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An investigation into the effect of New Zealand green-lipped mussel (*Perna canaliculus*) on non-haem iron absorption

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Abstract

The bioavailability of dietary non-haem iron can be influenced by the nutritional composition of a meal. Ascorbic acid and components within meat, fish and poultry, but particularly red meat and pork, all appear to enhance the absorption of non-haem iron when consumed together within a meal compared to other protein sources. However the promotion of red meat or pork is problematic due to their high saturated fatty acid content and alternative foods to red meat or pork that may enhance iron absorption such as oily fish or shellfish require further investigation. The aims of the present study were to investigate the effects of short-term and prolonged supplementation of New Zealand green-lipped mussel (*Perna canaliculus*) (NZGLM) on mucosal iron transport and iron retention. The mechanism(s) by which mucosal iron transport is affected during NZGLM supplementation was also investigated. When investigated *in vitro*, NZGLM and beef both enhanced iron absorption by a similar magnitude compared to egg albumin. The enhancing effect of NZGLM on iron absorption was repeatedly observed using two separate *in vitro* models; Caco-2 cells and mouse small intestine mounted on Ussing chambers. When investigated in iron-deficient mice, mucosal iron transport and extra-intestinal iron retention were significantly enhanced when an iron supplement was combined with NZGLM compared to egg albumin. Conversely, an inhibitory effect was observed when mice were supplemented with NZGLM for a prolonged period of time prior to consuming an iron supplement. The inhibitory effect of NZGLM was not associated with the dietary iron load. Prolonged NZGLM supplementation inhibits mucosal iron absorption by reducing brush border

iron transport. The inhibitory effect of prolonged NZGLM supplementation was observed to be associated with its high calcium content; however other competitive nutrients such as copper, manganese or zinc may also contribute to the inhibitory effect. The main inhibitory effect is proposed to be calcium-stimulated DMT1 internalisation into cytosolic vesicles. This occurs after prolonged or repeated NZGLM supplementation. The findings of this study suggest that NZGLM enhances mucosal iron transport and distribution to extra-intestinal tissues when consumed as a single dose with an iron supplement. For this reason NZGLM may be an alternative iron absorption enhancer to red meat or pork with additional cardio-protective properties. Repeated NZGLM supplementation may reduce mucosal transport; therefore repeated NZGLM supplementation should be moderated in order to ensure that mucosal iron transport is not compromised.

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List of abbreviations

%	Percent
Δ	Change
$^{\circ}\text{C}$	Degrees Celsius
μg	Micrograms
μL	Microliter
μM	Micromole/Litre
$\beta\text{-CPP}$	β -casein phosphopeptide
Ω	Ohms
Ω/cm^2	Ohms per centimetre squared
AA	Ascorbic acid
AHA	American Heart Association
ANOVA	Analysis of variance
AP	Alkaline phosphatase
Asc	Ascorbate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BMP (2,4,6)	Bone morphogenetic proteins (2,4,6)
CaCl_2	Calcium chloride
Caco-2	Colorectal carcinoma cell line
cm	Centimetre
cm^2	Square centimetre
CO_2	Carbon dioxide
cpm	Counts per minute
CS	Chondroitin sulfate
DcytB	Duodenal cytochrome B
DHA	Dehydroascorbic acid
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DMT1	Divalent metal transporter 1
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ERT	Extrinsic radionuclide tracer
FAK	Focal Adhesion Kinase
FBS	Foetal bovine serum
Fe	Iron
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
FeCl_3	Ferric chloride
FeSO_4	Ferrous sulphate
FLVCR	Feline leukemia virus subgroup C receptor-related protein
FOS	Fructooligosaccharide

