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THE *IN VITRO* ASSESSMENT OF THE BIOAVAILABILITY
OF IRON IN NEW ZEALAND BEEF

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ABSTRACT

The bioavailability of iron in New Zealand beef either alone or as part of a 'typical' New Zealand meal was investigated. The solubility of iron and its *in vitro* absorption by mouse intestinal tissue were used to evaluate iron bioavailability.

The solubility of haem and/or non-haem iron in meat (beef longissimus muscle), vegetables and meat-plus-vegetables was investigated. Samples were cooked and then subjected to *in vitro* gastrointestinal digestion with pepsin followed by a combination of pancreatic enzymes and bile. Cooking at 65°C for 90 minutes reduced the soluble iron concentration in meat by 81% and reduced the haem iron concentration by 27%, which coincided with a 175% increase in non-haem iron concentrations. However, gastrointestinal digestion increased the solubility of iron in cooked meat (333%), vegetables (367%) and meat-plus-vegetables (167%). A proportion (35%) of the haem iron in the meat was broken down by the action of pancreatic enzymes leading to a 46% increase in non-haem iron concentrations, although this was not the case for the meat-plus-vegetables.

Validation studies showed that mouse intestinal segments mounted in Ussing chambers maintained integrity and viability, and were responsive to glucose, theophylline and carbachol. Intestinal tissue from iron deficient mice was then used in the Ussing chambers to investigate the absorption of iron from ferrous gluconate and the soluble fractions of meat, vegetables and meat-plus-vegetables after gastrointestinal digestion. Results indicated a trend towards a higher absorption of iron from meat and ferrous gluconate, compared to vegetables and meat-plus-vegetables. However, iron absorption results were difficult to interpret due to the wide variation in the data. This variation was possibly due to errors associated with the sample processing and the analysis of iron, which was by inductively coupled-mass spectroscopy.

Overall, the present study showed that before estimations can be made on the bioavailability of food iron, the effects of the cooking and gastrointestinal digestion processes must be considered. Further, the use of *in vitro* gastrointestinal digestion followed by the use of Ussing chambers to assess intestinal absorption is a potentially valuable system for assessing mineral bioavailability.

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TABLE OF CONTENTS

CHAPTER ONE - Literature review	1
1.1 Physiological importance of iron	1
1.1.1 Iron requirements	2
1.1.2 Iron deficiency	3
1.2 Distribution and turnover of body iron	4
1.2.1 Iron storage	5
1.2.2 Iron transport	5
1.2.3 Recycling of body iron	6
1.2.4 Loss of body iron	6
1.3 Iron absorption	7
1.3.1 Non-haem iron: the DMT-1 pathway	8
1.3.2 Non-haem iron: the integrin-mobilferrin pathway	9
1.3.3 Haem iron	10
1.4 Maintenance of iron homeostasis	10
1.4.1 Cellular control of iron homeostasis	11
1.4.1.1 Iron-responsive elements	11
1.4.1.2 Iron-regulatory proteins	12
1.4.1.3 Interaction of IRPs with IREs	12
1.4.2 Iron homeostasis: communication with the small intestine	13
1.4.3 Intestinal iron transporter proteins and the IRE/IRP system	15
1.5 Bioavailability	16
1.5.1 Solubility and bioavailability	17
1.5.2 Nutrient impact on iron bioavailability	17
1.5.2.1 Ascorbic acid	18
1.5.2.2 Meat factor	19
1.5.2.3 Phytates	21
1.5.2.4 Polyphenols	22
1.5.2.5 Calcium	22
1.6 Assessment of food iron bioavailability	23
1.6.1 <i>In vivo</i> versus <i>in vitro</i> techniques	23
1.6.2 <i>In vitro</i> methods used to investigate iron bioavailability	24

1.6.2.1 Food iron solubility.....	24
1.6.2.2 Iron dialysability	25
1.6.2.3 Isolated cell lines	27
1.6.2.4 Membrane vesicles.....	28
1.6.2.5 Everted gut sacs	29
1.6.3 An <i>in vitro</i> method using Ussing chambers.....	30
1.7 Conclusions	32

