted mice (Reynolds *et al.*, 2006b) and the extent to which preparations enhance the uptake of iron into and across Caco-2 cells (Wolber *et al.*, 2006). Studies undertaken in

Caco-2 cells have demonstrated that the meat extract containing peptides and carbohydrates <0.5kDa sarcoplasmic fraction induced significant up regulation of both iron uptake and iron transport (Wolber *et al.*, 2006). It was therefore decided that this extract should be tested on iron absorption in humans.

The meat extract (<0.5kDa sarcoplasmic fraction) used in this study was prepared by Brian Wilkinson. The process of preparing this meat extract is seen in Figure 3.3 and has been described previously (Wilkinson *et al.*, 2006).

Figure 3.3 Flowchart showing the production of various meat fractions (Wilkinson et al., 2006)



The meat extract was tasted by the researchers to ensure its acceptability before giving it to the subjects. 3.073g of meat extract (the amount of meat extract found in 75g raw beef) was added to the meat extract meal. The meat extract was provided in a solution of 49.254%. 6.236g (3.073g meat extract) of the solution was pipetted using calibrated pipettors into 10mL sterile conical tubes (Geiner Bio-One International, NC, USA) and frozen at -20°C. On the day prior to giving subjects the test meal containing the meat extract, the tube containing the meat extract was removed from the freezer and placed in a beaker of boiling water for 5 minutes, then placed in the refrigerator overnight. The meat extract was added to the sodium caseinate meal before the meal was microwaved.

Analysis of the meals

The meat and sodium caseinate meals were homogenised and analysed in duplicate at the Nutrition Laboratory (Massey University, Palmerston North). Analyses of minerals and β-Carotene were sub contracted to other laboratories. The meat extract meal (Meal 2) was not analysed as its composition was assumed to be identical to that of the sodium caseinate meal (Meal 1). The meat extract provided in solution was analysed separately for iron content at Hill Laboratories, Hamilton, New Zealand and found to contain 3.2mg iron/kg. This equates to 0.02mg iron in the meat extract.

Gross energy was measured using bomb calorimetry, protein using the Leco total combustion method (AOAC 968.06) (AOAC, 2005), fat using Soxtec extraction (AOAC 920.39) (AOAC, 2005) and carbohydrate was calculated by difference. Dietary fibre was measured using the Enzymatic-gravimetric method (AOAC 991.43) (AOAC, 2005). Moisture was measured by drying the sample for 16 hours in a convection oven at 105°C, and ash was determined after the sample was furnaced in a muffle furnace for 16 hours at 550°C. Copper, zinc and magnesium were determined by a wet oxidation and the remaining minerals (iron, calcium, sodium, phosphorus and phosphate) were digested with various acids and measured using Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES). Vitamin A was measured as retinol using high pressure liquid chromatography (HPLC), Vitamin C using liquid chromatography (Lee & Coates, 1987),

and β-Carotene was measured using reversed-phase chromatography analysis (Zakaria *et al.*, 1979; Nells & De Leenheer, 1983). Non haem iron was measured using a spectrophotometer using a method developed by the Nutrition Lab for measuring the non haem iron content of meat.

Table 3.3 Estimated and measured compositions of the test meals

	Pasta, bread, sauce and meat		***Pasta, bread, sauce and sodium caseinate***	
	Measured	**Estimated**	**Measured**	**Estimated**
Weight (g)	357	371	312.98	312.98
% Moisture	75.65	72.92	72.31	68.75
% Ash	1.37	3.2	1.40	3.4
% Protein	7.64	7.21	8.19	8.55
kJ/g GE	4.69	3.96	5.28	4.50
% Carbohydrate	12.67	14.57	15.40	17.28
% Fat	1.22	0.93	0.91	0.64
% TDF	1.44	1.17	1.80	1.38
Total % DM	24.35	27.08	27.69	31.25
Iron (mg/kg)	11	10.30	7	6.81
Calcium (mg/kg)	283	150.30	379	188.45
Phosphorus (mg/kg)	893	863.48	901	922.9
Phosphate (mg/kg)	2.8	N/A	2.8	N/A
Sodium (mg/kg)	2419	2467.87	3204	3475.49
Copper (mg/kg)	1.1	1.64	1.0	N/A
Zinc (mg/kg)	11	10.78	6	6.55
Magnesium (mg/kg)	232	148.17	210	126.17
Vitamin A (IU/100g)	0.79	1.35	0.84	N/A
Vitamin C (µg/100g)	0.51	0.40	0.06	0.05
β-Carotene (mg/100g)	0.10	0.11	0.24	N/A
Non haem iron (mg/kg)	8.865	N/A	6.897	6.8

Table 3.4 Final composition of meals based (based on meal size)

	Pasta, bread, sauce and meat	*Pasta, bread, sauce and sodium caseinate*	*Pasta, bread, sauce, sodium caseinate and meat extract*
Weight (g)	357	312.98	319.22
Water (g)	270.07	226.32	232.56
Energy (kJ)	1375.12	1340.41	1340.41
Carbohydrate (g)	45.23	48.2	48.2
Protein (g)	27.27	25.63	25.63
Fat (g)	4.36	2.85	2.85
Iron (mg)	3.93	2.19	2.21
Calcium (mg)	101.04	118.62	118.62
Phosphorus (mg)	318.81	282.00	282.00
Phosphate (mg)	1.00	0.88	0.88
Sodium (mg)	863.62	1002.82	1002.82
Copper (mg)	0.39	0.31	0.31
Zinc (mg)	3.93	1.88	1.88
Magnesium (mg)	82.83	65.73	65.73
Vitamin A (IU)	2.82	2.63	2.63
Vitamin C (μg)	1.82	0.19	0.19
β-Carotene (mg)	0.36	0.75	0.75
Non haem iron (mg)	3.16	2.16	2.18
Haem iron (mg)	0.77	0.03	0.03

3.2.13 Analysis of iron in saline from the infusion set

Four random samples of saline collected from four subjects following the infusion of ^{58}Fe (2 from batch 1 and 2 from batch 2) were analysed for their iron content by Hill Laboratories (Hamilton, New Zealand). A sample of saline taken prior to the addition of any iron (saline only) was also analysed for iron content. These analyses were undertaken to ensure that all of the ^{58}Fe had been infused. All of the saline samples contained <0.10mg/kg of iron demonstrating that all of the ^{58}Fe had been infused.

3.2.14 Analysis of isotopic composition of blood sample

Iron isotope enrichment ratios were measured in the blood taken on Day 14 by Steven Abram's group (U.S. Department of Agriculture / Agricultural Research Service Children's Nutrition Research Center, Department of Paediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas) using high resolution inductively coupled plasma mass spectrometry (ICP-MS) (Chen *et al.*, 2005). The results were checked by the same group using thermal ionisation mass spectrometry (TIMS). Iron absorption was calculated from the isotopic enrichment of the red blood cells, the total iron concentration in the blood and an estimate of blood volume (Allen *et al.*, 1956). The absorption of ^{57}Fe from the meal was calculated by dividing the incorporation of ^{57}Fe in the red blood cells by the incorporation of ^{58}Fe in the red blood cells and multiplying by 100.

3.2.15 Correction for iron status

Iron absorption (^{57}Fe) was expressed in percentage form as unadjusted absorption and iron absorption adjusted to 40μg/L. This was done to control for the effects of iron status and to compare the results with other data.

Dietary absorption was corrected to 40μg/L using the following equation (Cook *et al.*, 1991a):

$$Log\ A_c = Log\ A_o + LogF_o - Log\ 40$$

Where A_c was corrected dietary absorption, A_o was observed absorption and F_o was the average serum ferritin from baseline and week 2.

Iron absorption was converted to mg of ^{57}Fe absorbed after adjusting for the amount of ^{57}Fe and non haem iron contained in each meal and ^{57}Fe added to the meal.

3.2.16 Statistical analysis

Statistical analysis was performed by the researcher using SPSS for Windows, release 14.0 (SPSS Inc, 2005). Normality of data was tested using Kolmogorov-Smirnov's test. One way-independent analysis of variance (ANOVA) was used to compare the means between groups for normally distributed data and the Kruskal-Wallis test was used to compare the means between groups for data that was not normally distributed. Homogenity of variance was tested using Levene's test. Where homogeneity of variance was not met, the Welch *F* ratio was used

Analysis of covariance (ANCOVA) was used to investigate the effect of batch and serum ferritin at baseline (covariants) on the absorption of ^{57}Fe from the three diet groups.

Evidence for a difference between dietary intakes before (using the 24 hour recall at screening) and after dietary education to follow a diet of low iron bioavailability (as measured by the food record) were tested using dependent t-tests for continuous variables that were normally distributed and the Wilcoxin signed rank test for data that was not normally distributed.

Dietary intakes of macro and micronutrients from the 5th and final day of the food record were compared with that from the 24 hour recall using Pearson's correlation coefficient (Vitamin C and fat intake) or Spearman's correlation coefficient (iron, energy, protein, carbohydrate, fat, total vitamin A and calcium intake) after testing for normality.

3.3 Results

Eighteen healthy women took part in the Meat Study. All women had a serum ferritin between 12 and 30μg/L and a Hb level >110g/L at the time of screening. All had an estimated blood volume of less than 4.3L. All subjects were non smokers and were not pregnant or lactating, and none of the subjects took any vitamin or mineral supplements in the 1 month prior to and during the study. Blood donation was not permitted during the study or for the 3 months prior to taking part in the study. There were no significant differences between groups in terms of subject's age, weight, height, serum ferritin, haemoglobin, estimated blood volume and estimated iron in blood at baseline (Table 3.5).

Table 3.5 Averages across groups at baseline

	Sodium caseinate (n=6)	***Meat extract (n=6)***	***Meat (n=6)***	***p value for difference***
Age* (years)	24.8 (4.9)	31.8 (8.8)	27.0 (9.0)	0.26
Weight (kg)	58.8 (6.1)	57.1 (7.1)	62.3 (11.3)	0.56
Height (cm)	165.6 (5.6)	163.1 (5.6)	167.1 (7.2)	0.55
Serum ferritin at baseline (μg/L)	24.3 (14.1)	22.0 (10.9)	19.5 (8.6)	0.94
Haemoglobin (g/L)	129.3 (8.0)	128.7 (8.9)	130.2 (20.0)	0.98
Blood volume (L)	3.8 (0.3)	3.6 (0.4)	3.9 (0.4)	0.45
Iron in blood (mg)*	1639.3 (179.6)	1562.0 (134.3)	1689.8 (223.5)	0.78

Reported as mean (standard deviation)

*Kruskal-Wallis test

3.3.1 Effect of the meat extract on iron absorption

Subject data and iron absorption data from week 2 for each group is displayed in tables 3.6, 3.7 and 3.8. For subject DD the serum ferritin at baseline (66μg/L) was replaced with the serum ferritin value taken at week 2 (33μg/L) due to an elevated C-RP and subject DD a having a cold at baseline.

Table 3.6 Subject data for the Sodium Caseinate meal

Subject	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*SF (µg/L) at baseline*	*SF (µg/L) at Week 2*	*Hb (g/L) base - line*	*Hb (g/L) at Week 2*	*Estd BV (L)*	*Estd iron in blood (mg)*	*Energy intake in 5 days prior (kJ)*	*Iron intake in 5 days prior (mg)*	*Vit C intake in 5 days prior (mg)*	*In ^{58}Fe (%)*	*In ^{57}Fe (%)*	*Abs ^{57}Fe (%)*	*Abs ^{57}Fe (%) adjusted to SF 40µg/L*	*mg ^{57}Fe absorbed from the meal[1]*
AN	31	58	163.5	20	20	129	124	3.68	1588.3	7878.1	15.6	43.3	120.66	7.69	6.37	3.18	0.53
BN	21	48	167	19	9	135	114	3.47	1567.7	5137.8	15.8	36.7	134.14	6.30	4.70	1.64	0.39
BQ	20	63	162	34	24	117	118	3.80	1485.4	7287.7	9.2	48.3	113.35	6.77	5.98	4.33	0.49
CY	23	66	167	48	30	138	129	4.06	1875.0	7871.1	14.0	32.5	114.99	3.43	2.98	2.91	0.25
AK	31	59	159	10	17	123	128	3.57	1468.1	4663.0	12.1	35.5	123.95	24.89	20.08	6.77	1.66
DN	23	59	175.2	15	10	134	127	4.13	1851.4	7824.1	8.5	30.4	153.83	20.12	13.08	4.09	1.08
Mean	**24.8**	**58.8**	**165.6**	**24.3**	**18.6**	**129.3**	**123.3**	**3.79**	**1639.3**	**6777.0**	**13.6**	**40.2**	**126.82**	**11.53**	**8.86**	**3.82**	**0.73**
SD	4.9	6.1	5.6	14.1	9.0	8.0	6.1	0.27	179.6	1478.0	3.2	6.8	15.17	8.75	6.48	1.73	0.53

[1]Adjusted for the ^{57}Fe in the meal (2.15%), added to the meal (96.44%), and the non haem iron content of the meal (2.16mg)
Estd = Estimated
Inc = Incorporation
Abs = Absorption
BV = Blood volume

Table 3.7 Subject data for the Meat Extract meal

Subject	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*SF (µg/L) at baseline*	*SF (µg/L) at Week 2*	*Hb (g/L) at base - line*	*Hb (g/L) at Week 2*	*Estd BV (L)*	*Estd iron in blood (mg)*	*Energy intake in 5 days prior (kJ)*	*Iron intake in 5 days prior (mg)*	*Vit C intake in 5 days prior (mg)*	*In ^{58}Fe (%)*	*In ^{57}Fe (%)*	*Abs ^{57}Fe (%)*	*Abs ^{57}Fe (%) adjusted to SF 40µg/L*	*mg ^{57}Fe absorbed from the meal[1]*
CT	27	46	153	11	12	133	129	2.96	1317.2	9257.1	16.8	22.6	122.48	22.58	18.44	5.31	1.52
OB	21	63.5	163.5	28	26	124	129	3.86	1601.5	9653.4	7.9	53.5	91.89	3.29	3.59	2.42	0.30
CA	40	52	162.5	28	29	144	146	3.45	1662.3	8437.1	8.8	31.7	128.46	10.74	8.36	5.96	0.69
AX	39	58	164.5	8	6	121	116	3.72	1503.3	9709.9	13.0	33.0	115.21	23.79	20.65	3.61	1.70
DC	40	65	170	21	23	121	133	4.14	1673.5	10919.7	17.0	59.2	98.31	14.34	14.58	8.01	1.20
CC	24	58	165.3	36	24	129	127	3.74	1614.3	5260.3	4.9	13.5	93.31	6.81	7.29	5.47	0.60
Mean	**31.8**	**57.1**	**163.1**	**22**	**20**	**128.7**	**130**	**3.64**	**1562.0**	**8872.9**	**11.4**	**35.6**	**108.27**	**13.59**	**12.15**	**5.13**	**1.00**
SD	8.8	7.1	5.6	10.8	9.0	8.9	9.7	0.4	134.3	1943.6	5.0	17.6	15.80	8.31	6.77	1.94	0.56