<i>g</i>	<i>Grams</i>
<i>g/L</i>	<i>Grams per litre</i>
<i>GAG</i>	<i>Glycosaminoglycan</i>
<i>GLUT (1,2,3)</i>	<i>Glucose transporter (1,2,3)</i>
<i>H⁺</i>	<i>Hydrogen</i>
<i>HA</i>	<i>Hyaluronic acid</i>
<i>HBSS</i>	<i>Hanks balanced salt solution</i>
<i>HCl</i>	<i>Hydrochloric acid</i>
<i>HFE</i>	<i>High iron gene</i>
<i>HHC</i>	<i>Hereditary hemochromatosis</i>
<i>HJV</i>	<i>Hemojuvelin</i>
<i>HO (1,2)</i>	<i>Haem oxidase (1,2)</i>
<i>ID</i>	<i>Iron deficiency</i>
<i>IDA</i>	<i>Iron deficiency anaemia</i>
<i>IL-6</i>	<i>Interleuken-6</i>
<i>IMP</i>	<i>Integrin mobilferrin paraferitin</i>
<i>IRE</i>	<i>Iron response element</i>
<i>IREG1</i>	<i>Iron regulating protein 1</i>
<i>IRP</i>	<i>Iron response protein</i>
<i>IRT</i>	<i>Intrinsic radionuclide tracer</i>
<i>K⁺</i>	<i>Potassium</i>
<i>KCl</i>	<i>Potassium chloride</i>
<i>Kg</i>	<i>Kilogram</i>
<i>LIP</i>	<i>Labile iron pool</i>
<i>LPS</i>	<i>Lipopolysaccharide</i>
<i>LSD</i>	<i>Least square difference</i>
<i>L-α</i>	<i>L-α-Glycerophosphocholine</i>
<i>M</i>	<i>Mole/litre</i>
<i>MAPK</i>	<i>Mitogen-activated protein kinase</i>
<i>mg</i>	<i>Milligram</i>
<i>mg/L</i>	<i>milligrams per litre</i>
<i>min</i>	<i>Minute</i>
<i>mL</i>	<i>Millilitre</i>
<i>mm</i>	<i>Millimetre</i>
<i>mM</i>	<i>Millimole/litre</i>
<i>mOsm</i>	<i>Milliosmole/kg</i>
<i>MPF</i>	<i>Meat poultry fish</i>
<i>MPF factor</i>	<i>Meat poultry fish factor</i>
<i>mRNA</i>	<i>Messenger ribonucleic acid</i>
<i>MUFA</i>	<i>Monounsaturated fatty acid</i>
<i>mV</i>	<i>Millivolts</i>
<i>n</i>	<i>Number</i>
<i>n-3 PUFA</i>	<i>Omega three polyunsaturated fatty acid</i>
<i>NA</i>	<i>Nitric acid</i>
<i>Na⁺</i>	<i>Sodium</i>
<i>Na⁺/K⁺ATPase</i>	<i>Sodium/potassium ATPase pump</i>
<i>NaCl</i>	<i>Sodium chloride</i>

NaHCO_3	<i>Sodium bicarbonate</i>
NHE	<i>Sodium hydrogen exchanger</i>
nM	<i>Nanometres</i>
NRAMP	<i>Natural resistance-associated macrophage protein</i>
NZBS	<i>New Zealand blood service</i>
NZGLM	<i>New Zealand green-lipped mussel</i>
O_2	<i>Oxygen</i>
OH	<i>Hydroxide</i>
P	<i>Probability</i>
PBS	<i>Phosphate buffered saline</i>
PC	<i>Polycarbonate</i>
PCFT/HCP1	<i>Haem carrier protein 1</i>
PE	<i>Polyethylene</i>
PET	<i>Polyethylene terephthalate</i>
ppm	<i>parts per million</i>
PUFA	<i>Polyunsaturated fatty acid</i>
RBC	<i>Red blood cell</i>
rpm	<i>Revolutions per minute</i>
SCFA	<i>Short chain fatty acids</i>
SEM	<i>Standard error of the mean</i>
SFA	<i>Saturated fatty acid</i>
SGLT (1,2)	<i>Sodium-dependent glucose co-transporter (1,2)</i>
SI	<i>Sucrose isomaltase</i>
SS	<i>Semi-synthetic</i>
STAT	<i>Signal Transducer and Activator of Transcription</i>
STEAP3	<i>STEAP family member 3</i>
TCA	<i>Trichloroacetic acid</i>
TEER	<i>Trans-epithelial electrical resistance</i>
TfR (1,2)	<i>Transferrin receptor (1,2)</i>
UTR	<i>Un-translated region</i>
w/w	<i>Weight for weight</i>
WBC	<i>White blood cell</i>
WHO	<i>World Health Organization</i>
ZIP (8, 14)	<i>Zrt- and Irt-like protein (8, 14)</i>
Zn	<i>Zinc</i>