CHAPTER TWO - Iron forms and solubility in foods: effects of cooking and <i>in vitro</i> pepsin and pancreatin-bile digestion	34
2.1 Introduction.....	34
2.2 Materials and methods	36
2.2.1 Experiment One.....	36
2.2.1.1 Preparation of meat, vegetables and meat-plus-vegetables.....	37
2.2.1.2 Sample collection	37
2.2.1.3 <i>In vitro</i> gastrointestinal digestion	38
2.2.1.4 Separation of soluble and insoluble iron fractions	38
2.2.1.5 Determination of total iron concentration.....	39
2.2.1.6 Determination of haem iron concentration	39
2.2.1.7 Determination of non-haem iron concentration	40
2.2.1.8 Validation of the assay for the total iron concentration	41
2.2.1.9 Validation of the assay for the haem iron concentration.....	41
2.2.1.10 Validation of the assay for non-haem iron concentration	42
2.2.1.11 Haem and non-haem iron concentrations in pepsin, pancreatin and bile.....	43
2.2.1.12 Statistical analysis	43
2.2.2 Experiment Two.....	44
2.2.2.1 Meat	44
2.2.2.2 Vegetables	44
2.2.2.3 Preparation of the homogenates	44
2.2.2.4 Sample collection	45
2.2.2.5 Statistical analysis.....	45
2.3 Results	46

2.3.1 Experiment One.....	46
2.3.1.1 Validation of the iron assay methods.....	46
2.3.1.2 Determination of total, haem and non-haem iron concentrations in undigested and digested meat, vegetables and meat-plus-vegetables ...	48
2.3.2 Experiment Two.....	50
2.3.2.1 Effect of cooking on meat haem and non-haem iron.....	50
2.3.2.2 Effect of gastrointestinal digestion on iron solubility.....	52
2.3.2.2.1 Meat.....	53
2.3.2.2.2 Meat-plus-vegetables	55
2.3.2.2.3 Comparison of meat and meat-plus-vegetable haem and non-haem iron concentrations	57
2.3.2.2.4 Vegetables	58
2.3.2.2.5 Total soluble iron	59
2.4 Discussion	59
2.4.1 Experiment One.....	59
2.4.2 Experiment Two.....	60
2.4.2.1 Iron concentrations in beef.....	61
2.4.2.2 Cooking effects.....	61
2.4.2.2.1 Haem and non-haem iron.....	62
2.4.2.2.2 Iron solubility	62
2.4.2.3 Effects of gastrointestinal digestion on iron solubility.....	64
2.4.2.3.1 Meat.....	64
2.4.2.3.2 Vegetables	66
2.4.2.3.3 Meat-plus-vegetables	66
2.4.2.3.4 Comparison between the soluble iron in the meat, vegetables and meat-plus-vegetables.....	67
2.5 Conclusions	68
CHAPTER THREE - Validation of an <i>in vitro</i> system using Ussing chambers for the assessment of iron bioavailability	70
3.1 Introduction.....	70
3.2 Materials and Methods.....	71
3.2.1 Animals.....	71

3.2.2 Tissue preparation	72
3.2.3 Ussing chamber experiments	72
3.2.4 Test reagents	74
3.2.5 Histological examination of intestinal tissues	75
3.2.6 Iron absorption experiments	76
3.2.6.1 Iron preparation	76
3.2.6.2 Sample collection	76
3.2.6.3 Iron analysis	76
3.2.7 Statistical analysis	76
3.3 Results	77
3.3.1 Tissue viability	77
3.3.1.1 Basal bioelectric measurements	77
3.3.1.2 Tissue response to the test reagents	77
3.3.1.3 Histology of intestinal tissues	79
3.3.2 Iron absorption measurements	80
3.4 Discussion	81
3.4.1 Tissue viability	81
3.4.2 Spontaneous oscillations	82
3.4.3 Iron absorption	82
3.5 Conclusions	84

CHAPTER FOUR - Estimation of the iron bioavailability of foods

using Ussing chambers	86
4.1 Introduction	86
4.2 Materials and Methods	87
4.2.1 Animals	87
4.2.1.1 Iron deficient mice	88
4.2.1.2 Iron replete mice	88
4.2.2 Haematology	89
4.2.3 Tissue preparation	89
4.2.4 Iron solutions	89
4.2.4.1 Digested meat, vegetable and meat-plus-vegetable solutions	89
4.2.4.2 Ferrous gluconate	90