[1]Adjusted for the ^{57}Fe in the meal (2.15%), added to the meal (96.44%), and the non haem iron content of the meal (2.18mg)
Estd = Estimated
Inc = Incorporation
Abs = Absorption
BV = Blood volume

Table 3.8 Subject data for the Meat meal

Subject	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*SF (µg/L) at baseline*	*SF (µg/L) at Week 2*	*Hb (g/L) base - line*	*Hb (g/L) at Week 2*	*Estd BV (L)*	*Estd iron in blood (mg)*	*Energy intake in 5 days prior (kJ)*	*Iron intake in 5 days prior (mg)*	*Vit C intake in 5 days prior (mg)*	*In ^{58}Fe (%)*	*In ^{57}Fe (%)*	*Abs ^{57}Fe (%)*	*Abs ^{57}Fe (%) adjusted to SF 40µg/L*	*mg ^{57}Fe absorbed from the meal[1]*
CG	37	60	177	12	11	114	130	4.23	1614.0	7817.0	10.2	29.7	121.84	12.52	10.28	2.96	0.85
CK	21	66	167.5	13	9	114	109	4.08	1555.5	11368.4	18.1	53.4	110.79	9.78	8.83	2.43	0.73
BI	23	49.5	162	22	21	137	139	3.35	1536.4	6464.3	8.8	35.7	138.95	10.55	7.59	4.08	0.63
DD	21	60	169	33	33	162	150	3.94	2132.4	8256.4	8.8	46.7	116.58	8.63	7.40	6.11	0.61
DH	20	56	170.5	25	20	141	134	3.59	1692.1	6945.1	8.5	31.4	109.35	9.23	8.44	4.75	0.70
DE	40	82.5	156.3	12	7	113	112	4.26	1608.5	7214.4	14.6	37.0	111.77	14.35	12.84	3.05	1.06
Mean	**27**	**62.3**	**167.1**	**19.5**	**16.8**	**130.2**	**129**	**3.91**	**1689.8**	**8010.9**	**11.5**	**39.0**	**118.21**	**10.84**	**9.23**	**3.90**	**0.76**
SD	9.0	11.3	7.2	8.6	9.8	20.0	15.8	0.37	223.5	1762.5	4.0	9.2	11.15	2.19	2.05	1.37	0.17

[1]Adjusted for the ^{57}Fe in the meal (2.15%), added to the meal (96.44%), and the non haem iron content of the meal (3.16mg)
Estd = Estimated
Inc = Incorporation
Abs = Absorption
BV = Blood volume

The differences (mean ± standard deviation) between each group including the absorption of ^{57}Fe are displayed in Table 3.9. Iron absorption has been expressed as a percentage as unadjusted absorption and absorption adjusted to a serum ferritin concentration of 40μg/L. Iron absorption has also been expressed in mg of ^{57}Fe absorbed.

Table 3.9 Averages across groups at baseline

	Sodium caseinate (n=6)	***Meat extract (n=6)***	***Meat (n=6)***	***p value for difference***
Serum ferritin at baseline (μg/L)	24.3 (14.1)	22.0 (10.9)	19.5 (8.6)	0.94
Serum ferritin at Week 2 (μg/L)	18.3 (8.1)	20.0 (9.0)	16.8 (9.8)	0.83
Average serum ferritin (μg/L)	21.3 (10.6)	21.0 (9.6)	18.2 (9.2)	0.83
Haemoglobin (g/L)	129.3 (8.0)	128.7 (8.9)	130.2 (20.0)	0.98
Energy intake (kJ)	6777.0 (1478.0)	8872.9 (1943.6)	8010.9 (1762.5)	0.15
Iron intake (mg)	12.5 (3.2)	11.4 (5.0)	11.5 (4.0)	0.87
Vitamin C intake (mg)	37.8 (6.8)	35.6 (17.6)	39.0 (9.2)	0.89
Absorption ^{57}Fe (%)	8.86 (6.48)	12.15 (6.77)	9.23 (2.05)	0.63
Adjusted absorption ^{57}Fe to SF <40μg/L (%)	3.82 (1.73)	5.13 (1.94)	3.90 (1.37)	0.35
Absorption ^{57}Fe (mg)	0.73 (0.53)	1.00 (0.56)	0.76 (0.17)	0.63

Reported as mean (standard deviation)

*Kruskal-Wallis test

There were no significant differences between groups in subject's serum ferritin and haemoglobin levels at baseline or serum ferritin at week 2. There were no significant differences between groups in intakes of energy, iron or vitamin C in the five days prior to subjects eating the test meal. Iron absorption (^{57}Fe) was 8.9% from the sodium caseinate meal, 12.2% from the meat extract meal and 9.2% from the meat meal. These values were not significantly different from one another, F (2, 7.94) = 4.32, p >.05.

When the results were adjusted to a serum ferritin level of 40μg/L, iron absorption (^{57}Fe) was 3.8% from the sodium caseinate meal, 5.1% from the meat extract meal and 3.9% from the meat meal. These values were also not significantly different from one another, F (2, 15) = 1.13, p >.05.

Iron absorption after adjusting for the amount of ^{57}Fe and non haem iron contained in the meal and ^{57}Fe added to the meal was 0.73mg for the sodium caseinate meal, 1.00mg for the meat extract meal and 0.76mg for the meat meal. Again, these values were not significantly different from one another, F (2, 7.77) = 4.88, p >.05.

The batch in which the iron was given had no effect on the absorption of ^{57}Fe. Serum ferritin at baseline was significantly related to the absorption of ^{57}Fe, F (1, 14) = 8.02, p<0.05, r = .58. A higher serum ferritin level was associated with a decreased level of ^{57}Fe absorption.

There was no significant effect of diet on ^{57}Fe absorption after controlling for the effects of serum ferritin statistically. When adjusted for levels of serum ferritin at baseline, iron absorption was 8.98% from the sodium caseinate meal, 11.78% from the meat extract meal and 9.49% from the meat meal.

3.3.2 Effect of education on lowering dietary iron bioavailability

Table 3.10 displays dietary intakes of participants at screening as determined by a 24 hour recall and following dietary education to follow a diet of low iron bioavailability for 5 days (as measured by the food record).

Table 3.10 Dietary intakes before and after dietary education

Nutrient	*Before education (as measured by 24 hour recalls)*	*After education (as measured by food records)*	*p-value for difference*
Total iron (mg)	11.30 (3.73)	11.81 (3.89)	0.684
Haem iron (mg)*	0.80 (0.14, 1.12)	0.0 (0.0, 0.0)	0.001
Non haem iron (mg)	10.50 (3.69)	11.80 (3.89)	0.270
Vitamin C (mg)*	139.94 (61.12, 211.85)	35.6 (31.15, 47.10)	0.001
Energy (kJ)*	7213.91 (6484.58, 8151.07)	7847.60 (6824.90, 9356.18)	0.112
Protein (g)*	75.31 (55.72, 85.65)	64.95 (52.3, 73.3)	0.472
Fat (g)	56.09 (20.34)	60.53 (16.81)	0.431
Carbohydrate (g)	224.70 (69.71)	262.42 (69.26)	0.027
Fibre (g)*	22.96 (15.29, 28.85)	23.10 (16.78, 28.85)	0.396
Vitamin A (µg)*	848.73 (389.01, 1371.64)	714.4 (520.2, 878.53)	0.145
Calcium (mg)*	863.76 (498.33, 1088.20)	995.8 (857.98, 1171.90)	0.053

Values are expressed as mean (standard deviation) or median (interquartile range)

* Wilcoxin signed rank test

Following dietary education, vitamin C intakes were significantly lower (median = 35.6mg) than prior to dietary education (median = 139.9mg), z = -3.288, $p<0.01$, r = -.55. Haem iron intakes were significantly lower (median = 0mg) than prior to dietary education (median = 0.80mg), z = -3.479, $p<0.01$, r = -.58. Carbohydrate intake was significantly higher (mean = 262.4g) following dietary education compared with intakes before dietary education (mean = 262.42g), t (17) = -2.418, $p<0.05$, r = 0.51. There were no significant differences between total iron, non haem iron, energy, protein, fat, fibre, vitamin A and calcium intakes ($p>.05$).

3.3.3 Subject compliance with a low iron diet

As a means of checking subject compliance with the diet of low iron bioavailability the 24 hour recall taken on the day of subjects came in for testing was compared with the data recorded on the final day of the food record. A significant positive relationship was observed for iron (r=.98, p(one-tailed) <.001), Vitamin C (r=.97, p(one-tailed) <.001), energy (r=.97, p(one tailed) <.001), protein (r=.96, p(one-tailed) <.001), fat (r=.98, p(one-tailed) <.001), carbohydrate (r=.96, p(one-tailed) <.001), fibre (r=.94, p(one-tailed) <.001), total vitamin A (r=.96, p(one-tailed) <.001) and calcium (r=.90, p(one-tailed) <.001).

3.4 Discussion

3.4.1 Comparison of results with those of other studies

There were no significant differences in iron absorption between the meat meal, the sodium caseinate meal and the meal containing the meat extract. Unadjusted iron absorption (^{57}Fe) was 8.9% from the sodium caseinate meal, 9.2% from the meat meal, and 12.2% from the meat extract meal. When converted to mg ^{57}Fe absorbed, 0.73mg ^{57}Fe was absorbed from the sodium caseinate meal, 1.0mg ^{57}Fe was absorbed from the meat extract meal and 0.76mg ^{57}Fe was absorbed from the meat meal. These differences were not significant. When adjustments were made for iron status using either baseline serum ferritin or serum ferritin adjusted to 40μg/L there were still no significant differences between the three meals. While the differences observed between the three meals were not significant, iron absorption was 31% higher from the meal containing the meat extract and 6% higher from the meat meal compared with iron absorption from the sodium caseinate meal after adjusting for baseline serum ferritin levels.

The majority of studies have shown a 50-300% increase in non haem iron absorption with the addition of meat to a meal (Baech *et al.*, 2003a). No studies have directly compared the effects of sodium caseinate and meat when added to a meal containing pasta and pasta sauce. Several studies have however investigated the effect of replacing beef with another

protein source in a meal. These studies have used radioiron isotopes to measure iron absorption in one group of subjects. This differs to the present study where sodium caseinate and meat were compared across different groups of subjects when added to the same meal in protein equivalent quantities. Cook and Monsen (1975) found iron absorption progressively rose as increased amounts of beef were substituted for egg albumin in protein equivalent quantities in a semi synthetic meal. The addition of 75g beef (equivalent to the amount of meat used in our study) increased iron absorption by 111% when substituted for egg albumin (Cook & Monsen, 1975). When 100g beef replaced a protein equivalent amount of egg albumin in a similar semi synthetic meal (egg albumin, dextrimaltose and corn oil), iron absorption increased by 200%. The nutrient quantities of this meal including iron were matched. Replacing egg albumin in protein equivalent quantities, but not nutrient equivalent quantities with beef in a standard meal containing potatoes, corn, bread, margarine, ice milk and peaches increased iron absorption significantly by 156% (Cook & Monsen, 1976). When 70g beef and 30g textured soy flour or 30g soy flour were replaced with 100g lean beef in a hamburger type meal iron absorption increased by 112 and 117% (Cook *et al.*, 1981; Lynch *et al.*, 1985). Hurrell et al (2006) found that when egg albumin was replaced with beef muscle in protein equivalent quantities in a semi synthetic meal iron absorption increased significantly 181% from 11.21 to 31.52%.

Only one study has found the addition of beef to have less of an effect than that of another non meat protein source when added to a baseline meal. Hurrell et al (1988) found that adding 92g beef to a 60g baladi bread prepared from 82% extraction wheat flour non significantly increased iron absorption by 7% while the addition of 30g bovine serum albumin significantly increased iron absorption by 61%. In contrast, when 92g of beef was added to a 60g corn meal iron absorption significantly increased 222%, while the addition of 30g bovine serum albumin increased iron absorption non significantly by 15% (Hurrell *et al.*, 1988). All meals contained equivalent quantities of protein.

Other studies have compared the effects of adding protein equivalent quantities of beef and non meat proteins in different groups of subjects. For example, the addition of 75g beef

(20g protein) to a maize meal increased iron absorption significantly by 280% in one group of subjects while the addition of 100g egg albumin (20g protein) increased iron absorption non significantly by 56% in another group of subjects (Bjorn-Rasmussen & Hallberg, 1979). The addition of 39.4g cooked freeze dried beef to a meal consisting of corn starch and corn oil increased iron absorption by 148%. In different groups of subjects the addition of egg albumin and soy protein isolate to the same meal decreased iron absorption (Reddy *et al.*, 1996).

