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

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LISA ROCHELLE WALKER

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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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LIST OF ABBREVIATIONS

%	percent
µAmps	microamperes
µAmps/cm^2	microamperes per square centimetre
µg	micrograms
µg/g	micrograms per gram
µg/g/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
°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
g	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_2EDTA	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	millliosmoles 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_2PO_4	sodium phosphate dibasic
Na_2HPO_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).