4.2.5 Sample collection	90
4.2.6 Sample processing and iron analysis.....	91
4.2.6.1 Acid digestion of mucosal solutions and tissue samples.....	91
4.2.6.2 Wash samples	91
4.2.6.3 Iron analysis.....	91
4.2.7 Baseline iron levels.....	92
4.2.8 Calculations	92
4.2.9 Statistical analysis	93
4.3 Results	95
4.3.1 Body weight and iron status.....	95
4.3.2 Basal bioelectric parameters	96
4.3.3 Changes in bioelectric measurements in response to the digested meat, vegetable and meat-plus-vegetable solutions	96
4.3.4 Resistance of the mucosal solution	98
4.3.5 Iron absorption.....	98
4.3.5.1 Iron removed from the mucosal solution	98
4.3.5.2 Data variation.....	102
4.3.6 Total recovery	103
4.4 Discussion	103
4.4.1 Iron deficient diet	104
4.4.2 Iron status of the mice	104
4.4.3 Bioelectric parameters.....	106
4.4.4 Iron absorption.....	108
4.5 Conclusions	113
CHAPTER FIVE - General discussion.....	114
5.1 Iron solubility.....	114
5.2 Estimation of food iron bioavailability: the use of Ussing chambers	118
5.3 Future directions.....	120
APPENDIX ONE	123
APPENDIX TWO	124
APPENDIX THREE	125

APPENDIX FOUR..... 126

APPENDIX FIVE..... 128

APPENDIX SIX..... 131

BIBLIOGRAPHY 134

LIST OF FIGURES

	Page
Figure 1.1: Model of non-haem iron absorption across enterocytes of the small intestine.	8
Figure 1.2: Model of the integrin-mobilferrin pathway for ferric iron absorption.	9
Figure 1.3: Changes in the solubility of ferric chloride (FeCl_3), ferrous chloride (FeCl_2), and haem iron with increasing pH.	18
Figure 1.4: Diagram of digestion/Caco-2 cell culture model.	27
Figure 1.5: Diagram of the everted gut sac technique.	29
Figure 1.6: Diagram of the Ussing chamber apparatus with intestinal tissue mounted between the two half chambers.	31
Figure 2.1: Mean (+ SEM) percentage iron concentrations in the soluble and insoluble fractions of haem (H) and non-haem (NH) iron in raw meat (n=4) and cooked meat (n=6).	51
Figure 2.2: Mean (+ SEM) percentage iron concentrations as soluble haem, insoluble haem, soluble non-haem and insoluble non-haem in meat (n=6) after cooking, after pepsin digestion, and after pepsin and pancreatin-bile digestion.	53
Figure 2.3: Mean (+ SEM) percentage iron concentrations as soluble haem, insoluble haem, soluble non-haem and insoluble non-haem in meat-plus-vegetables (n=3) after cooking, after pepsin digestion, and after pepsin and pancreatin-bile digestion.	55
Figure 2.4: Mean (+ SEM) percentage iron concentrations as soluble non-haem and insoluble non-haem in vegetables (n=3) after cooking, after pepsin digestion, and after pepsin and pancreatin-bile digestion.	58
Figure 2.5: The effect of pepsin and pancreatin-bile digestion on the soluble iron in meat-plus-vegetables, vegetables and meat.	59
Figure 3.1: Diagram of the Ussing chamber apparatus with intestinal tissue mounted between the two half chambers.	73
Figure 3.2: A typical recording of basal short-circuit current in mouse proximal small intestinal tissue exhibiting spontaneous oscillations.	77
Figure 3.3: Typical recording of the change in short-circuit current in response to carbachol, theophylline or glucose to mouse proximal small intestinal tissue mounted in Ussing chambers.	78

	Page
Figure 3.4: Photomicrographs of a cross section of mouse jejunum after being mounted in an Ussing chamber.	79
Figure 3.5: Linear regression line (\pm 95% confidence intervals) for the removal of iron from the mucosal solution of Ussing chambers containing proximal mouse intestinal tissue.	80
Figure 4.1: Typical recordings of the changes in short-circuit current after the addition of digested meat (Mt), vegetable (Vg) or meat-plus-vegetable (Mt + Vg) solutions to mouse small intestinal tissues mounted in Ussing chambers.	97
Figure 4.2: Box and whisker plots for the percent iron removed from the mucosal solution (top) and apparent absorption (bottom) after mouse duodenal and jejunal tissues were incubated for 90 minutes with ferrous gluconate (Fe glu) or digested meat (Mt), vegetables (Vg) or meat-plus-vegetables (Mt + Vg).	101