Only two studies in humans have investigated the effect of casein on non haem iron absorption. These studies have compared casein with that of egg albumin. Cook et al (1981) found equivalent amounts of casein and egg albumin (based on protein content) had similar effects on non haem iron absorption from a semi synthetic meal consisting of dextrimaltose and corn oil, while isolated soy protein decreased iron absorption significantly when compared with egg albumin. Hurrell et al (1989) compared the effect of egg albumin and casein in protein equivalent quantities on non haem iron absorption when added to a semi synthetic meal containing maize starch and corn oil. In the same group of subjects replacing egg albumin with sodium caseinate (Alanate 110®) reduced mean iron absorption to approximately half that of egg albumin, although the difference was not statistically significant. Prior 84% enzyme hydrolysis of the casein removed much of the inhibitory effect with the difference in iron absorption between meals prepared with sodium caseinate (Alanate 110®) and enzyme hydrolysed casein being statistically significant. In vitro studies in semi purified meals under simulated gastrointestinal conditions found that the replacement of egg albumin with casein reduced dialysable iron (Hurrell *et al.*, 1989). Kane and Miller (1984) found similar results with dialysability being high for beef and bovine serum albumin, intermediate for egg albumin and low for casein, soy flour and soy protein isolate. In vitro studies have also shown that the greater the extent of protein hydrolysis of casein the more the dialysable iron increased (Hurrell *et al.*, 1989). Yeung et al (2002) found that iron absorption was higher in the presence of casein phosphopeptides (casein digested with proteolytic enzymes) than in the presence of sodium caseinate when measured using an in vitro digestion / Caco-2 cell culture model. Iron uptake from beef

was several times higher than that from casein when measured using caco-2 cell monolayers (Glahn *et al.*, 1996).

The literature suggests that a meal containing meat would enhance iron absorption to a greater extent than a meal containing sodium caseinate. Using a model proposed by Monsen et al (1978), the sodium caseinate meal used in this study is a meal of low iron bioavailability (contains 0g meat and <25 mg ascorbic acid), and the meat and meat extract meals have medium bioavailability (meat content 30-90g and ascorbic acid content <25mg).

Using the algorithm *(Absorption Ratio = 1 + 0.00628 x amount of meat (in g))* developed by Reddy et al (2000) to measure the effect of meat on iron absorption it would be expected that the absorption ratio from the meat meal would be 1.47 times that of a meal that did not contain meat. This was not found in this study where the absorption ratio from the meat meal was 1.06 times that of the sodium caseinate meal. Iron absorption from the meat extract meal was 1.31 times higher than that of the sodium caseinate meal. Absorption from the meat extract meal was 1.24 times higher than that of the meat meal. The algorithm does not take into account other inhibitors or enhancers of iron absorption that may be found in the meal. However, these were kept fairly constant between the meals used in this study.

3.4.2 The meat extract

The amount of meat extract added to this meal was equivalent to that contained in 75g beef. While the meat extract meal did not appear to increase iron absorption significantly, iron absorption from the meat extract meal was higher than that from the meat meal and sodium caseinate meal. It is possible that the meat extract tested is the factor in meat that enhances non haem iron absorption and further research will be required to determine this. Based on the work using Caco-2 cells, this meat extract may work by enhancing iron uptake by DMT-1 or transporting non haem iron out of the erythrocyte (Wolber *et al.*, 2006). However, it is also possible that the meat extract fraction contains inhibitors as well as

enhancers of non haem iron absorption. Further work and testing needs to be done to positively identify the meat extract.

3.4.3 Composition of the meals

The sodium caseinate and meat extract meal were almost identical in their composition. The meat extract meal contained 0.02mg more iron than the sodium caseinate meal with the addition of the meat extract. There may have been other differences in the content of the meal due to the addition of the meat extract but these would have been minimal.

The iron content of the meat meal was 1.74mg higher than that of the sodium caseinate meal and 1.72mg higher than that of the meat extract meal. When these iron contents were adjusted for, there were still no significant differences in iron absorption between the three meals. The meat meal contained 0.77mg haem iron (24.4% of total iron), with the meat extract and sodium caseinate meals containing minimal haem iron. The small amount that appeared is likely to be due to rounding errors, or to differences in the results obtained from the two laboratories. Extreme care was taken to ensure that no contaminant iron was added to any of the meals during and following their preparation (Brittin & Nossaman, 1986; Park & Brittin, 1997).

The macronutrient contents of the three meals were virtually identical and micronutrient differences between the three meals were minor. Calcium was higher in the sodium caseinate and meat extract meal by 17.6mg compared with the meat meal. This amount is unlikely to be large enough to affect iron absorption and if so would tend to inhibit rather than enhance non haem iron absorption. 40mg calcium reduced iron absorption by 40% when added to the dough of white wheat rolls, but had no significant effect on iron absorption when added to white wheat rolls after cooking (Hallberg *et al.*, 1991). The difference in the vitamin C (an enhancer of iron absorption) content of each meal was minimal and also not likely to affect iron absorption (1.82mg in the meat meal and 0.19mg in the other two meals). Vitamin C in amounts less than 25mg is unlikely to have an effect on iron absorption (Bjorn-Rasmussen & Hallberg, 1974; MacPhail *et al.*, 1981; Gillooly *et*

al., 1984b; Fairweather-Tait *et al.*, 2000). The zinc, copper, magnesium, phosphorus, vitamin A and β-carotene contents of the three meals were similar. Polyphenol, phytate, organic acid and oxalate contents of the meals were not measured, but were assumed to be similar between the meals because sodium caseinate, meat and the meat extract are unlikely to differ in their content of polyphenols, phytate, organic acids or oxalate.

It is unknown why the meat extract meal and the meat meal did not increase iron absorption to a greater extent than the sodium caseinate meal. Hurrell et al (1988) found that beef added to a baladi bread meal non significantly increased iron absorption compared with bovine serum albumin. This may be due in part to meal composition. White wheat products (found in pasta and bread) are less inhibitory to iron absorption than other food products such as whole wheat, maize, rice and sorghum. It has been suggested that meat enhances iron absorption only when ingested with markedly inhibitory foods (Bjorn-Rasmussen & Hallberg, 1979). For example, the addition of 50g meat to a meal containing wheat buns prepared from 60% extraction white wheat flour (25mg phytate) did not significantly increase iron absorption (Hallberg *et al.*, 1989), but when added to the same meal, containing 250mg phytate, iron absorption increased by 86% (Hallberg *et al.*, 1989). Adding 100g beef to a semi purified meal containing corn syrup, corn oil and either egg albumin or soy protein increased iron absorption by 25 and 300% (Morck *et al.*, 1982).

Sodium caseinate has an inhibitory effect on iron absorption (Hurrell *et al.*, 1989). Therefore the addition of the meat extract to a sodium caseinate meal should enhance iron absorption due to sodium caseinate's inhibitory nature. However, a more significant increase in iron absorption may have been seen if the meat extract was added to a more inhibitory protein such as soy protein isolate (Cook *et al.*, 1981).

3.4.4 Population group

Studies using stable isotopes are extremely expensive, restricting the number of subjects that can be tested. Power calculations estimate that three more subjects will need to be tested in each group to observe a significant difference between the three diets. The three

groups did not differ significantly in iron status. There were no significant differences between groups in terms of subjects' age, weight, height, estimated blood volume and estimated iron in blood at baseline. There were no significant differences between groups in intakes of energy, iron or vitamin C in the five days prior to subjects eating the meal. New Zealand is a heterogeneous society and the population used in this study was possibly more heterogeneous than populations used in other studies. While ethnicity was not documented four women were known to be of Asian ethnicity (two were of Chinese descent, one of Japanese descent and one of Malaysian descent). One woman was of African descent. Genetic factors have been shown to influence iron status (Whitfield *et al.*, 2003) however it is not know if genetic factors have any independent effect on iron absorption. If genetic factors including ethnicity do influence iron absorption the heterogeneity of the population studied may have masked the effects of the three meals on iron absorption. Including more subjects would help to counteract this effect. Other studies investigating the MFP factor have not reported the ethnicity of the subjects tested (Hurrell *et al.*, 2006; Storcksdieck genannt Bonsmann *et al.*, 2007).

3.4.5 Iron status and iron absorption

The main factors that determine the amount of non haem iron absorbed from a meal include the iron content of the meal, the meal composition and the iron status of the subject.

Differences in iron absorption from meals is due more to variations in their iron bioavailability than to differences in their iron content (Hallberg & Hulthen, 2000). Monsen et al (1978) suggested that absorption of non haem iron in iron deficient subjects may be as much as 20% in meals of high bioavailability. Two subjects in this study had an iron absorption level of greater than 20%. One of these subjects was in the sodium caseinate group and the other in the meat extract group. Both of these subjects had a serum ferritin of <10μg/L. In an iron replete individual served a meal of low iron bioavailability non haem absorption may be around the 2% mark (Monsen *et al.*, 1978). The subject with the highest serum ferritin level (48μg/L) in the sodium caseinate group had an iron absorption value of only 2.98%. For most typical meals the bioavailability ranges from 5-

15% in subjects with small but not fully absent iron stores (FAO/WHO, 1988). The average iron absorption from our meals was within this range.

In an iron deficient state, iron requirements increase and more iron is absorbed (Hallberg & Rossander-Hulten, 1991). This study clearly demonstrated that subjects with a low serum ferritin concentration at baseline absorbed more iron than those subjects with a higher serum ferritin level. Several other studies have shown a strong inverse relationship between serum ferritin concentration and iron absorption (Cook *et al.*, 1974; Taylor *et al.*, 1988; Hallberg *et al.*, 1995; Hallberg *et al.*, 1997; Hunt & Roughead, 2000; Roughead & Hunt, 2000). Hallberg et al (1997) reported an inverse association between total iron absorption and serum ferritin up to a serum ferritin concentration of about 60μg/L. Another report suggests that dietary factors are only important in subjects with low iron stores, and when serum ferritin values are greater than 25μg/L there is no difference in absorption of iron from foods of different bioavailability (Fairweather-Tait, 1995a).

Iron status may be a more important factor in determining iron absorption than dietary bioavailability (Cook *et al.*, 1991a). Results from this study showed that serum ferritin accounted for 33% of the variation in iron absorption. Reddy et al (2000) found that serum ferritin accounted for 32% of the overall variability in iron absorption from a range of meals while dietary factors accounted for 16.4% of the variation. This group had a wider range of serum ferritin values than our study, with a mean serum ferritin of 41μg/L. Using women with depleted iron stores (SF<20μg/L) rather than women with a SF<30μg/L would have reduced the variation in iron status and may have meant that a more significant difference in iron absorption between the three diets was seen.

3.4.6 Other factors affecting iron absorption

Reddy et al (2000) suggested that there may be some unknown physiologic factors contributing to the variation in iron absorption. Other factors known to affect non haem iron absorption include the secretion of hydrochloric acid from the stomach which is important for the solubilisation of non haem iron in food. Hydrochloric acid secretion is

affected by diet, genetic factors and stress (Fairweather-Tait, 1995a). The longer food is in the stomach the greater the opportunity for hydrochloric acid and dietary factors to reduce ferric iron (Fe^{3+}) to the more soluble ferrous (Fe^{2+}) iron. There is no evidence however that iron absorption is affected by the length of time food is in the small intestine (Fairweather-Tait & Wright, 1991). Pancreatic secretions and the rate of erythropoiesis may also have an effect on iron absorption (Cook *et al.*, 1991a). Finally, the level of iron to which the intestinal mucosal cells have been previously exposed may influence iron absorption (Fairweather-Tait, 1995a). A study by Barrett et al (1992) found that healthy individuals, including those with low iron stores had reduced non haem iron absorption from food following 12 weeks iron supplementation. The current study minimised this effect by having subjects follow a diet low in iron bioavailability for five days prior to the study and through subjects avoiding the use of iron supplements for one month prior to and throughout the duration of the study (until the last blood sample was taken).

3.4.7 Low iron diet for the five days prior to the study

The subjects were given individual dietary advice to decrease the iron bioavailability of their diets in the 5 days leading up to the study. The aim of this was to stabilise iron intakes between subjects and optimise iron absorption. The average iron intakes in the five day period before the study were all lower than the RDI and ranged from 11.4 to 12.5mg across the three groups. In other studies subjects have consumed less than 5mg iron per day in the three days prior to taking part in the study (Cook *et al.*, 1991a; Barrett *et al.*, 1992; Whittaker *et al.*, 2001). This would have been difficult to achieve in the present group without compromising the energy intakes of subjects during the lead in period. For example, the average energy and iron intakes of the sodium caseinate group were 6777kJ and 12.5mg/day during the five day lead in period. The vitamin C intakes across all groups during this time were lower than the RDI.

Vitamin C and haem iron intakes were significantly lower during the five days leading up to the study compared with that measured using a 24 hour recall at screening. There were no significant differences between total iron intakes before and after receiving dietary

advice. In fact the average iron intake increased from 11.30 to 11.81mg per day. Only one other study has documented a diet designed to inhibit iron absorption (Cook *et al.*, 1991a). In this diet prescribed by Cook et al (1991a) red meat was not permitted, although limited amounts of fish and chicken were able to be consumed. Ascorbic acid intake was reduced by the avoidance of fresh vegetables, fruits, citrus juices and foods fortified with ascorbic acid. The subjects included legumes, cereals, and food rich in bran, eggs and dairy products in the diet. At least one cup of tea or coffee was taken with each meal. The nutrient content of the diet was not reported.

Dietary intake for the five days prior to the present study was assessed using a 5 day food record. Where possible it was asked that subjects weigh their food. A food record is useful for assessing the nutrient intake of individuals, however its accuracy depends on the subject's motivation, conscientiousness and ability to weigh or estimate food portion sizes accurately. As with all methods of dietary assessment there was likely to have been some reporting errors. The final day of the food record was also assessed using a 24 hour recall. There was an extremely high correlation between intakes of iron, vitamin C and energy from the final day of the food record and that of the 24 hour recall.

All subjects fasted for 12 hours prior to coming into the Human Nutrition Laboratory to reduce the effect of food eaten previously on non haem iron absorption. The absorption of non haem iron is greatest when given to an iron deficient individual in a fasting state (Lynch, 1997). However, other studies have found that iron absorption was not significantly different when a meal was served in the morning after an overnight fast or if eaten during the day (Taylor *et al.*, 1995). Iron absorption from a hamburger meal at lunch time was not affected by a high calcium containing breakfast eaten 2 or 4 hours prior (Gleerup *et al.*, 1993).

3.4.8 Assessment of iron status and adjustment for iron status

Iron absorption was adjusted for iron status using a number of methods including adjustments made on the basis of serum ferritin levels. Making these adjustments is

dependent on the reliability of serum ferritin as a measure of iron status. Serum ferritin is regarded as one of the best measures of iron status due to its high sensitivity and relationship to body iron stores. A concentration of 1μg/L corresponds to iron stores of about 8-10mg stored iron (Finch, 1994). Over several weeks the day to day coefficient of variation for serum ferritin within subjects is approximately 15% (Gibson, 2005b). Haemoglobin represents the amount of iron in erythrocytes and is a relatively insensitive measure, falling only in iron deficiency anaemia (Gibson, 2005b).

