# 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**

**Kathryn Louise Beck**

**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.

# Table of Contents

**Introduction and organisation of thesis** ..... 1

**Chapter 1. Review of literature** ..... 2
**1.1 Iron – an introduction** ..... 2
**1.2 Functions of iron** ..... 2
**1.3 Iron distribution in the body** ..... 2
**1.4 Iron requirements for adult females** ..... 3
1.4.1 Recommended dietary iron intakes for adult females ..... 4
**1.5 Iron deficiency anaemia** ..... 5
1.5.1 Prevalence of iron deficiency ..... 5
1.5.2 Stages of iron deficiency anaemia and assessment of iron status ..... 5
1.5.3 Iron status and intakes of New Zealand females ..... 6
1.5.4 Risk factors for developing iron deficiency ..... 10
1.5.5 Consequences of iron deficiency ..... 11
1.5.6 Treatment of iron deficiency ..... 11
**1.6 Iron excess** ..... 12
**1.7 Mechanism of iron absorption** ..... 13
**1.8 Factors regulating iron absorption** ..... 15
1.8.1 Host related factors ..... 15
1.8.2 Diet related factors ..... 17
**1.9 Methods for estimating non haem iron absorption** ..... 19
**1.10 Methods that do not use isotopes** ..... 20
1.10.1 Chemical balance technique ..... 20
1.10.2 Rate of haemoglobin repletion ..... 21
1.10.3 Changes in serum ferritin/haemoglobin ..... 21
1.10.4 Plasma appearance/disappearance ..... 22
**1.11 Methods that use isotopes** ..... 22
1.11.1 Whole body counting ($^{59}$Fe) ..... 23
1.11.2 Faecal monitoring (radio and stable isotopes) ..... 23
1.11.3 Plasma appearance/disappearance ..... 24
1.11.4 Haemoglobin incorporation (radio and stable isotopes) ..... 24
**1.12 Analytical methods for stable isotope analysis** ..... 26
1.12.1 Thermal ionisation mass spectrometry ..... 26
1.12.2 Inductively coupled plasma mass spectrometry ..... 27
**1.13 Issues related to the measurement of iron absorption** ..... 28
1.13.1 Iron status of subjects ..... 28
1.13.2 Single meals versus whole diet ..... 29
1.13.3 Composition of meals being tested ..... 29
**1.14 Dietary factors affecting non haem iron absorption** ..... 29
**1.15 Meat, fish and poultry** ..... 30
1.15.1 Studies using single meals ..... 30
1.15.2 In vitro studies ..... 39
1.15.3 Studies using complete diets ..... 39
1.15.4 Effect of meat on iron status – intervention studies ..... 40
1.15.5 Effect of meat on iron status – observational studies ..... 40
1.15.6 The New Zealand Situation ..... 42

**1.16 Meat, fish, poultry factor (MFP factor)** ..... 42
**1.17 Ascorbic acid** ..... 47
1.17.1 Studies using single meals ..... 47
1.17.2 Ascorbic acid versus meat ..... 62
1.17.3 Studies using complete diets ..... 62
1.17.4 Effect of ascorbic acid on iron status – intervention studies ..... 63
1.17.5 Effect of ascorbic acid on iron status – observational studies ..... 64
1.17.6 The New Zealand Situation ..... 64
**1.18 Organic acids** ..... 65
**1.19 Alcohol** ..... 69
1.19.1 Studies using single meals ..... 69
1.19.2 Effect of alcohol on iron status – observational studies ..... 72
1.19.3 The New Zealand situation ..... 72
**1.20 Vitamin A and β-Carotene** ..... 73
**1.21 Fermented products** ..... 77
**1.22 Polyphenols** ..... 79
1.22.1 Studies using single meals ..... 79
1.22.2 Effect of polyphenols on iron status – observational studies ..... 89
1.22.3 The New Zealand Situation ..... 89
**1.23 Oxalate/oxalic acid** ..... 90
**1.24 Calcium** ..... 90
1.24.1 Studies using single meals ..... 90
1.24.2 Studies using complete diets ..... 98
1.24.3 Effect of calcium on iron status – intervention studies ..... 99
1.24.4 Effect of calcium on iron status – observational studies ..... 99
1.24.5 The New Zealand Situation ..... 100
**1.25 Protein** ..... 100
1.25.1 Studies using single meals ..... 100
- Eggs and egg albumin ..... 100
- Bovine serum albumin ..... 101
- Milk proteins ..... 101
- Nuts and legumes ..... 102
- Soy protein ..... 102
1.25.2 Effect of protein on iron status – intervention studies ..... 115
1.25.3 Effect of protein on iron status – observational studies ..... 115
1.25.4 The New Zealand Situation ..... 115
**1.26 Phytates/fibre** ..... 116
1.26.1 Studies using single meals ..... 116
1.26.2 Studies using complete diets ..... 132
1.26.3 Effect of phytate/fibre on iron status – intervention studies ..... 132
1.26.4 Effect of phytate/fibre on iron status – observational studies ..... 132
1.26.5 The New Zealand Situation ..... 133
**1.27 Haem iron** ..... 133
**1.28 Summary of dietary factors affecting iron absorption** ..... 134
**1.29 Justification of assessment methods used** ..... 135