LIST OF TABLES

	Page
Table 1.1: Major iron-containing proteins and their functions in the body.	2
Table 1.2: Recommended total daily dietary intake of iron adopted by New Zealand.	2
Table 1.3: Approximate distribution of iron in adult males and females.	5
Table 1.4: Losses of iron from a healthy adult male.	7
Table 1.5: Dietary constituents that affect the absorption of non-haem iron.	17
Table 2.1: Weights of the cooked meat (Mt) and cooked vegetables (Vg) prepared as homogenates and the amount of water added to achieve a liquid consistency.	45
Table 2.2: Statistical data for the goodness-of-fit, sensitivity and variability of the total iron, haem iron and non-haem iron assays.	47
Table 2.3: The iron concentration in the Certified Reference Material (CRM) as determined by the total iron assay method.	47
Table 2.4: Comparison of the calculated and assayed haem iron concentrations in three haematin solutions.	48
Table 2.5: Means (\pm SEM) for the total and haem iron concentrations in two haemoglobin samples.	48
Table 2.6: Haem (H) and non-haem (NH) iron concentrations in cooked, but undigested meat (Mt), vegetables (Vg) and meat-plus-vegetables (Mt + Vg), and digested Mt + Vg.	48
Table 2.7: Percent contribution of the potatoes, carrots and peas to the soluble and insoluble haem iron fraction of the vegetable mix.	49
Table 2.8: Non-haem iron concentrations in the potatoes, carrots and peas.	49
Table 2.9: Means (\pm SEM) for the total iron and total haem and non-haem iron concentrations in cooked, but undigested meat (Mt), vegetables (Vg) and meat-plus-vegetables (Mt + Vg).	50
Table 2.10: Means (\pm SEM) for the haem and non-haem iron concentrations and soluble and insoluble iron concentrations in raw (n=4) and cooked (n=6) meat.	51

	Page
Table 2.11: General linear model results for the absolute values ($\mu\text{g/g}$) of haem and non-haem iron.	52
Table 2.12: General linear model results for haem and non-haem iron concentrations expressed as a percentage of the total iron concentration.	52
Table 2.13: Mean (\pm SEM) concentrations of soluble and insoluble haem and non-haem iron in meat ($n=6$) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	54
Table 2.14: Means (\pm SEM) for the total haem and non-haem iron concentrations in meat ($n=6$) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	54
Table 2.15: Mean (\pm SEM) concentrations of soluble and insoluble haem and non-haem iron in meat-plus-vegetables ($n=3$) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	56
Table 2.16: Means (\pm SEM) for the total haem and non-haem iron concentrations in meat-plus-vegetables ($n=3$) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	56
Table 2.17: Statistical comparisons between meat (Mt) and meat-plus-vegetables (Mt + Vg) for concentrations of soluble haem and insoluble non-haem iron after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	57
Table 2.18: Mean (\pm SEM) concentrations of soluble and insoluble non-haem iron in vegetables ($n=3$) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	58
Table 3.1: Effects of theophylline, glucose and carbachol on the secretion and/or absorption of charged ions by intestinal epithelium.	70
Table 3.2: Composition of Rodent Diet 83.	71
Table 3.3: Composition of the Ringer's solution (A.G. Butt, personal communication).	72
Table 3.4: Test reagent concentrations and final bath concentrations in 10ml Ringer's solution.	75
Table 3.5: Method for staining intestinal sections for histological analysis.	75

	Page
Table 3.6: Baseline bioelectric measurements for mouse proximal small intestinal tissue mounted in Ussing chambers.	77
Table 3.7: Means (\pm SEM) for short-circuit current of mouse proximal small intestinal tissue mounted in Ussing chambers and exposed to theophylline, glucose or carbachol.	78
Table 3.8: Percentage of iron as ferrous gluconate removed from the mucosal solution (starting iron concentration of 55 μ g/g) in Ussing chambers containing mouse proximal small intestinal tissue.	80
Table 4.1: Composition of the powdered low iron diet.	88
Table 4.2: Means (\pm SEM) for the haematological indices, tissue iron concentrations and body weights of iron replete and iron deficient mice.	95
Table 4.3: The baseline bioelectric measurements for mouse duodenum and jejunum mounted in Ussing chambers.	96
Table 4.4: Means (\pm SEM) for changes to short-circuit current of mouse small intestinal tissue after the replacement of the mucosal Ringer's solution with digested meat (Mt), vegetable (Vg) or meat-plus-vegetable (Mt + Vg) solutions.	96
Table 4.5: Means (\pm SEM) for changes to tissue resistance of mouse small intestinal tissue after the replacement of the mucosal Ringer's solution with digested meat (Mt), vegetable (Vg) or meat-plus-vegetable (Mt + Vg) solutions.	97
Table 4.6: Means (\pm SEM) for the resistance (Ω) of the Ringer's solution and the digested meat, vegetable and meat-plus-vegetable solutions.	98
Table 4.7: Means (\pm SEM) for the percent iron removed from the mucosal solution and apparent absorption of iron from ferrous gluconate (Fe glu) or digested meat (Mt), vegetables (Vg) or meat-plus-vegetables (Mt + Vg).	99
Table 4.8: Means (\pm SEM) for the iron concentrations (μ g/g dry weight) in intestinal segments after incubation with Ringer's solution (baseline), ferrous gluconate (Fe glu) or digested meat (Mt), vegetables (Vg) or meat-plus-vegetables (Mt + Vg).	100
Table 4.9: Means, standard deviations (SD) and coefficient of variation (CV) for the starting iron concentrations in the ferrous gluconate (Fe glu) solutions and digested meat (Mt), vegetable (Vg) or meat-plus-vegetable (Mt + Vg) solutions.	102