In the present study, the iron provided in the meals was not expected to affect serum ferritin concentrations, as the amounts provided in the meal including the stable isotopes were less than half that of the RDI.

All subjects at screening had a serum ferritin of 30μg/L or less. However at baseline, 4 subjects had a serum ferritin of >30μg/L. For example, one subject's serum ferritin at screening was 29μg/L. Twenty six weeks later (at baseline) her serum ferritin was 34μg/L. At week 2 her serum ferritin was 24μg/L. Even with less time between screening and baseline there were large differences in some subjects serum ferritin levels. One subject had a serum ferritin of 26μg/L at screening, a serum ferritin of 36μg/L at baseline (10 weeks later) and a serum ferritin of 24μg/L at Week 2.

Fifteen subjects had normal C-RP values at baseline and at week 2. Of these, eight subjects had less than 2μg/L difference in their serum ferritin levels between baseline and week 2. Four subjects had serum ferritin differences of 4-5μg/L, and three subjects had serum ferritin differences of 10μg/L. Three subjects had slightly increased CRP values at either baseline or week 2. A high CRP indicates the presence of infection or inflammation. Ferritin is an acute phase reactant and in the presence of infection or inflammation the ferritin may be high (Hulthen *et al.*, 1998). Subjects who currently have or who have had a recent, even minor infection usually have an increased serum ferritin that may remain for a considerable time, even after the infection has disappeared (Hulthen *et al.*, 1998). One subject had a serum ferritin of 66μg/L at baseline, despite having a screening serum ferritin of 23μg/L and a serum ferritin at week 2 of 33μg/L, suggesting that the measure at baseline

was increased due to infection. As her C-RP was raised slightly at baseline and she had a cold, her serum ferritin at week 2 was used when adjusting for iron status. The serum ferritin values in the other two subjects with an increased C-RP were not hugely different for the subject's other serum ferritin values and therefore were used when correcting for iron status.

All subjects were given the meal within one week following the last day of their menstrual period. One study found differences in serum ferritin and haemoglobin across the menstrual cycle (Kim *et al.*, 1993). However, a study in iron deficient women found no difference in iron status across the menstrual cycle as determined by serum ferritin, haemoglobin and serum transferrin receptors (Belza *et al.*, 2005).

Early studies have found serum ferritin to have a relatively large intra-individual variance (Borel *et al.*, 1991; Kim *et al.*, 1993). Studies have suggested that repeat blood samples (figures ranging from three to ten days) are necessary to determine serum ferritin accurately in young women (Borel *et al.*, 1991; Cooper & Zlotkin, 1996). Conversely, one blood sample has been suggested as sufficient to accurately determine haemoglobin (Borel *et al.*, 1991). A study in young women with depleted iron stores (SF between 12 and 30μg/L and an Hb>119g/L) found that only one blood sample was necessary to accurately determine serum ferritin and haemoglobin concentrations (Belza *et al.*, 2005). In this study, the lower day to day variations were possibly due to a more homogenous groups of subjects and a stricter standardised study design (Belza *et al.*, 2005).

Other factors that may have an influence on serum ferritin include alcohol consumption (Leggett *et al.*, 1990), variations in red cell turnover and physical activity, with serum ferritin increasing after exercise and remaining elevated for several days after maximal exertion (Malczewska *et al.*, 2000a). Ideally, the present study would have recommended no moderate or intensive physical activity or alcohol in the 24 hour period prior to the ingestion of the test meal and prior to any blood tests. However, subjects in this study were not consumers of excessive amounts of alcohol so this is unlikely to have influenced serum ferritin concentrations. No subject consumed excess alcohol in the 24 hours prior as

determined by the food record and 24 hour diet recall. No record was made of physical activity prior to eating the test meal.

3.4.9 Use of stable isotopes to measure iron absorption

Differences in the results between this study and others may be based on various techniques used to measure iron bioavailability and methods of isotope labelling. In many studies using stable isotopes an assumption must be made (usually 80-100% depending on iron status) as to the amount of isotope incorporated into the erythrocytes in order to calculate iron absorption. The use of a double isotope technique eliminates many of the errors associated with these assumptions. The intravenous administration of a second isotope is able to account for the redistribution of orally absorbed iron between the erythrocytes and the rest of the body (1981; Barrett *et al.*, 1992; Whittaker *et al.*, 2001).

The incorporation of ^{58}Fe into red blood cells in the present study ranged from 91.89 to 153.83%. This was expected to be 90-100% as our population included iron depleted subjects. The concentration of the infused ^{58}Fe was measured by laboratories in Ipswich and Houston, as well as rechecked by Hills Laboratories to ensure that it was not in fact higher than originally measured. Blood volume was also recalculated using a number of different algorithms, but was not found to alter the amount of ^{58}Fe that was absorbed. The incorporation of ^{58}Fe at levels higher than 100% has been observed previously but not documented (personal correspondence with Ian Griffins, U.S. Department of Agriculture / Agricultural Research Service Children's Nutrition Research Center and Section of Neonatology, Department of Paediatrics, Baylor College of Medicine, Houston, Texas). It is not known why this occurs but may be due to differences in body handling of the ^{57}Fe and ^{58}Fe. It is generally assumed that they are handled the same way but the incorporation of the isotope given orally into the red blood cells may differ from that of the intravenous isotope (Roughead & Hunt, 2000). The ^{57}Fe absorbed from the small intestine will be transferrin bound and go straight to the liver via the hepatic portal vein for metabolism, while ^{58}Fe enters the venous circulation before reaching the liver.

The ^{58}Fe used in this study was prepared in two batches as described in the methodology section (see page 183). The batch subjects were given was not found to have any significant effect on iron absorption. It was not possible to mix the ^{58}Fe from the two batches together due to sterility issues. As the ^{58}Fe was to be given intravenously it was essential to avoid excess handling and the potential for contamination.

One of the difficulties in this study was accurately measuring the amount of ^{58}Fe that was administered. The ^{58}Fe was provided in 2.5mL sterilised ampoules with 0.2mL overage. These were opened and the solution was drawn into a syringe to provide either 9 or 6.3mL of solution. These measures were based on reading from a syringe. It was difficult to do this more accurately without compromising the sterility of the isotope.

Providing four saline flushes at the end of the iron infusion ensured that the subject received as much of the ^{58}Fe as possible. Measurements taken by Hill Laboratories on saline alone and samples of saline collected from four subjects following the infusion of ^{58}Fe contained minimal amounts of iron, indicating that all of the ^{58}Fe had been infused.

3.4.10 Studies using stable isotopes to investigate iron absorption

Only one other study has used stable isotopes to investigate the effect of meat on non haem iron absorption. In this study, 25g lean beef was added to a vegetable puree meal and given to infants. The meals were given on 2 consecutive days and labelled with ^{57}Fe or ^{58}Fe. The energy and iron contents of the two meals were the same. Non haem iron absorption increased significantly (1.5 times) when meat was added to the vegetable meal (Engelmann *et al.*, 1998).

Only a few other studies have used the double isotope technique to provide ^{57}Fe orally and ^{58}Fe intravenously to measure iron absorption. Whittaker et al (2001) provided 8.5mg ^{57}Fe and 0.5mg ^{58}Fe to compare iron absorption in non pregnant women and women in early and late pregnancy. Other studies in normal healthy women have investigated iron absorption through the provision of 5mg ^{57}Fe and 250μg of ^{58}Fe (Barrett *et al.*, 1992), 8.5mg ^{57}Fe and

0.5mg ^{58}Fe (Whittaker *et al.*, 2001), 10mg ^{57}Fe and 0.5mg ^{58}Fe (Whittaker & Barrett, 1992) and 23.3mg of ^{57}Fe and 448.5μg of ^{58}Fe (van den Heuvel *et al.*, 1998a). Two studies have compared iron absorption from different meals using the administration of ^{57}Fe orally and ^{58}Fe intravenously (Tondeur *et al.*, 2004; Roe *et al.*, 2005). Roe et al (2005) found evidence of increased iron absorption from a high bioavailability diet fed over 2 days compared with iron absorption from fortified cereal products fed over 3 days. Tondeur et al (2004) found no effect of two different doses of iron from sprinkles added to a maize based porridge when fed to infants.

3.4.11 Potential use of the MFP Factor

The present study showed that addition of a meat extract to a sodium caseinate meal increased iron absorption in comparison to the sodium caseinate meal alone but not significantly. Identification of the MFP factor would raise the possibility of extracting it from meat or synthesising it for use by several groups of people, particularly those with or at risk of iron deficiency. The MFP factor may be useful in both developing and developed countries.

Approximately two billion people in the world suffer from anaemia, mostly iron deficiency anaemia. The cost of iron deficiency anaemia is huge including lost productivity, health costs and costs related to the permanently impaired cognitive development of young children who develop iron deficiency anaemia (FAO/WHO, 1998).

Solutions for iron deficiency, particularly in the developing world need to be investigated. The three main strategies to combat iron deficiency include iron supplementation, iron fortification or food and nutrition education to improve the bioavailability of iron in the diet (Baker & DeMaeyer, 1979). Several factors determine the feasibility and effectiveness of these strategies.

Iron supplementation is effective in changing a population's iron status (Umbreit, 2005) and can be targeted at those in need including pregnant women and pre school children

(International Food Policy Research Institute, 2000). Iron supplementation is essential when iron requirements are high and iron deficiency acute (International Food Policy Research Institute, 2000). However, iron supplementation requires an effective system of delivery, may cause unpleasant gastrointestinal side effects (Cook & Reusser, 1983) and is dependent on subject compliance (Baker & DeMaeyer, 1979; Cook & Reusser, 1983).

Food fortification is a longer term approach to alleviating iron deficiency which does not rely on individual motivation, can reach all segments of the population and costs less than supplementation (Baker & DeMaeyer, 1979). Technical difficulties associated with iron fortification include the choice of an appropriate food vehicle and identifying a form of iron that is adequately absorbed which does not alter the taste or appearance of the food in which it is delivered (International Food Policy Research Institute, 2000). There are possible risks of toxicity with food fortification as the entire population is exposed to iron fortified foods including people with haemochromatosis. Breeding rice to contain a higher level of iron may be one method of combating iron deficiency in some parts of the world. Using dietary data from Bangladeshi women and assuming that iron enhanced rice reached half of the Bangladeshi population (65 million people) over ten years the cost was estimated to be US 1 cent per person per year (International Food Policy Research Institute, 2000). Iron supplementation was estimated to be 3.5 times more effective in improving the population's haemoglobin level. However, the cost per person was estimated to be 100 times more expensive, with the cost of iron supplementation during pregnancy estimated at $1.70 US (International Food Policy Research Institute, 2000).

Alternatively people can be educated to increase their intake of foods high in iron and foods that increase iron bioavailability. This however is unlikely to be effective in developing countries where the cost of even basic foods is prohibitive. In addition, many staple foods contain high levels of dietary inhibitors of iron.

In Bangladesh households spend 70% of their total expenditure on food, with 25% of this allocated to meat (International Food Policy Research Institute, 2000). It has been estimated that meat consumption would need to increase three times to have the same effect

in increasing haemoglobin levels as increasing the iron content of rice (International Food Policy Research Institute, 2000). Increasing meat consumption was estimated to cost $70 US per year per person (International Food Policy Research Institute, 2000). Both food fortification and iron supplementation are more cost effective than increasing meat consumption in a population (Umbreit, 2005). While iron supplementation and fortification trials have been shown to increase blood indices of iron status, an increased intake of meat has not always been associated with an increase in serum ferritin levels (Hunt *et al.*, 1995; Harvey *et al.*, 2005).

The addition of a preparation containing the MFP factor to meals could be used alongside any of the above strategies to increase iron absorption in the population. However, for this to be of benefit the MFP factor would need to be affordable and accessible to those people most in need. Work to identify the MFP factor is funded by groups who are driven by profit. Successfully identifying the MFP factor and patenting it, is likely to be worth billions of dollars. If increasing meat consumption is more expensive than food fortification or iron supplementation, it is unlikely that the production of a preparation containing the MFP factor will be much cheaper. Either governments will need to subsidise their populations or social inequality will mean that those people who would really benefit from a preparation containing the MFP factor will not be able to afford it. Like many supplements it may be the "worried well" who spend their money on such a preparation. A country faced with the prospect of paying millions of dollars to obtain a preparation containing the MFP factor may decide to put this money into alleviating food insecurity, food fortification programmes or iron supplementation programmes or the purchase of meat. Meat not only contains the MFP factor, but iron and haem iron which are well absorbed and other nutrients including zinc, vitamin A and vitamin B12. These nutrients are often lacking in the diets of people living in the developing world.

Consideration would need to be given to how costly a preparation containing the MFP factor would be to produce, storage needs (for example, many people do not have access to refrigeration), taste and acceptability. Acceptability is likely to depend on how a preparation containing the MFP factor is presented.

The meat extract in its current form is provided in solution which could be added to meals or made into a meal supplement or drink such as soup. It has a salty taste therefore is likely to work better in savory type foods. Alternatively it could be converted to a powder and sprinkled over meals or made into a paste similar to pate to be spread on bread and crackers. Market research would need to be conducted to determine how the MFP factor is best presented based on the population groups it is targeted at. Another option would be to co package the MFP factor with or without a dose of iron in supplement form.

A preparation containing the MFP factor is unlikely to be acceptable to vegetarians. However, identification of the structure may enable the MFP factor to be produced in a synthetic form which may be more acceptable.

There are several groups within developed countries that may benefit from adding the MFP factor to their meals. These may include people who have iron deficiency but are not yet anaemic, people who restrict their dietary or meat intake for health, cultural, religious, ethical, environmental or other reasons (American Dietetic Association, 2003), people who are reluctant to increase their energy intake, or people who are reluctant to use iron supplements due to their unpleasant side effects (Gibson *et al.*, 1997). For some people a preparation containing the MFP factor may be more easily consumed than a large serving of meat. The preparation could therefore be given in amounts that provide greater quantities of the MFP factor than an average serving of meat.