1.29.1 Assessment of iron status ..... 135
1.29.2 Assessment of dietary intake ..... 136
1.29.3 Anthropometric assessment ..... 137
**1.30 Study justification and aims** ..... 138
1.30.1 Study justification ..... 138
1.30.2 Study aims ..... 138

**Chapter 2. Dietary intakes and iron status of non vegetarian women aged 18-40 years living in the Manawatu region** ..... 140
**2.1 Introduction** ..... 140
**2.2 Methods** ..... 141
2.2.1 Participant recruitment ..... 141
2.2.2 Dietary interview ..... 142
2.2.3 Dietary analysis ..... 143
2.2.4 Measurement of iron status ..... 144
2.2.5 Statistical analysis ..... 145
**2.3 Results** ..... 147
2.3.1 Study Participants ..... 147
2.3.2 Iron status of study participants ..... 147
2.3.3 Dietary iron intakes of study participants ..... 148
2.3.4 Nutrient intakes of study participants ..... 149
2.3.5 24 hour recall versus food frequency questionnaire ..... 152
2.3.6 Factors affecting serum ferritin ..... 152
**2.4 Discussion** ..... 155
2.4.1 Study participants ..... 155
2.4.2 Iron status of study participants ..... 155
2.4.3 Dietary intakes of iron ..... 157
2.4.4 Intakes of other nutrients ..... 159
2.4.5 Dietary factors affecting iron status ..... 161
2.4.6 Other factors affecting iron status ..... 163
2.4.7 Dietary assessment and analysis ..... 165
2.4.8 Estimation of the haem iron content of foods ..... 167
2.4.9 Limitations of this study ..... 168
**2.5 Conclusions** ..... 168

**Chapter 3. The effect of a meat extract on iron absorption in young women** ..... 170
**3.1 Introduction** ..... 170
**3.2 Methods** ..... 171
3.2.1 Subject recruitment ..... 171
- Dietary interview ..... 171
- Measurement of iron status ..... 171
- Medical interview ..... 172
3.2.2 Overview of the study ..... 173
3.2.3 Calibration of equipment ..... 175
3.2.4 Instructions for subjects prior to taking part in the study ..... 176
3.2.5 Set up of the IV infusion set ..... 178
3.2.6 Procedure for the IV infusion of $^{58}Fe$ and blood sampling ..... 178
3.2.7 Serving of the meal ..... 180
3.2.8 Other measurements taken ..... 181

3.2.9 Blood samples – days 7, 14 and 21 ..... 182
3.2.10 Subject's iron status ..... 182
3.2.11 Preparation of stable isotope labels ..... 183
- Preparation of $^{58}$Fe-citrate solution, IFNHH – Batch 1 ..... 183
- Preparation of $^{58}$Fe-citrate solution, IFNHH – Batch 2 ..... 184
- Preparation of $^{58}$Fe-citrate solution, Ipswich Hospital, NHS Trust Pharmacy – Batches 1 and 2 ..... 184
- Preparation of $^{57}$Fe-citrate solution, IFNHH ..... 184
3.2.12 Test meals ..... 185
- Designing the meals ..... 185
- Preparation of meals ..... 187
- Sodium caseinate meal ..... 188
- Meat meal ..... 188
- Meat extract ..... 188
- Analysis of the meals ..... 191
3.2.13 Analysis of iron in saline from the infusion set ..... 193
3.2.14 Analysis of isotopic composition of blood sample ..... 194
3.2.15 Correction for iron status ..... 194
3.2.16 Statistical analysis ..... 195
**3.3 Results** ..... 196
3.3.1 Effect of the meat extract on iron absorption ..... 196
3.3.2 Effect of education on lowering dietary iron bioavailability ..... 201
3.3.3 Subject compliance with a low iron diet ..... 203
**3.4 Discussion** ..... 203
3.4.1 Comparison of results with those of other studies ..... 203
3.4.2 The meat extract ..... 206
3.4.3 Composition of the meals ..... 207
3.4.4 Population group ..... 208
3.4.5 Iron status and iron absorption ..... 209
3.4.6 Other factors affecting iron absorption ..... 210
3.4.7 Low iron diet for the five days prior to the study ..... 211
3.4.8 Assessment of iron status and adjustment for iron status ... 212
3.4.9 Use of stable isotopes to measure iron absorption ..... 215
3.4.10 Studies using stable isotopes to investigate iron absorption ..... 216
3.4.11 Potential use of the MFP factor ..... 217
**3.5 Conclusions** ..... 222