	Page
Table 4.10: Repeat ICP-MS reading of three acid digested mucosal samples.	103
Table 4.11: Means (\pm SD) for the total recovery of iron (%) for ferrous gluconate solutions or digested meat, vegetable or meat-plus-vegetable solutions.	103
Table A2.1: The concentrations of haem iron in the insoluble and soluble fractions of meat (Mt) and meat-plus-vegetables (Mt + Vg) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	124
Table A2.2: The concentrations of non-haem iron in the insoluble and soluble fractions of meat (Mt), vegetables (Vg) and meat-plus-vegetables (Mt + Vg) after cooking (Cook), after pepsin digestion (Pep) and after pepsin and pancreatin-bile digestion (Panc).	124
Table A4.1: Osmolarity of the Ringer's solution and the digested meat, vegetable and meat-plus-vegetable solutions at an iron concentration of $5\mu\text{g/g}$.	126
Table A5.1: Comparison of the statistical data for the goodness-of-fit, sensitivity and variability of analysis of total iron concentration by atomic absorption spectroscopy (AA) and inductively coupled plasma-mass spectroscopy (ICP-MS).	128
Table A5.2: Iron concentrations ($\mu\text{g/g}$) in replicate samples of digested vegetable (Vg) and meat-plus-vegetable (Mt + Vg) solutions after dilution or digestion with nitric acid.	129
Table A6.1: Means (\pm SEM) for the apparent absorption and total recovery of haem iron from a digested haemoglobin solution.	132
Table A6.2: Means (\pm SEM) for the apparent absorption and total recovery of non-haem iron from the mucosal Ringer's solution containing ferrous gluconate.	132

LIST OF ABBREVIATIONS

%	percent
μAmps	microamperes
$\mu\text{Amps}/\text{cm}^2$	microamperes per square centimetre
μg	micrograms
$\mu\text{g}/\text{g}$	micrograms per gram
$\mu\text{g}/\text{g}/\text{min}$	micrograms per gram per minute
μm	micrometre
μl	microlitre
Ω	ohm
Ω/cm^2	ohms per square centimetre
AA	atomic absorption spectroscopy
ANOVA	Analysis of Variance
ATP	adenosine triphosphate
$^{\circ}\text{C}$	degrees Celsius
CaCl_2	calcium chloride
Caco-2	human colonic adenocarcinoma cell line
cAMP	cyclic adenosine monophosphate
cm	centimetre
cm^2	square centimetre
CO_2	carbon dioxide
Cook	after cooking
CRM	Certified Reference Material
CV	coefficient of variation
DCT-1	divalent cation transporter-1
Dcyt b	duodenal cytochrome b
df	degrees of freedom
DMT-1	divalent metal transporter-1
DNA	deoxyribose nucleic acid
f	f statistic
Fe^{2+}	ferrous iron
Fe^{3+}	ferric iron
FeCl_2	ferrous chloride
FeCl_3	ferric chloride
Fe glu	ferrous gluconate
[Fe-S]	iron-sulphur
fL	femtolitre
FP-1	ferroportin-1
g	grams
<i>g</i>	g force
g/L	grams per litre
g/kg	grams per kilogram
GLM	general least squares model
H	haem iron
ICP-MS	inductively coupled plasma-mass spectrometry
IRE	iron-responsive element