Infants and toddlers are at risk of becoming iron deficient because of their increased needs for growth and limited food choices (Dallman *et al.*, 1980), with iron deficiency anaemia resulting in poor growth and developmental problems. Iron deficiency is a well recognised health problem among New Zealand children. In a study of 53 healthy children aged 9-24 months, 13.2% of children had iron deficiency (SF<10μg/L) and 20% had iron deficiency anaemia (SF<10μg/L and Hb<110g/L) (Wham, 1996). In a study of 323 healthy 6-24 month old children in the South Island, 15% had a SF<10μg/L (Soh *et al.*, 2002). In 391 hospitalised children aged 8-23 months old with an acute illness, 56.3% had iron deficiency anaemia, 18.7% had iron deficiency and 25.1% were not iron deficient (Grant *et al.*, 2003).

Infants and toddlers are often reluctant and fussy eaters. The addition of a preparation containing the MFP factor to their meals could help ensure an adequate intake of bioavailable iron is received.

Adolescents are vulnerable to low iron stores and iron deficiency due to high physiological requirements for growth, losses of iron through menstruation and an inadequate dietary intake of iron (Beard, 2000). In New Zealand females aged 11-14 years, the prevalence of iron deficiency was 4.3% and iron deficiency anaemia was 1.2% (Parnell *et al.*, 2003). A preparation containing the MFP factor may be a useful supplement to the diet of adolescents, particularly in those who are restricting their energy intake.

Women of child bearing age are vulnerable to iron deficiency (Russell *et al.*, 1999). The iron requirements of pregnant women increase markedly during the third trimester (Whittaker *et al.*, 2001). The RDI for pregnant women in New Zealand and Australia is 27mg iron/day (Commonwealth Department of Health and Ageing Australia, 2006). Iron requirements in pregnancy need to provide for the growing fetus and increased maternal blood volume. Fetal nutrient requirements are met at the expense of the mother. A regional study of pregnant New Zealand women found that 6.2% of women had iron deficiency anaemia (using a cut off value of Hb<100g/L) and 31.5% of women had serum ferritin levels less than 10μg/L (Watson & McDonald, 1999). A preparation containing the MFP factor could be a simple way to increase the absorption of non haem iron for premenopausal and pregnant women.

Iron requirements are lower and iron deficiency is less common among elderly individuals (Fleming *et al.*, 1998). Dietary inadequacy is rarely the main cause of iron deficiency in this age group. A preparation containing the MFP factor however, may be of benefit in elderly people with restricted appetites due to nausea, in elderly who find chewing difficult due to poor dentition or in elderly with increased iron requirements.

A preparation containing the MFP factor may be a useful addition to the diets of those athletes who have a restricted energy intake, or for those athletes competing in ultra

endurance events over several weeks where it is not possible to carry large quantities of food including meat due to perishability or weight issues.

The identification and subsequent production of the MFP factor could have beneficial effects in many groups including young women if acceptable to the end consumer. It is possible that the MFP factor could be used alongside other strategies to increase the iron status of varying population groups including food fortification, iron supplementation and food and nutrition education. However, one of the main concerns associated with the production of the MFP factor is its affordability and whether it would reach those in need. In addition, it must be remembered that an increased intake of meat provides other important nutrients that are not likely to be contained in a preparation containing the MFP factor.

3.5 Conclusions

Despite our results showing no significant impact of the meat extract on iron absorption, it is still possible that the meat extract does enhance iron absorption. This is based on previous research that has been undertaken in the IFNHH (Reynolds *et al.*, 2006a; Reynolds *et al.*, 2006b; Wolber *et al.*, 2006) and the fact that both the meat extract and the meat meal enhanced iron absorption to a greater extent than the sodium caseinate meal. There was however only a slight difference in iron absorption between the meat meal and the sodium caseinate meal, which was unable to be explained. Serum ferritin was significantly related to iron absorption, with those subjects who had lower levels of serum ferritin showing increased levels of iron absorption. This study will be expanded to include three more subjects in each study group to determine whether the meat extract does have any effect on iron absorption.

Chapter 4
Concluding Discussion

4.1 Summary of key findings

- The mean and median serum ferritin level of women aged 18-40 years living in the Manawatu region was 39.7μg/L and 36.0μg/L.

- The majority of women (87.2%) had normal iron stores (SF>20μg/L). Two women (2.4%) had iron deficiency anaemia (SF<12μg/L and Hb<120g/L). Nine women (10.6%) had depleted iron stores (SF<20μg/L).

- Seventy one women (83.5%) consumed less than the recommended dietary intake (RDI) of 18mg iron per day. Eighteen women (21.2%) did not meet the Estimated Average Requirement for iron of 8mg/day, while 45 women (53%) consumed less than two thirds of the RDI.

- The daily mean and median iron intakes were 12.7±6.2mg and 10.8mg. The estimated mean haem iron intake was 0.99±0.91mg/day or 7.8% of total dietary iron.

- There were no significant differences between age, BMI or dietary intakes of those subjects who had a serum ferritin <20μg/L and those with a serum ferritin >20μg/L.

- Serum ferritin was positively associated with age and total dietary iron intake. No statistically significant relationship was found between serum ferritin and Body Mass Index, total exercise or hours spent running per week or daily intakes of

energy, protein, haem iron, red meat, total meat, vitamin C, vitamin A, total tea, coffee, alcohol, fibre or calcium.

- 7.1% of women in this study reported using iron supplements, 27.1% of women used multivitamin/mineral supplements and 29.4% of women reported the use of other nutritional supplements.

- Mean and median intakes of carbohydrate, protein and fat were all within the Acceptable Macronutrient Distribution Range (AMDR) to reduce the risk of chronic disease. Alcohol contributed on average 0.7% of total energy and saturated fats contributed to 11.99% of total energy intake. Energy intakes were below the recommended range of estimated energy requirements.

- Mean and median intakes of protein, vitamin C, thiamin, riboflavin, niacin equivalents, vitamin B6, vitamin B12, phosphorus, zinc, copper, potassium and sodium were all above their respective RDI and AIs. Sodium intakes were above the suggested dietary target of 1600mg/day.

- Mean and median intakes of calcium, folate, selenium, fibre and manganese were below their respective RDIs and AIs. Over half of the women consumed less than the EAR for folate and calcium, and over three quarters of the women consumed less the than EAR for selenium.

- Iron absorption was not significantly different between the sodium caseinate group, the meat extract group or the meat group even after adjusting for serum ferritin levels. However, both the meat extract and the meat meal enhanced iron absorption to a greater extent than the sodium caseinate meal.

- Serum ferritin at baseline was significantly related to iron absorption accounting for 33% of the variation in iron absorption. A higher serum ferritin level was associated with a decreased level of iron absorption.

4.2 Discussion of findings

Thirteen percent of the women included in the first study had depleted iron stores or iron deficiency anaemia. This finding supports previous research which has shown iron deficiency to be a problem in developed countries (Fawcett *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001). The prevalence of iron deficiency in this population was lower than that seen in other groups (Brussard *et al.*, 1997; Looker *et al.*, 1997; Rangan *et al.*, 1997; Fawcett *et al.*, 1998; Galan *et al.*, 1998; Russell *et al.*, 1999; Heath *et al.*, 2001). This may be explained by the exclusion of vegetarians and blood donors. The population on which this research was conducted is unlikely to be representative of New Zealand women aged 18-40 years of age. Several groups were excluded from the study including women who had been pregnant or were breastfeeding in the past year, women planning pregnancy, smokers and women who consumed large amounts of alcohol. In addition a large amount of recruiting was undertaken at Massey University, which may have meant the population was biased towards a student population. Data was not collected on occupation, education levels, socio economic status or ethnicity, all factors which may impact on a person's dietary intake and iron status. Several subjects were nutrition students which may have added further bias to the study group.

The prevalence of iron deficiency was much lower than the number of women who consumed less than the RDI or EAR for iron. The EAR and RDI for iron may be set too high for New Zealand women. In May 2006, the Nutrient Reference Values for Australia and New Zealand were published which included an increased RDI for iron, calcium and folate (Commonwealth Department of Health and Ageing Australia, 2006). Over half of the women surveyed did not meet the estimated average requirement for folate, calcium and selenium. Investigating biochemical levels of folate and selenium would determine whether low dietary intakes were reflected by a low body status of these nutrients. The limitations of using a 24 hour recall to assess dietary intakes of nutrients must also be considered.

Total dietary intake of iron and age were the only factors found to be associated with iron status. For some nutrients such as vitamin C or calcium the bioavailability of dietary iron depends on the amount of these nutrients consumed with the iron rather than the total dietary intakes of these nutrients (Cook & Monsen, 1977; Gleerup *et al.*, 1993; Gleerup *et al.*, 1995). Only one study investigating the dietary factors which affect iron status has taken this into account (Razagui *et al.*, 1991). It would be useful to look further at the nutrients consumed with dietary iron and whether this had an effect on the iron status of subjects.

It is recommended that future studies investigating factors that affect iron status use a validated food frequency questionnaire such as an updated version of the food frequency questionnaire developed by Heath et al (2000). One of the major limitations of this study was that we did not investigate oral contraceptive use, blood loss through menstruation or nose bleeds, parity or previous diagnosis of iron deficiency anaemia. Future studies should investigate these factors. In addition socio demographic factors and ethnicity should also be investigated to determine their impact on iron status.

Identifying the factor in meat which enhances non haem iron absorption may be one solution to correcting the world wide problem of iron deficiency. The investigation of the meat extract on iron absorption was a well controlled study using stable isotopes to determine iron absorption. However no significant differences in iron absorption were found between the meat meal, the meat extract meal and the sodium caseinate meal even after adjusting for iron status. Iron status was found to have a large influence on iron absorption. By including only subjects with depleted iron stores (serum ferritin <20μg/L) the effects of iron status on iron absorption would be minimised. Fewer subjects would need to be tested to obtain significant results. However, a larger number of subjects would need to be screened initially to identify those women with depleted iron stores. Adjusting iron absorption for iron status based on serum ferritin levels is dependent on the reliability of serum ferritin values. Serum ferritin levels fluctuated within subjects over a two week period (up to 18μg/L in one case). It is unknown why this occurred, however including more stringent guidelines regarding physical activity levels in the days prior to eating the

meal may have helped reduce this variance. Serum ferritin levels may increase in the days following strenuous exercise (Malczewska *et al.*, 2000a). Finally a more significant increase in iron absorption may have been seen if the meat extract was added to a protein such as soy protein isolate that inhibited iron absorption to a greater extent than sodium caseinate.

4.3 Concluding remarks

Iron deficiency is a problem in developing countries. The prevalence of iron deficiency anaemia in this population group was similar to that seen in other New Zealand studies, but lower than that seen in other Western countries. The incidence of iron deficient erythropoiesis and depleted iron stores was lower compared with similar groups both in New Zealand and other Western countries. This may have been due to the exclusion of subjects who had donated blood in the past 12 weeks and the exclusion of women who did not eat meat. Total dietary intake of iron and age were found to be positively associated with iron status. Dietary intakes of iron appeared low with the majority of women not consuming the RDI for iron and over one fifth of the women not meeting the EAR for iron. Despite this only 13% of women had depleted iron stores or iron deficiency anaemia. The women in this study also appeared to be at high risk of consuming inadequate amounts of folate, calcium and selenium.

Solutions need to be found to improve the iron status of women in both developing and developed countries. Possible solutions include improving the iron bioavailability of the diet by identifying the factor in meat which enhances non haem iron absorption. This research found no significant differences in iron absorption between a meat, meat extract or sodium caseinate meal. However iron absorption was higher from the meat extract and meat meal compared with the sodium caseinate meal. Women with low iron stores showed increased levels of iron absorption compared with women who had higher serum ferritin levels. Further testing with more subjects is needed to determine whether in fact the <0.5kDa sarcoplasmic fraction does enhance non haem iron absorption significantly.

4.4 Further work

Further work will be undertaken to determine whether the <0.5kDa sarcoplasmic fraction increases non haem iron absorption. Nine more subjects with low iron stores will be recruited to enhance the statistical significance of our findings. If the findings show that the meat extract does enhance iron absorption significantly, further research should be undertaken. This should include finding out in which form the meat extract is most acceptable to people and whether the meat extract is able to improve a population's iron status. One research possibility involves providing iron depleted subjects with an iron supplement containing the meat extract and seeing whether this increases iron status as opposed to an iron supplement alone.

The National Nutrition Survey planned for 2008 will provide further up to date data on the prevalence of iron deficiency in New Zealand women. However, further work should be undertaken to determine the factors which determine iron status in a sample of women who are more representative of the New Zealand population. Levels of blood loss through nose bleeds and menstruation should be investigated and a validated food frequency questionnaire used.

Ongoing work continues at Massey University to identify and test the MFP factor and its mechanisms of increasing non haem iron absorption. Extracts which show promising results using in vitro, animal and Caco-2 cell methodology will ultimately need to be tested in human subjects.

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Appendix 5.1

Advertisement and article for the meat study

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Meat Study

The Institute of Food, Nutrition and Human Health at Massey University invites you to take part in a groundbreaking research project investigating iron absorption. We are investigating how eating meat affects the absorption of iron from other food sources. You will get a **free meal** out of this!

Who?

We are looking for young **women aged 20-40** who are not pregnant or breastfeeding or who are not vegetarian. If this sounds like you then we would love to hear from you.

Why?

Iron deficiency is one of the most important nutritional problems in the world, to which young women of reproductive age are particularly susceptible. A major cause of iron deficiency is an inadequate supply of absorbable iron in food. Including meat in the diet somehow increases the absorption of iron from all possible food sources, not just from the meat. We are investigating how this happens.

How?

If you would like to participate, you will first need to answer some simple questions about your normal diet and food habits, and have a blood sample taken by MedLab Central in Palmerston North, to check that your iron stores are within the normal range. You will be briefly examined and interviewed by a qualified medical doctor to make sure you have no conditions which could interfere with the study's results. All information is confidential.

Once accepted into the study you will be asked to eat a diet that is low in iron, based on simple nutritional guidelines appropriate to your own personal requirements, for 5 days. After this you will be given a meal with either no meat, meat, or a meat extract, and have an infusion of a very small amount of iron. We will need to take 5 small blood samples from you over the course of the trial.

Contact

If you are interested please contact the researcher, Kathryn McNaughten, by phoning (06) 350 5904 or emailing K.L.McNaughten@massey.ac.nz or Dr Jane Coad, (06) 350 5962 or J.Coad@massey.ac.nz.