**Chapter 4. Concluding Discussion** ..... 223
**4.1 Summary of key findings** ..... 223
**4.2 Discussion of findings** ..... 225
**4.3 Concluding remarks** ..... 227
**4.4 Further work** ..... 228

**References** ..... 229

**Appendices**

**5.1 Advertisement and article for the Meat Study** ..... 257
**5.2 Information sheet and consent form for the Meat Study** ..... 260
**5.3 Interview schedule for the Meat Study including the food frequency questionnaire** ..... 267
**5.4 Letters to participants regarding iron status and inclusion / exclusion to the Meat Study** ..... 278
**5.5 Dietary guidelines for the low iron diet** ..... 283
**5.6 Instructions for keeping the food record** ..... 293
**5.7 Copy of the product analysis certificate and product bulletin Tatua 100 (sodium caseinate)** ..... 298

# List of Figures

Figure 1.1 Iron distribution (mg) and metabolism within the body ..... 3
Figure 1.2 Iron absorption in normal subjects ..... 14
Figure 3.1 Overview of the study protocol ..... 174
Figure 3.2 Intravenous infusion set up ..... 175
Figure 3.3 Flowchart showing the production of various meat fractions ..... 190

# List of Tables


Table 1.1 Recommendations for iron intake for women aged 19-50 years ..... 4
Table 1.2 Stages in the development of iron deficiency and overload ..... 6
Table 1.3 Percentage of New Zealand females with low iron stores, iron deficiency, and iron deficiency anaemia and inadequate dietary intakes of iron ..... 8
Table 1.4 In vivo methods for measuring iron absorption ..... 20
Table 1.5 Effect of meat on iron absorption (single meal studies) ..... 33
Table 1.6 Effect of ascorbic acid on iron absorption (single meal studies) .... 51
Table 1.7 Effect of organic acids on iron absorption (single meal studies) ... 67
Table 1.8 Effect of alcohol on iron absorption (single meal studies) ..... 70
Table 1.9 Effect of vitamin A and β-Carotene on iron absorption (single meal studies) ..... 74
Table 1.10 Effect of fermented food on iron absorption (single meal studies) ..... 78
Table 1.11 Effect of polyphenols on iron absorption (single meal studies) ..... 82
Table 1.12 Effect of calcium on iron absorption (single meal studies) ..... 93
Table 1.13 Effect of protein on iron absorption (single meal studies) ..... 106
Table 1.14 Effect of phytate/fibre on iron absorption (single meal studies) ..... 120
Table 1.15 Factors influencing dietary iron absorption ..... 135
Table 2.1 Serum ferritin and haemoglobin levels of the included women (n=85) ..... 147
Table 2.2 Iron status of women aged 18-40 years living in the Manawatu region ..... 148
Table 2.3 Dietary iron intake of women aged 18-40 years living in the Manawatu area ..... 148
Table 2.4 Mean and median intakes of macronutrients ..... 149
Table 2.5 Mean and median intakes of carbohydrates and lipids ..... 150
Table 2.6 Mean and median intakes of iron and other nutrients ..... 151
Table 2.7 Characteristics of study participants with and without a serum ferritin of < 20μg/L ..... 153
Table 2.8 Multiple regression to determine factors which influence serum ferritin ..... 154
Table 3.1 Final composition of the test meals ..... 186
Table 3.2 Estimated composition of test meals as determined by the New Zealand Food Composition Tables ..... 187
Table 3.3 Estimated and measured compositions of the test meals ..... 192
Table 3.4 Final composition of meals based (based on meal size) ..... 193
Table 3.5 Averages across groups at baseline ..... 196
Table 3.6 Subject data for the sodium caseinate meal ..... 197
Table 3.7 Subject data for the meat extract meal ..... 198
Table 3.8 Subject data for the meat meal ..... 199
Table 3.9 Averages across groups ..... 200
Table 3.10 Dietary intakes before and after dietary intervention ..... 202

# 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.