



Murdoch
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The Effects of Dietary Iron Concentration on Colonic Inflammation.

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Declaration

I declare that this thesis is my own account of my research and contains work that has not been previously submitted for a degree at any tertiary educational institution.

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Abstract

Colorectal cancer (CRC) is highly prevalent in Western society with more than 14,000 diagnoses and 4,000 deaths in Australia annually. CRC is caused by both genetic and environmental risk factors, with a number of population studies linking high dietary iron and elevated body iron stores with increased CRC risk. The iron overload disorder, hereditary haemochromatosis, is associated with increased risk of CRC, as are inflammatory bowel diseases including ulcerative colitis and Crohn's disease. A better understanding of the pathogenesis of CRC is required to develop improved medical therapies for individuals most at risk, such as those with hereditary haemochromatosis or inflammatory bowel diseases. Improved disease prevention and management is also important from a public health perspective, and will potentially decrease the incidence and mortality of this malignancy.

In this study, a mouse model of inflammation-associated CRC was used to evaluate the effects of increasing dietary iron concentrations on tissue iron loading and colonic inflammation. Two strains of mice (C57BL/6 and FVB/N) were evaluated to compare their responses to increased dietary iron levels.

The results demonstrated that increased dietary iron increased colonic and liver iron deposition and colonic inflammation via an IL-6/STAT3 signalling pathway in a dose-dependent manner. Both colitis scores and plasma IL-6 levels were positively correlated with increased liver iron levels. Both strains of mice showed similar trends in response to increased dietary iron concentration, though the magnitude of the responses varied between strains.

In conclusion, the results from this study showed that increasing dietary iron concentrations exacerbate colonic inflammation, suggesting that high dietary iron consumption may be linked to an increased risk of CRC.

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List of Abbreviations

ACF	Aberrant crypt foci
ANOVA	Analysis of variance
AOM	Azoxymethane
APC	Adenomatous polyposis coli
APO-TF	Apo-transferrin
ARBP	Acidic ribosomal phosphoprotein PO
β-actin	Beta actin
β-catenin	Beta catenin
BAX	Bcl-2-like protein 4 apoptosis regulator
BCA	Bicinchoninic acid
BCL-xL	B-cell lymphoma-extra large (Bcl-2-like 1)
bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic protein
BMP-R	Bone morphogenetic protein receptor
BPS	Bathophenanthroline disulfonic acid
B-Raf	B-Raf oncogene
BSA	Bovine serum albumin
CDC4	Cell division control protein 4
cDNA	Complementary DNA
CIN	Chromosomal instability
CRC	Colorectal cancer
DAB	3,3'-diaminobenzidine
DcytB	Duodenal cytochrome B

DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
DPX	Distyrene plasticiser xylene
DSS	Dextran sulphate sodium salt
DTT	DL-dithiothreitol
ECL	Enhanced chemiluminescence
EDTA	Ethylenediamine tetracetic acid
ELISA	Enzyme-linked immunosorbent assay
FAP	Familial adenomatous polyposis
Fe	Iron
FPN	Ferroportin
Fwd	Forward
GI	Gastrointestinal
gp130	Glycoprotein 130
H&E	Haematoxylin and eosin
Hamp	Hepcidin antimicrobial peptide
HCP1	Haem carrier protein
HCl	Hydrochloric acid
HFE	Human haemochromatosis protein
HIF	Hypoxia inducible factor
HJV	Haemojuvelin
HMOX	Haem oxygenase
HNPCC	Hereditary non-polyposis colorectal cancer

HOLO-TF	Holo-transferrin
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
ID1	Inhibitor of DNA binding 1
IGF2R	Insulin-like growth factor 2 receptor
IgG	Immunoglobulin G
IKK	Inhibitor of kappa B (I κ B) kinase
IL	Interleukin
IP-10	Interferon- γ -inducible protein-10
IRE	Iron responsive element
IRP	Iron regulatory protein
IκB	Inhibitor of kappa B
JAK	Janus kinase
JNK	c-Jun-N-terminal kinase
K-Ras	Kirsten rat sarcoma viral oncogene
MAPK	Mitogen-activated protein kinase
MIN	Multiple intestinal neoplasia
MLH-1	MutL homologue 1
MMR	Mismatch repair
MOPS	4-morpholine propanesulfonic acid
mRNA	Messenger RNA
MSH-2	MutS protein homologue 2
MSI	Microsatellite instability
NaCl	Sodium chloride

NaOH	Sodium hydroxide
NFκB	Nuclear factor kappa B
NO	Nitric oxide
OD	Optical density
p53	Tumour suppressor gene p53
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PPIA	Peptidylprolyl isomerase A
pSTAT	Phosphorylated STAT
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RAGE	Receptor for advanced glycation end product
RANTES	Regulated on activation / normal T-cell expressed and secreted
RAS	An abbreviation of “rat sarcoma” oncogene
RBC	Red blood cell
Rev	Reverse
RIPA	Radioimmunoprecipitation buffer
RNA	Ribonucleic acid
RNase	Ribonuclease
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
SMAD	Mothers against decapentaplegic homologue
SOCS-3	Suppressor of cytokine signalling 3
STAT	Signal transducer and activator of transcription
TAE	Tris acetate EDTA

TBST	Tris-buffered saline and tween
TFR	Transferrin receptor
TGF	Transforming growth factor
TGFBR2	Transforming growth factor β type II receptor
TLR	Toll like receptor
TNF	Tumour necrosis factor
UTR	Untranslated region
VEGF	Vascular endothelial growth factor
Wnt	Wingless-related integration site

List of Units:

°C	degrees Celsius	M	molar
µg	microgram	mA	milliampere
µL	microlitre	mg	milligram
µm	micrometre	mL	millilitre
µM	micromolar	mm	millimetre
µmol	micromole	mM	millimolar
g	gram	ng	nanogram
kb	kilobase	nm	nanometre
kg	kilogram	pg	picogram
kDa	kilodalton	rpm	revolutions per minute
L	litre	U	units
m	metre	V	volts