This project has been reviewed and approved by the Manawatu-Wanganui Human Ethics Committee No. 04/02/002.

This project has been reviewed and approved by the Massey University Human Ethics Committee, PN Protocol No. 03/145.

If you have any concerns about the conduct of this research, please contact Professor Sylvia V Rumball, Chair, Massey University Campus Human Ethics Committee: Palmerston North, telephone 06 350 5249, email humanethicspn@massey.ac.nz

Local volunteers wanted for study on iron deficincy

The Human Nutrition Unit at Massey University has become so popular that the unit has set up a database for potential volunteers living in the area, lecturer Kathryn McNaughten says.

She is currently gathering volunteers for a study on iron absorption and meat.

Iron deficiency is one of the most important nutritional problems in the world – young women are particularly susceptible, she says.

Massey's Institute of Food, Nutrition and Human Health is undertaking research to investigate the effect of including a meat extract on the absorption of iron.

There are two sorts of iron in foods, Kathryn says.

Haem iron occurs in animal foods like meat, poultry and fish and is much better absorbed than non-haem iron found in plant and animal foods.

Volunteers will eat a meal containing either no meat, meat or a meat extract. The meat extract contains an unidentified "meat factor" which enhances the body's ability to absorb both types of iron from that meal.

Kathryn is looking for young, local women who are neither vegetarian nor pregnant.

If you are between 18 and 40 years and would like to find out more about the study which has received ethical approval contact her on 350 5904 or K.J.McNaughten@masseyac.nz

IRON INTAKE – Kathryn McNaughten (right) conducts a dietary interview with study participant Sarah Arts to assess much iron is in her diet.

Appendix 5.2

Information sheet and consent form for the meat study

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Effect of meat and meat extract on iron absorption in young women

Information Sheet

Introduction

This is a staff research project being conducted by Kathryn McNaughten (a dietitian), Dr Jane Coad (a human nutritionist) and Dr Roger Lentle (a medical doctor and nutritionist). We all work in the Institute of Food, Nutrition and Human Health, at Massey University. The purpose of this project is to investigate the effect of including a meat extract in a non-meat meal on the absorption of a certain type of iron called non-haem iron.

Iron deficiency is one of the most important nutritional problems in the world, to which young women of reproductive age are particularly susceptible. A major cause of iron deficiency is an inadequate supply of iron in food. Including meat in the diet somehow increases the absorption of iron from all possible food sources, not just from the meat. We are not yet sure how this happens.

The best method of telling how much iron is absorbed from the diet and how the body uses it is to measure the amount of iron that is incorporated into red blood cells. If the iron atoms in the food are a naturally occurring type which is usually found only in very low concentrations, then the passage of this special type of iron can be followed easily as it moves around the body. The iron atoms in the food are effectively "marked".

The meal you will receive in the trial will have iron added to it that has been enriched with this special type of "slightly heavier than normal" iron atom i.e. with a stable isotope of iron. Stable isotopes occur naturally in the environment, are never radioactive, are completely safe and are the same types of atoms that your body uses normally. Stable isotopes have been used as markers to measure nutrition in a number of studies including ones using pregnant women and young babies.

Volunteers for this study will be required to eat a meal containing either no meat, meat, or a meat extract, as well as have an infusion of a solution containing a very small amount of iron. We will need to take 5 small blood samples from you to measure your existing iron stores as well as to see how well the iron we give you in the meal and in the infusion has been absorbed into your red blood cells.

Participant recruitment and selection

We are looking for 18 women aged 20-40 to participate in our study. If you would like to participate, we will first need you to answer some simple questions about your normal diet and food habits so we can estimate the amount of iron you would normally consume. Your weight and height will also be measured at this time. You will need to have a sample of your blood taken by MedLab Central in Palmerston North. This is so we can check that your iron stores are within the normal range. We will also test whether you have had a recent infection that could affect the accuracy of other measurements.

Provided your results are suitable, we will invite you to visit the Human Nutrition Laboratory at Massey University where our doctor will briefly examine and interview you to check your general health. All clinical data will be held by our doctor, who will assign identity numbers to all subjects and is responsible for maintaining your absolute confidentiality.

Some facts about you may mean that we cannot use you in our study. These are:

- you are vegetarian / vegan;
- you have cultural or religious reasons for not eating meat or blood products;
- you are anaemic;
- you have or have had hepatitis;
- you have an allergy to iron containing products;
- you have donated blood during the past 12 weeks or plan to during the duration of the study;
- you smoke;
- you consume more than 2 glasses of wine or 1 pint of beer a day;
- you have been pregnant or breastfeeding in the last year, or plan to become pregnant during the study (as iron absorption is affected by pregnancy);
- you are currently taking iron supplements, and would not be willing to stop using these during the trial as well as for a month before the trial period begins.

If you have any queries about these, please contact us by phone.

All subjects accepted into the study will receive reimbursement for their time and input. This will be in the form of gift vouchers to the value of $50 on completion of the study.

What the study will involve

If the results of your blood test, medical examination and dietary interview are suitable, we will ask you to come into the Human Nutrition Laboratory where we will ask you some further questions about your diet and provide you with dietary education on how to keep your diet low in iron. We will also organise suitable times for you to take part in the trial.

Five days before the meal we will ask you to go on a diet that is low in iron. This diet will be based on your normal diet with a few adjustments. We would like you to keep a record of what you have eaten during this period. We would like you to eat nothing for a 12 hour period before you have the meal. For most of this time you should be asleep anyway, and then just avoiding breakfast on the day of the meal should bring your no-food time up to 12 hours.

On the day of the meal, you will first come to the Human Nutrition Laboratory at Massey University where we will take a blood sample. You will then be given a small infusion of a solution containing iron into the vein of your arm. After the infusion you will then be given the meal to eat. It will be a pasta-based meal with either a meat sauce, a non-meat sauce, or a sauce made with a meat extract added to it depending on which group we have allocated you to.

You will remain at the Human Nutrition Laboratory as a safety precaution for two hours after this, so that we can make sure you have no adverse reactions to the iron infusion. During this time you will be given no other food or drink (other than water) as eating or drinking (especially tea!) can interfere with the absorption of the iron we are trying to measure. While you are waiting we will ask you about what you have eaten over the previous 24 hours, and measure your height and weight again. After the 2 hour waiting period you will be free to go.

We will ask you to come back again after 7 days, 14 days and 21 days, to have another blood sample taken on each of these days in order to measure how well the iron we gave you has been absorbed and used in your body.

The total of your time that we need from you will be around 10 hours, which breaks down as follows:

- Measurements and dietary recall interview: 1 hour
- Initial blood screening: 20 minutes
- Interview and examination with the medical practitioner: 30 minutes
- Dietary questions and education for low iron diet: 1 hour
- Keeping a dietary record: about 15 minutes for each of the 5 days
- Blood sampling, iron infusion, meal consumption and monitoring: 5 ½ hours
- Blood samples at days 7, 14 and 21: 10 minutes x 3

We recognise that this is a considerable amount of time and for this reason we will reimburse you for your time in the manner described above.

All participants in the study will be assigned an anonymous identity number which will be held by the medical practitioner. The original data will be destroyed after a period of 5 years during which time it will be locked up. We hope to publish the information about the overall study findings in a scientific journal but individual participants will not be mentioned. We will make the findings of the study available to individual participants who request them as soon as they are available.

Participant's rights

As a participant in this study, you have the right to:

- decline to participate;
- decline to answer any particular questions (although if you do not want to answer the questions about your health before the study begins we will not be able to recruit you);
- withdraw from the study;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

It is important for you to understand that if, at any point during the study, you decide that you no longer wish to continue, you may withdraw and you do not need to explain to us the reasons. However we would like for you to inform us of this decision, and also to inform us if you are withdrawing because you are unhappy or dissatisfied with the study in any way.

Support processes

If at any time we discover facts about your health that require further action, the study doctor will contact you regarding them and ask whether you would like him to communicate them to your own doctor.

Project contacts

You may have several questions relating to this study, whether or not you subsequently choose to participate. If you wish to discuss any aspect of this study further, or you have any concerns or queries, please contact either:

Kathryn McNaughten
Massey University
(06) 350 5904
K.L.McNaughten@massey.ac.nz

or

Dr Jane Coad
Massey University
(06) 350 5962
j.coad@massey.ac.nz

If you do wish to participate, please contact Kathryn McNaughten within a week of receiving this information sheet and she will arrange a time for you to visit the Human Nutrition Lab to sign the enclosed consent form in the presence of one or more of the researchers. This form simply confirms that you have read and understood the information about this study, and that you have agreed to participate. At this time we will also complete the dietary interview and measurements, and give you a lab form to take to MedLab Central.

If your results of the above are suitable for the study we will ask you to attend a medical screening with our doctor. Following this, we will contact you again to let you know that if you have been accepted into the study and to organise suitable times for taking part in the remainder of the study.

This project has been reviewed and approved by the Manawatu-Wanganui Human Ethics Committee No. 04/02/002.

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Effect of meat and meat extract on iron absorption in young women

CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR A PERIOD OF FIVE (5) YEARS

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

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

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

Full Name – printed: .....

Postal Address for Correspondence: .....

.....

.....

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Appendix 5.3

Interview schedule for the Meat Study including the food frequency questionnaire

Meat Study - Interview Schedule

Name

Code

Contact Address

Contact Phone Number

Meat Study – Screening Questionnaire

Thank you volunteering to take part in the meat study. The meat study consists of 3 screening processes – a lifestyle assessment, a blood test to select people with low iron stores and a medical screening. If you are accepted into and complete the study we will give you gift vouchers to the value of $50.

The lifestyle assessment will take place today. If you fit the criteria for our study we will arrange for you to have a blood test taken at Med Lab Central. We are looking for women with normal haemoglobin levels and serum ferritin levels in the range between the 10^{th} and 50^{th} percentile normal for your population group.

Subjects with serum ferritin values below the 10^{th} percentile (or less than 12g/L) will be excluded as such values indicate clinical iron deficiency. Women with serum ferritin levels above the 50^{th} percentile will also be excluded as they have adequate iron stores and therefore will absorb less measurable amounts of iron.

We will inform you of the results of your iron tests and whether you meet the criteria to continue with the study.

The lifestyle assessment consists of some questions regarding your dietary habits and lifestyle, a height and weight measurement, and a 24 hour diet recall. You may decline to answer any question at any time. I will begin the lifestyle assessment now.

Are you between 20 and 40 years of age?
What is your date of birth?

Are you pregnant or breastfeeding or have you been pregnant / breastfeeding in the past year?

Are you planning on becoming pregnant in the next 6 months?

Are you vegetarian or vegan?

Do you eat meat and meat products?

Do you eat pasta and tomato based pasta sauces?

Do you consume more than 2 glasses of wine or 1 pint of beer per day?

If so, would you be prepared to stop this for the duration of the trial?

Do you smoke?

Are you taking iron supplements?

If so, would you be willing to stop them for a month before the trial period begins and during the trial period?

Are you taking multivitamins?

If so, would you be willing to stop them for a month before the trial period begins and during the trial period?

Do you take any other supplements?

Do you have any allergies to iron containing products?

Have you donated blood in the past 12 weeks or do you plan to donate blood during the next 6 months?

How many hours per week do you exercise and what form of exercise is this?

How many hours per week would you run?

24 hour recall

The next part of the dietary screening involves a 24 hour recall. This will provide us with information on your diet and enable us to get a picture of what women aged 20 – 40 years are eating. The dietary recall asks you to recall all you have eaten in the past 24 hours.

I now have some further questions about your diet....

How often do you eat meat or meat products (excluding chicken, fish, seafood, liver and kidneys or pate)?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat chicken?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat fish / seafood?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat liver or kidneys?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you consume pate?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat eggs?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat soy products (for example soy milk, tofu)?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you drink tea?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

What type of tea do you drink?

How often do you drink coffee?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you consume milk, yoghurt, dairy foods, milk puddings and cheeses?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

If 2 or more times per day, how many serves of these products would you have per day? (a serve equals 1 glass of milk, 1 pottle yoghurt, 2 slices of cheese or 2 scoops of ice cream)

On average, how many servings of fruit (fresh, frozen, canned or stewed) do you eat per day? (a serving = 1 medium piece Or 2 small pieces of fruit OR ½ cup stewed fruit) eg. 1 apple + 2 small apricots = 2 servings

- o I don't eat fruit
- o Less than 1 per day
- o 1 serving
- o 2 servings
- o 3 or more servings

How often do you eat the following fruits: oranges, tangelos, mandarins, grapefruits, kiwifruit, strawberries?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat dried fruit?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you drink fruit juices or cordial?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

On average, how many servings of vegetables (fresh, frozen, canned) do you eat a day? (a serving = 1 medium potato / kumara OR ½ cup cooked vegetables OR 1 cup of salad vegetables). eg. 2 medium potatoes + ½ cup peas = 3 servings)

- o I don't eat vegetables
- o Less than 1 per day
- o 1 serving
- o 2 servings
- o 3 or more servings

How often do you eat the following vegetables: broccoli, spinach, silverbeet, cabbage, capsicum, brussel sprouts, or cauliflower?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat marmite or vegemite?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

On average, how many servings of breads and cereals do you eat per day?
(a serving = 1 medium slice of bread, 1 roll, 1 cup cornflakes, ½ cup muesli or porridge, 1 cup cooked pasta or rice, 2 plain sweet biscuits)

- o Less than 1 per day
- o 1-2
- o 3-4
- o 5-6
- o 7 or more

Do you eat cereals and breads that you know are fortified with iron?

- o Yes
- o No
- o Unsure

How often do you eat nuts, including peanut butter?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat seeds including sesame seeds and poppy seeds?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you eat wheat bran cereal or wheat germ?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

How often do you consume beans, pulses and legumes (including baked beans)?

- o 2 or more times per day
- o Once per day
- o 5-6 times per week
- o 2-4 times per week
- o 1-3 times per month
- o Less than once a month
- o Never

Thank you for that information. I will now take your height and weight.

Height

Weight

The next part of the screening process involves a blood screening at MedLab Central. You will need to take this form to Med Lab (you do not need to be fasted).

