THESIS TITLE:

Effects of dietary fish oil and fibre on contractility of gut smooth muscle

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To be submitted for: PhD by prior publication

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And

CSIRO Human Nutrition

- August 2007 -

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ABSTRACT

From animal experimentation, and studies using *in vitro* models, there was evidence in the literature to suggest that dietary fibre may influence contractility and motility of the gastrointestinal tract and long chain (LC) n-3 polyunsaturated fatty acids (PUFAs) from marine sources may influence contractility of smooth muscle cells in blood vessels. The hypothesis of this thesis was that dietary fish oil and/or fibre influence the contractility of isolated intact sections of gut smooth muscle tissue from small animal models. Methodology was established to measure *in vitro* contractility of intact pieces of guinea pig ileum with the serosal side isolated from the lumen. It was demonstrated that four amino acid peptides from κ-casein (casoxins) applied to the lumen overcame morphine-induced inhibition of contraction. Using this established technology, the guinea pig was used to investigate the effects of dietary fibre and fish oil supplementation on gut *in vitro* contractility. In separate experiments, changes in sensitivity to electrically-driven and 8-*iso*-prostanglandin (PG)E_2-induced contractility were demonstrated for dietary fibre and fish oil. A modified, isolated gut super-perfusion system was then established for the rat to validate these findings. It was subsequently shown that LC n-3 PUFA from dietary fish oil significantly increased maximal contraction in response to the G-protein coupled receptor modulators, acetylcholine and the eicosanoids PGE_2, $PGF_{2\alpha}$, 8-*iso*-PGE_2 and U-46619 in ileum but not colon, without changes in sensitivity (EC_{50}), when n-3 PUFA as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) had been incorporated to a similar degree into the gut total phospholipid membrane pool. It was further established that the spontaneously hypertensive rat (SHR) had a depressed prostanoid (PGE_2 and $PGF_{2\alpha}$) response in the gut that could be restored by dietary fish oil supplementation (5% w/w of total diet) in the ileum but not the colon. Importantly,

the muscarinic response in the colon of the SHR was increased by fish oil supplementation with DHA likely to be the active agent. Dietary fish oil dose experiments deduced differential increases in response occurred at fish oil concentrations of 1% for muscarinic and 2.5% (w/w) for prostanoid stimulators of the ileum with no difference in receptor-independent KCl-induced depolarization-driven contractility. Studies combining high amylose resistant starch (HAMS, 10% w/w) and fish oil (10% w/w) fed to young rats demonstrated a low prostanoid response that was enhanced by dietary fish oil but not resistant starch. There was however, an interactive effect of the HAMS and fish oil noted for the muscarinic-mimetic, carbachol. Generally, resistant starch increased the large bowel short chain fatty acid pool with a subsequent lower pH. Binding studies determined that while the total muscarinic receptor binding properties of an isolated ileal membrane fraction were not affected in mature rats by dietary fish oil, young rats had a different order of muscarinic receptor subtype response with a rank order potency of $M_3 > M_1 > M_2$ compared to mature animals of $M_3 > M_2 > M_1$ with fish oil altering the sensitivity of the M_1 receptor subtype in isolated carbachol-precontracted ileal tissue. In conclusion, experiments using the guinea pig and rat gut models demonstrated that dietary fish oil supplementation, and to a lesser degree fibre, increased receptor-driven contractility in normal and compromised SHR ileum and colon. Further, changes in responsiveness were demonstrated in the developing rat gut prostanoid and muscarinic receptor populations that could be altered by dietary fish oil. Preliminary evidence suggested that fish oil as DHA may alter receptor-driven gut contractility by mechanisms involving smooth muscle calcium modulation. Defining the role that dietary fibre and fish oil, and other nutrients, play in normal and diseased states of bowel health such as

inflammatory bowel disease (IBD), where contractility is compromised, are among the ongoing challenges.

DECLARATION:

'I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of the thesis being made available to the university library.

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Glen Stephen Patten (August 2007)

"All great truths begin as blasphemies." ***George Bernard Shaw***

ACKNOWLEDGEMENTS

I thank Professor Peter Clifton and Associate Professor Ted McMurchie for offering their valuable time and extensive expertise to be supervisor and co-supervisor respectively for this thesis.

I acknowledge the Faculty of Sciences, School of Molecular Biosciences, Discipline of Physiology of the University of Adelaide, and in particular Associate Professor Mike Nordstrom the Postgraduate Coordinator, for facilitating the opportunity for me to become a candidate for PhD by Prior Publication.