IREG-1	iron-regulating protein-1
IRP-1	iron-regulatory protein-1
IRP-2	iron-regulatory protein-2
K ₂ EDTA	potassium ethylenediaminetetraacetic acid
KCl	potassium chloride
kDa	kilodalton
kg	kilogram
M	molar
MCV	mean cell volume
mg	milligrams
MgCl ₂	magnesium chloride
mg/day	milligrams per day
mg/kg	milligrams per kilogram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
mOsm/L	milliosmoles per litre
mRNA	messenger ribonucleic acid
MS	mean square
Mt	meat
Mt + Vg	meat-plus-vegetables
MTP-1	metal transport protein-1
MUAEC	Massey University Animal Ethics Committee
mV	millivolts
n	number
NaCl	sodium chloride
NaHCO ₃	sodium bicarbonate
NaH ₂ PO ₄	sodium phosphate dibasic
Na ₂ HPO ₄	sodium phosphate monobasic
ng/g	nanograms per gram
NH	non-haem iron
nm	nanometre
Nramp2	natural resistance-associated macrophage protein 2
NS	not significant
O ₂	oxygen
p	probability statistic
p.	page
Panc	after pepsin and pancreatin-bile digestion
PCV	packed cell volume
Pep	after pepsin digestion
USF-2	upstream stimulatory factor-2
UTR	untranslated region
r ²	coefficient of determination
RDI	Recommended Daily Intake
RNA	ribonucleic acid
rpm	revolutions per minute
RSD	residual standard deviation
SD	standard deviation
SEM	standard error of the mean

TCA	trichloroacetic acid
SS	sum of squares
III SS	type III sum of squares
Vg	vegetables

INTRODUCTION

Iron is the most abundant trace element in the body and is vital in the nutrition of all mammalian species. The ability of this transition metal to exist in two redox states makes it useful at the catalytic centre of many biochemical reactions, including DNA synthesis, transport of oxygen and respiration. However, the same properties that make iron useful also make it toxic. Free iron is able to catalyse the formation of highly toxic oxidative free radicals that damage many important biological components, such as lipids, proteins and DNA. Despite such toxic potential, there is no physiological regulatory mechanism for excretion of iron from the body. Thus, the body must 'sense' its internal iron load and respond appropriately by altering iron absorption and storage processes.

The body is economical in its handling of iron. The iron from senescent, damaged or malformed erythrocytes is recycled and reutilised by the body. Extra iron, which is utilised at times of increased iron requirements, can be stored by a specially designated protein (ferritin) and is transported within the body between sites of absorption, storage and utilisation by transferrin. Furthermore, iron absorption is regulated by the requirements of the body. In spite of the variety of mechanisms that control iron metabolism and participate in the conservation and recycling of iron, iron deficiency is the most common deficiency disorder in the world. In most cases, attempts to remedy iron deficiency have been by way of orally administered iron supplements. However, improving the diet may play a more important role in the prevention of iron deficiency.

One of the leading causes of iron deficiency is low bioavailability of dietary iron. Bioavailability is defined as the proportion of the total mineral intake that is potentially available for absorption and utilisation for normal body functions (Wienk *et al.*, 1999). Thus, when evaluating foods for nutritional significance to the human body, knowledge of iron intake alone is of little value without an understanding of iron bioavailability.

Meat is a good source of iron, which *in vivo* and *in vitro* studies suggest is highly bioavailable. Furthermore, meat has been consistently shown to enhance the bioavailability of iron from other foods by increasing the solubility and intestinal absorption of iron. However, the effects of cooking and gastrointestinal digestion on the bioavailability of meat iron have not yet been fully investigated. Thus, the main aim of the research reported in this Thesis was to study the bioavailability of iron in New Zealand beef either alone or as part of a 'typical' New Zealand meal. Iron bioavailability was evaluated in two phases: firstly, by measuring the solubility of iron after cooking and gastrointestinal digestion and secondly, by measuring the *in vitro* absorption of iron by mouse intestinal tissue. Specifically, the objectives of the present study were:

1. To validate the use of total, haem and non-haem iron assays to determine these iron forms in the soluble and insoluble fractions of meat, vegetables and a combination of meat-plus-vegetables ('typical' New Zealand meal) (Chapter Two).
2. To investigate the effects of pepsin and pancreatin-bile digestion on the solubility of haem and/or non-haem iron in meat, vegetables and meat-plus-vegetables (Chapter Two).
3. To validate an *in vitro* technique using Ussing chambers for measuring iron absorption (Chapter Three).
4. To use the Ussing chamber model to investigate the absorption of iron, by mouse intestinal tissue, from ferrous gluconate and the soluble fractions of meat, vegetables and meat-plus-vegetables after gastrointestinal digestion (Chapter Four).