We will contact you once we have received the results and inform you of your results and whether we would like you to continue to the medical screening process and arrange a suitable time for this. Following acceptance into the study, we will ask some further dietary questions and provide dietary education for keeping your diet low in iron for the 5 days before the study.

Thank you for your time. If you have any questions please don't hesitate to contact me.

Appendix 5.4

Letters to participants regarding iron status and inclusion / exclusion to the Meat Study

Massey University
COLLEGE OF SCIENCES

INSTITUTE OF FOOD, NUTRITION
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Private Bag 11 222
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T 64 6 350 4336
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www.massey.ac.nz

Kathryn Beck
Institute of Food, Nutrition and Human Health
Massey University
K.L.Beck@massey.ac.nz
Ph (09) 414 0800 ext 41210

Date

Name
Address

Re: Meat Study

Dear

Thank you for attending the dietary interview and blood screening for the Meat Study.

Your results were as follows:

Serum ferritin	ug/L	(20-200)
Haemoglobin	g/L	(115-155)

Your serum ferritin results are between the 10^{th} and 50^{th} percentile for your age group. We would like to invite you to continue with the Meat Study and attend a medical screening at the Institute of Food Nutrition and Human Health. I will contact you in the next few days to organise a suitable time to attend this appointment. Please contact me if you have any further questions.

Thank you once again.

Yours sincerely

Kathryn Beck

Massey University
COLLEGE OF SCIENCES

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AND HUMAN HEALTH
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Kathryn Beck
Institute of Food, Nutrition and Human Health
Massey University
K.L.Beck@massey.ac.nz
Ph (09) 414 0800 ext 41210

Date

Name
Address

Re: Meat Study

Dear

Thank you for attending the initial dietary screening and blood screening for the Meat Study.

Your blood results were as follows:

Serum Ferritin	ug/L	(20-200)
Haemoglobin	g/L	(115-155)

These results show that your serum ferritin levels are above the 50th percentile for your age group and that you have adequate iron stores. Because of this you are less likely to absorb iron as well, so will not be suitable for our study.

Thank you once again for your time and for volunteering to take part in the study. Please contact me if you have any further questions.

Yours sincerely

Kathryn Beck

Massey University
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Massey University
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Ph (09) 414 0800 ext 41210

Date

Name
Address

Re: Meat Study

Dear

Thank you for attending the dietary interview and blood screening for the Meat Study.

Your results which are attached to this letter are suggestive of iron deficiency. If you would like to see our medical doctor at the Institute of Food, Nutrition and Human Health to discuss the possible relevance of these results please contact me. Otherwise we would recommend that you visit your own GP with this letter and results. Please contact me if you have any further questions.

Thank you once again.

Yours sincerely

Kathryn Beck

Massey University
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Date

Name
Address

Re: Meat Study

Dear

Thank you for attending the initial dietary screening and blood screening for the Meat Study.

I have enclosed a copy of your blood results. These results show that your serum ferritin levels are above the 50th percentile for your age group and that you have adequate iron stores. Because of this you are less likely to be efficient in absorbing iron, so will not be suitable for our study.

Your serum ferritin levels are somewhat high. The high iron level is most likely to be due to you taking more iron than you need, for example, taking dietary supplements or foods fortified with iron. There is one medical condition, haemochromatosis that can do this. If your family history suggests this might be a possibility you should take these results to your own GP.

Thank you once again for your time and for volunteering to take part in the study. Please contact me if you have any further questions

Thank you once again.

Yours sincerely

Kathryn Beck

Appendix 5.5

Dietary guidelines for the low iron diet

Meat Study - Guidelines for keeping your diet low in iron for the 5 days prior to the study

For the 5 days before eating the study meal we would like you to keep your intake of iron low. Following a diet that is low in iron for a short period of time is not harmful. By keeping your intake of iron low you are more likely to absorb the iron in the test meal. The instructions below will help you to keep your intake of iron low. If you have any questions please contact Kathryn McNaughten on 350 5904.

1) Avoid eating all red meats, liver and kidneys, chicken, fish and seafood. This includes products made out of red meat including ham, bacon and pate. To ensure you obtain adequate protein in your diet you can substitute meat with eggs, beans, chickpeas, tofu, peas, lentils, nuts and seeds. Dairy products are very low in iron. Some recipes are included below to give you ideas.

2) Avoid eating iron enriched breads, crackers and breakfast cereals. The easiest way to do this is to avoid foods that claim to be a good source of iron.

3) Breads that are fortified with iron include Tip Top 'Mighty White', Tip Top Holsums bread (all varieties) and Quality Bakers 'Vita Rich' bread – avoid eating these.

4) Crackers that are fortified with iron include VitaLife 'Well Grain crackers' – avoid eating these.

5) The table below gives a guideline of cereals that should be avoided and cereals that can be eaten freely.

	Cereals to avoid	**Cereals that can be eaten freely**
Cornflakes	Kelloggs Cornflakes Skippy Cornflakes Budget Cornflakes Pams Cornflakes Kelloggs Crunchy Nut Cornflakes	Sanitarium Kornies
Rice Bubbles	Sanitarium Ricies Pams Rice Snaps Kelloggs Rice Bubbles	
Honey Puffs		Sanitarium Honey puffs Hubbards Honey Bumbles Pams Honey snaps Kelloggs Honey Krispex
Coco Pops	Hubbards Bugs 'n' Mud Hubbards Coco Crunch Kelloggs Coco pops Nestle Milo Kelloggs Fruit Loops Kelloggs Frosties	Lowans Whole foods Coco Bombs Pams Coco Snaps

Weet Bix	Sanitarium Weet Bix Crunch Sanitarium Weet Bix	Pams Wheat Biskits
Nutri Grain	Kelloggs Nutrigrain Pams Nutra Bites	
Light / Fruity cereals	Sanitarium Light 'n' Tasty Kelloggs Sustain Kelloggs Just Right Kelloggs Special K Hubbards Manuka and Honey Flakes	Hubbards Light & Right Pam's Fruit and Fibre Pam's Lite and Fruity Hubbards Very Fruitful Flakes Hubbards Fruitful Lite Hubbards Fruitful Breakfast Hubbards Berries, Apples and Cinnamon Hubbards Berry Berry Nice Hubbards Yours Fruitfully Healtheries Simple Tropical Cereal Weight Watcher's Cereal Tasti Cereal Sunreal Tri Grain Vogels Wildberry and Apple Flakes
Brans	Kelloggs Body Smart Wheat Bran Flakes Sanitarium Bran and Sultanas Kelloggs Sultana Bran Sanitarium SanBran Hubbards Bran and Apricot Kelloggs All Bran Sanitarium Bran Flakes	Pams Bran and Sultana Vogels Bran with Soy and Linseed Hubbards Oat Bran Muesli Sanitarium Bran flakes,
Muesli's		Vogels Natural Muesli Vogels Café Style Luxury Muesli Sunreal Muesli Healtheries Simple Apricot and Coconut Muesli Healtheries Simple Tropical Cereal Pam's Natural Breakfast Muesli Pam's Toasted Breakfast Muesli Naked Muesli Hubbards Simply Toasted Muesli Budget 98% Fat Free Tropical Fruit muesli Pam's Deluxe Muesli Organic Crunchy Granola Muesli Rutherford and Meyer's Toasted Fruit Muesli (tropical and orchard flavour) Sanitarium Muesli's (golden toasted, simply natural, toasted lite, fruity delight, naturally unsweetened)
Other Cereals	Kelloggs Crunchy Nut Clusters Vogels Summer Crunch	Pams Nutty Crunch Pams Nutberry Crunch Hubbards Outward Bound Cereal Sanitarium Puffed Wheat Alison Holst Cereal Lowan Soyflakes Vogels Soy Grain and Flakes
Oats / porridge	Oaties Instant Hot Oats Original	Uncle Toby's Flemings (Oats,

	flavour	Rolled Oats, Milk Oaties, Fine and Medium Ground Creamota Oats, Wholegrain Oats) Flemings OatBran Vogels Rolled Oats Vogels Summer fruit and Mixed grain porridge Vogels Manuka Honey and Apricot Porridge Vogels Café Style Porridge Hubbards Fruitful Porridge Lowan Rice porridge with orchard fruit Harraways Scotch Oats Harraways Oats – Singles Harraways Rolled Oats Pams MacOaties Creamy Porridge Pams Rolled Oats Oaties Instant Hot Oats – apple, apricot and grains, wild berry flavours

6) Other breakfast ideas include porridge, bran, toast, muffins, or crumpets, pancakes, baked beans, spaghetti, banana smoothie, yoghurts.

7) Avoid eating dried fruits (except for those in cereals) – these contain high levels of iron.

8) Vitamin C enhances iron absorption. We would like you to keep your intake of Vitamin C to a minimum. To do this, avoid drinking juice and cordial drinks. Have no more than 2 serves of fruit per day (a serve is the amount that fits into the palm of your hand) and no more than 3 serves of vegetables per day. Avoid the following fruits and vegetables as they contain high levels of Vitamin C.

Fruits to Avoid

Citrus fruits	Mango
Pineapple	Pawpaw
Melon	Fejoas
Passionfruit	Tamarillo
Kiwifruit	Strawberries
Cherries	Currants
Other berries	

Vegetables to Avoid

Capsicum	Parsley
Basil	Spinach
Silver beet	Brussel sprouts
Cabbage	Cauliflower

Broccoli	Chinese green vegetables
Kumara	Leeks
Tomato	Cassava
Spring onion	Radishes
Broad beans	Garlic (up to 1 clove/day acceptable)
Leeks	Pumpkin (acceptable if boiled)
Swede	Taro leaves
Turnip	Water cress

9) Avoid Milo and Bournvita (high in vitamin C) – Ovaltine, chocolate drinking powder and Cocoa are good substitutes. If you do have Milo, have no more than 1 teaspoon per day.

10) Avoid Marmite, Bovril, treacle and the use of meat based Oxo cubes in cooking. Vegemite is OK.

11) Avoid cooking with cast iron cook ware. Stainless steel pots and pans are OK to use.

Summary Table of Foods to Eat / Foods to Avoid

Food groups	Foods to avoid	Foods to eat freely
Breads and cereals	Iron fortified breakfast cereals, breads and crackers – see above	Cereals in eat freely column muffins, crumpets, pancakes, piklets, spaghetti, rice, pasta, couscous, cakes, biscuits
Vegetables	Spinach, silver beet, cabbage, Chinese green vegetables, broccoli, parsley, basil, capsicum, brussel sprouts, cauliflower, kumara, cassava, tomato, spring onion, radishes, broad beans, garlic, leeks, pumpkin, swede, taro leaves, turnip, watercress	All other vegetables including artichoke, asparagus, beans, beetroot, carrots, celery, chicory, choko, courgettes, cucumber, gherkin, lentils, lettuce, marrow, mushrooms, onion, parsnip, peas, squash, taro, yam, potatoes, corn
Fruit	Citrus fruits, kiwifruit, strawberries, other berries, cherries, currants, mango, pineapple, pawpaw, melon, dried fruit, cherries, fejoas, passionfruit, tamarillo	All other fruits including apples, apricots, avocadoes, banana, figs, grapes, lychees, nectarines, olives, peaches, pears, persimmon, plums, rhubarb, watermelon
Meat	Red meat, chicken, fish, seafood, ham, bacon, salami, sausages, pate, liver, kidneys	Beans, chickpeas, tofu, peas, lentils, nuts, seeds, eggs
Dairy products	-	Eat all freely
Drinks	Milo, Bournvita, orange, citrus, tomato and blackcurrant juices, cordials, orange flavoured soft drinks	Ovaltine, chocolate drinking powder, cocoa, coffee, tea, water, milk based drinks, soft drinks
Other	Marmite, Bovril, treacle, Meat based oxo cubes	Vegemite, peanut butter, honey, jam, margarine, butter, cooking oils, sugar, salt, pepper

Recipes for the low iron diet

Burritos - serves 4-6

Olive or canola oil spray
450g can refried beans
2x 425g cans of chilli beans
375g enchilada sauce
Chilli powder (optional)
2 cups cooked rice
10 burrito tortillas
½ cup grated cheese
½ cup lettuce, shredded
3 carrots (grated or cut into thin strips)
2 cucumbers, cut into thin strips

Preheat oven to 180C. Lightly spray a large ovenproof dish with oil. Combine refried beans, chilli beans, and half the enchilada sauce in a large mixing bowl. Add a pinch of chilli powder, if desired. Divide both the bean mixture and the rice into 10 equal portions. Place a portion of the bean mixture across the centre of a tortilla. Place a portion of rice next to the beans, and roll up the tortilla. Place the burrito in the prepared dish. Repeat until all tortillas are filled. Pour remaining enchilada sauce over the burritos, and sprinkle with cheese. Bake for 25 minutes, until cheese is melted and golden. Combine remaining ingredients to make a salad. Serve burritos with salad on the side.

Penne primavera – serves 4-6

500g penne
2 large carrots, halved lengthways and thinly sliced
2 large zucchini, halved lengthways and thinly sliced
125g snow peas, halved diagonally
1 Tbsp corn flour
375ml can Carnation Light and Creamy evaporated milk
2 Maggi Vegetable stock cubes
1 ½ Tbsp finely grated parmesan cheese, optional

Cook the pasta according to packet instructions. Steam or microwave the vegetables until just tender and brightly coloured, taking care not to overcook. Put corn flour in a small bowl and gradually add 1/3 cup evaporated milk, stirring until smooth. Add remaining evaporated milk to a small saucepan, and crumble in the stock cubes. Add corn flour mixture to the saucepan and stir over medium heat until the mixture boils and thickens. When the pasta is cooked, drain and serve with sauce, topped with the vegetables and a sprinkle of Parmesan cheese. Serve immediately.

Macaroni cheese

1½ cups macaroni
4 Tbsp butter
4 Tbsp flour
2 cups milk
1 tsp dry mustard
salt and pepper
½ - 1/3 cup grated cheese

Melt butter in pan. Add flour and dry mustard, cook for 1 minute and stir in the milk. Stir until boiling. Reduce heat. Add cooked macaroni, cheese, seasonings and any desired optional ingredients (eg. onion). Mix well together and serve.

Bean and nut burgers – makes 4 burgers

½ cup cooked peanuts
½ cup soya beans
2 Tbsp toasted sesame seeds, ground in blender
¼ cup toasted sunflower seeds
1 onion, grated
1 carrot, grated
1 celery stalk, chopped finely
2 garlic cloves, crushed or minced
1 egg
Pinch of salt

Place cooked peanuts and soya beans in a blender and process until smooth, to produce 1 cup. Add remaining ingredients and bind together with the egg. Shape into patties and cook in a little oil. Serve with a hamburger bun, lettuce and a small amount of tomato sauce.

Fried rice – serves 4

1 Tbsp olive / peanut oil
1 clove garlic - optional
1-2 onions, finely diced
½ cup frozen peas
1 cup chopped mushrooms
1 whole egg, lightly beaten
2 cups cooked rice
1 Tbsp soy sauce
2 tsp sesame seeds
Vegetables – a selection of green beans, carrot sticks, celery slices, courgettes

Heat oil in a large pan. Add onion and garlic and lightly stir fry for 2 – 3 minutes. Add remaining vegetables and stir fry for 2 – 3 minutes. Move vegetables to the side and pour in egg. Allow the egg to cook and scramble throughout the vegetables. Finally, stir in the rice and heat thoroughly. Add soy sauce and sesame seeds just before serving.

Vegetarian quick chilli tacos – serves 4

8 taco shells
1 x 400g can chilli beans, pureed, mashed or whole
1 Tbsp corn flour
½ lettuce
1 grated carrot
1 cup grated cheese

Place beans in a saucepan and heat gently. Mix corn flour with a little water and stir into beans. Allow to cook for 5 minutes or until thickened. Place taco shells in the oven at 150C for 5 minutes (do not allow them to soften). Remove from oven and place bean mixture in base of shell. Top with lettuce, carrot and cheese. Return to oven just to melt the cheese (1-2 minutes) and serve immediately. Serve with sour cream or avocado mashed with lemon juice.

Nachos

Nacho chips
Spray of oil
1x 400g can chilli beans
½ onion
2 cloves garlic
1 grated carrot

Stir fry onion and garlic in a saucepan. Add chilli beans and grated carrot (if desired) and heat. Serve with nacho chips and sour cream / avocado.

Penne with pumpkin sauce – serves 4

500g penne pasta

500g pumpkin, chopped
2 onions, finely chopped
1 tsp ground cumin
½ tsp nutmeg
½ tsp chilli powder
1 cup / 250ml reduced fat milk
2 Tbsp grated parmesan cheese
Freshly ground black pepper

Cook pasta in boiling water following packet instructions. Drain, set aside and keep warm. To make sauce, cook pumpkin in boiling water for 5 minutes or until soft. Drain well and mash. Place onions, cumin, nutmeg and chilli powder in a non stick frying pan and cook over a medium heat for 4 minutes or until onions are soft. Add pumpkin, stir in milk and cook for 3 minutes or until sauce is heated through. Spoon sauce over pasta and sprinkle with parmesan cheese and black pepper to taste.

Pita pizzas

8 pita rounds
425g spaghetti
1 cup grated cheese
Add cooked vegetables, olives, onions, spring onions

Toast the pita breads on one side by placing them under a hot grill for about 2 minutes. Turn them over and top with spaghetti, cheese and other fillings. Grill in a hot oven for 5 minutes until the cheese has melted and is golden brown.

Rice salad – 4-6 servings

2-3 cups cooked brown or white rice
1-2 carrots, grated
1-2 stalks celery, sliced
2-3 spring onions, thinly sliced
½ cup oil
¼ cup wine or cider vinegar
1 Tbsp mixed mustard
2 tsp sugar
1 tsp salt

Leave freshly cooked rice to stand for at least 10 minutes, then toss lightly with a fork to separate the grains. Fold in the carrot and celery, and the white and green parts of the spring onion. Add fresh herbs if desired. Shake the remaining ingredients together in a screw top jar. Toss about half this dressing through the salad, stopping when you like the flavour. Serve immediately or refrigerate in a covered container for up to 2 days. Add more dressing and extra vegetables before serving, if necessary.

Variations – add cucumber, avocado, bean sprouts, or chopped roasted peanuts.

Wholemeal Quiche

Base
250g wholemeal flour
125g butter or margarine
¾ cup grated cheese
2 Tablespoons cold water (approx)

Rub butter into whole meal flour, add cheese and water mixing to a stiff dough. Refrigerate ½ hour. Roll out and line a 23 x 30cm pie dish.

Filling
1 onion chopped
1 Tbsp butter
1 vegetable stock cube
Tin drained asparagus
2 egg
1 cup milk
1 cup grated cheese

Saute onion in butter, crumble in vegetable stock cube and spread over pastry. Spread asparagus on top. Beat eggs and milk and pour over. Sprinkle with cheese and bake at 220C for 10 minutes then at 180C for 30 minutes, until filling is set.

Other tips

- Mince can be replaced with brown or green lentils or textured vegetable protein
- Tofu is a great substitute for chicken
- Canned lentils, kidney beans and 3-bean mixes are quick and easy to use in cooking.

Appendix 5.6

Instructions for keeping the food record

Meat Study - Five Day Diet Record

- Record all that you eat and drink for the five days prior to coming in to eat the meal.
- If possible record food at the time of eating.
- Include all meals, snacks, and drinks.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.

Describing Food and Drink

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

- Example: Cheese – Mainland, Edam
  Milk – Pam's whole milk
  Breakfast cereal – Sanitarium Natural Muesli
  Pasta – Wholegrain pasta

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

- Record recipes of home prepared dishes where possible and the proportion of the dish you ate

Recording the amounts of food you eat

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

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

- By weight marked on the packages – eg. a 425g tin of baked beans, a 32g cereal bar, 600ml Coke

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

- For bread – describe the size of the slices of bread (eg. sandwich, medium, toast)

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

Please eat as normally as possible while following the instructions for the low iron diet. That is, don't adjust what you would normally eat just because you are keeping a diet record.

Date_____

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
Exanple: 7:55 AM	Pizza, vegetarian, home made- (recipe attached) microwaved	1 slice (1/8 of pizza)
" "	Orange juice (Citrus Tree with added calcium- nutrition label attached)	1 glass (275 ml)
10 AM	Raw Apple (gala)	Ate all of apple except the core, Apple was 125g

Date_____

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

Appendix 5.7

Copy of the product analysis certificate and product bulletin, Tatua 100 (sodium caseinate)

TATUA CO-OPERATIVE DAIRY COMPANY LIMITED

Tatua NUTRITIONALS

PRODUCT ANALYSIS CERTIFICATE
TATUA 100

Product Description: Sodium Caseinate Commodity Number: R0519 PAC ID: T1000-P01-04
Batch Number: B5072 Manufacture Date: 3 October 2005
Place of Manufacture: Tatua Co-Operative Dairy Company, State Highway 26, Tatuanui, Morrinsville, New Zealand

Parameter	Unit of Measure	Analysis
Chemical Analysis		
Protein (Dry Basis)	% m/m	94.9*
Fat	% m/m	0.8*
Moisture	% m/m	4.6
pH		6.7
Ash	% m/m	3.3*
Heavy Metals as Lead	mg/kg	<5**
Arsenic	mg/kg	<0.20**
Inhibitory Substances	IU/ml	Not Detected
Pesticides	mg/kg	Not Detected**
Physical Properties		
Appearance		White Powder
Typical product meets flavour/aroma standard: Slight sweet, slight to moderate barny, slight sour. No strong barny. No phenolic.		Typical
Foreign Matter	/50g	Not Detected
Scorched Particles	/50g	A
Bulk Density	g/ml	0.49
Microbiological Analysis		
Aerobic Plate Count	cfu/g	10
Coliforms	/g	Not Detected
E. Coli	/g	Not Detected
Yeasts and Moulds	cfu/g	<1
Coagulase Positive Staph aureus	/g	Not Detected
Salmonella	/750g	Not Detected
Listeria	/25g	Not Detected

*Attributes tested periodically. Results taken from: B0080
**Attributes tested periodically. Results taken from: B5004
This report may not be altered or reproduced except in full.
Tatua Co-operative Dairy Company Ltd. hereby certifies that this batch has been sampled and tested in accordance with the specification by IANZ registered laboratories. All facilities and systems that ensure the delivery of safe product, truth of labelling and compliance with NZFSA standards are subject to regular inspection by NZFSA Dairy. Similarly, the same facilities and systems are subject to external Quality Systems audits in accordance with ISO 9001(2000).

Providing the product is stored in conditions below 25°C and less than 65% Relative Humidity, the above product may be kept for 24 months from the manufacturing date.

New Zealand BSE/TSE Status
New Zealand is free from bovine spongiform encephalopathy, scrapie and chronic wasting disease. These diseases are known as transmissible spongiform encephalopathies, or TSEs. The New Zealand Ministry of Agriculture and Forestry have a comprehensive TSE preventive/surveillance programme in place to prevent the entry and spread of TSE agents. There is also targeted surveillance of susceptible livestock - cattle, sheep, goats and deer. Contingency plans for dealing with any suspect cases in livestock have also been developed.

I hereby declare that this is a true, complete and accurate copy of this record.

Prepared By: Title: LAB TECHNICIAN Date: 31 October 2005

Released By: Title: LABORATORY SUPERVISOR Date: 31 October 2005

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PRODUCT BULLETIN

TATUA 100 - SODIUM CASEINATE

Tatua 100 is a spray dried milk protein with excellent flavour properties. The extensive list of product characteristics for Tatua 100 attests to the flexibility of this sodium caseinate in a wide range of applications. Notably, Tatua 100 has a bland flavour and excellent flavour stability therefore its uses extend to flavour sensitive formulations.

Characteristics

- Very good emulsifying properties
- Good retort stability
- Low flavour profile
- Good alcohol stability
- Excellent water solubility
- Excellent whipping properties
- Fine particle size

Applications

- Bakery glazes
- Coffee whiteners
- Cream liqueurs
- Whipped toppings
- Gravy and sauce mixes
- Yoghurt
- Bakery mixes
- Pharmaceutical applications
- Confectionary coatings

Typical Compositional Data

Chemical Analysis	Unit	Value
Energy	kj/100g	1610
Protein (TN x 6.38)	%	93.3
Protein (dry basis) (TN x 6.38)	%	97.6
Moisture	%	4.3
Ash	%	3.6
Fat	%	0.6
Lactose	%	0.1
pH (5 % at 20°C)		6.7
Antibiotics as inhibitory substances	iu/ml	<0.003
Trans fatty acid content	% of total fat	0.3

Mineral Analysis	Unit	Value
Calcium	mg/100g	32
Iron	mg/100g	0.4
Magnesium	mg/100g	1.6
Phosphorus	mg/100g	770
Potassium	mg/100g	2.9
Sodium	mg/100g	1200
Zinc	mg/100g	4.3

Microbiological Analysis	Unit	Value
Aerobic plate count	cfu/g	<1000
Coliforms	/g	ND
E. coli	/g	ND
Yeasts and moulds	cfu/g	<10
Coagulase +ve S. aureus	/g	ND
Salmonella	/750g	Absent
Listeria	/25g	Absent

Nutritional Data

	Unit	Value
Calories	kcal/100g	385
Calories from fat	kcal/100g	5
Total fat	g/100g	0.6
Saturated fat	g/100g	0.5
Cholesterol	mg/100g	28.7
Sodium	mg/100g	1200
Total carbohydrate	g/100g	<0.5
Dietary fibre	g/100g	0
Sugars	g/100g	<0.5
Protein	g/100g	93.3
Vitamin A	iu/100g	<10
Vitamin C	mg/100g	<1
Calcium	mg/100g	32
Iron	mg/100g	<1

Vitamins	Unit	Value
Vitamin A	iu/100g	<10
Thiamine (Vitamin B_1)	mg/100g	<0.01
Riboflavin (Vitamin B_2)	mg/100g	0.3
Niacin (Vitamin B_3)	mg/100g	1.5
Pyridoxine (Vitamin B_6)	mg/100g	0.1
Folic acid	µg/100g	30.3
Vitamin C	mg/100g	<1
Vitamin D_3	µg/100g	<0.5
Vitamin E	mg/100g	<0.1

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PRODUCT BULLETIN

TATUA 100 - SODIUM CASEINATE

Amino Acid Composition (mg/g of sample)

Amino Acid	Total
Alanine	29.0
Arginine	35.0
Aspartic acid	69.5
Cystine	7.3
Glutamic acid	231.7
Glycine	17.3
Histidine	26.3
Isoleucine	41.0
Leucine	91.7
Lysine	75.0
Methionine	28.0
Phenylalanine	48.7
Proline	106.7
Serine	56.3
Threonine	41.7
Tryptophan	12.0
Tyrosine	53.7
Valine	52.7
Total	**1023.5**

Fatty Acid Profile (mg/100g)

Fatty Acid Profile		Value
Butyric	C4:0	24.0
Caproic	C6:0	15.2
Caprylic	C8:0	9.6
Capric	C10:0	26.4
Lauric	C12:0	34.4
Myristic	C14:0	92.8
Pentadecanoic	C15:0	8.8
Palmitic	C16:0	217.6
Margaric	C17:0	4.0
Stearic	C18:0	80.8
Arachidic	C20:0	8.0
Decenoic	C10:1	1.6
Myristoleic	C14:1	8.8
Pentadecenoic	C15:1	1.6
Hexadecenoic	C16:1	10.4
Heptadecenoic	C17:1	1.6
Oleic	C18:1n-9	132.0
Linoleic	C18:2n-6	14.4
Alpha Linolenic	C18:3n-3	8.0

Physical Properties

Appearance:	White to pale cream coloured powder
Bulk density:	0.49 g/ml (35 tap method)
Sediment (50g):	A disc

Quality Manufacturing Process

- This caseinate is produced under food grade conditions to accredited ISO9001 standards
- The final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognized procedures
- Each package is identified enabling trace back

Packaging and Storage

Standard pack size is 20.0 kg net. The pack is a multi-wall bag that incorporates a moisture barrier and an inner polyethylene bag liner. No staples or metal fasteners are used. For adequate protection on storage, it is recommended to maintain temperatures below 25°C, relative humidity below 65%, and an odour free environment. Packages should not be in direct contact with walls or floors. Stock should be used in rotation. Product has a minimum shelf-life of 24 months.