I would also like to thank the following senior CSIRO staff for their scientific input and support:

Dr Mahinda Y. Abeywardena

Professor Richard J. Head

Dr David L. Topping FTSE

Dr Anthony R. Bird

Dr Michael A. Conlon

Dr Wayne Leifert

And acknowledge further other CSIRO collaborators:

Mr Michael J. Adams

Mrs Julie A. Dallimore

Mr Paul F. Rogers

Dr Anisa Jahangiri

I dedicate this thesis to my late parents, father, Frank and mother, Dulcie, and siblings, Arthur, Bruce (deceased) and Louise. Finally, to Sue Hicks, all my dearest thanks for your support, love, and encouragement past, now and in the future.

LIST OF PUBLICATIONS AND ABSTRACTS THAT CONTRIBUTED TO THIS THESIS

Full papers

1. **Patten GS**, Head RJ, Abeywardena MY and McMurchie EJ. (2001) An apparatus to assay opioid activity in the infused lumen of the intact isolated guinea pig ileum. *J Pharmacol Toxicol Methods*, **45**: 39-46.

2. **Patten GS**, Bird AR, Topping DL and Abeywardena MY. (2002a) Dietary fish oil alters the sensitivity of guinea pig ileum to electrical driven contractions and 8-*iso*-PGE_2. *Nutr Res*, **22**: 1413-26.

3. **Patten GS**, Abeywardena MY, McMurchie EJ and Jahangiri A. (2002b) Dietary fish oil increases acetylcholine- and eicosanoid-induced contractility of isolated rat ileum. *J Nutr*, **132**: 2506-13.

4. **Patten GS**, Bird AR, Topping DL and Abeywardena MY (2004a) Effects of convenience rice congee supplemented diets on guinea pig whole animal and gut growth, caecal digesta SCFA and in vitro ileal contractility. *Asia Pac J Clin Nutr*, **13**: 92-100.

5. **Patten GS**, Adams MJ, Dallimore JA and Abeywardena MY (2004b) Depressed prostanoid-induced contractility of the gut in spontaneously hypertensive rats (SHR) is not affected by the level of dietary fat. *J Nutr*, **134**: 2924-29.

6. **Patten GS**, Adams MJ, Dallimore JA, Rogers PF, Topping DL and Abeywardena MY (2005a) Restoration of depressed prostanoid-induced ileal contraction in spontaneously hypertensive rats by dietary fish oil. *Lipids*, **40**: 69-79.

7. **Patten GS**, Adams MJ, Dallimore JA and Abeywardena MY (2005b) Dietary fish oil dose-response effects on ileal phospholipid fatty acids and contractility. *Lipids*, **40**:925-29.

8. **Patten GS**, Conlon MA, Bird AR, Adams MJ, Topping DL and Abeywardena MY (2006) Interactive effects of dietary resistant starch and fish oil on SCFA production and agonist-induced contractility in ileum of young rats. *Dig Dis Sci*, **51**:254-61.

Abstracts

1. **Patten GS**, McMurchie EJ, Abeywardena MY and Jahangiri A (2001) Dietary fish oil supplementation increases the in vitro contractility of rat ileum. NSA, Canberra, Dec 3-5. *Asia Pac J Clin Nutr*, **10** Suppl: S10.

2. **Patten GS** and Abeywardena MY (2003) Dietary n-3 PUFA on indices of bowel health. GESA, Adelaide Meeting. *J Gastroenterol Hepatology*, **17** [Suppl.]: 160.

3. **Patten GS** and Abeywardena MY (2003) Fish oil feeding increases gut contractility in spontaneous hypertensive rat (SHR) model. NSA, Hobart, Nov 30 – Dec 3. *Asia Pac J Clin Nutr*, **12** [Suppl]: S64.

4. **Patten GS** and Abeywardena MY (2003) High saturated fat diet does not affect gut contractility in the rat. NSA, Hobart, Nov 30 – Dec 3. *Asia Pac J Clin Nutr*, **12** [Suppl]: S65.

5. **Patten GS** and Abeywardena MY (2004) Role for long chain n-3 PUFA in gut contractility. AOCS Australasian Section. Biennial Workshop. Fats and oils – their role in food and health. Adelaide Nov 30 – Dec 1.

STATEMENT OF JOINTLY AUTHORED PAPERS ON THE CONTRIBUTIONS MADE BY EACH AUTHOR AS LISTED.

Glen S. Patten was involved in the study design and formulation of dietary regimens for papers (as listed above) 1, 2, 4, 5, 6, and 7, and designed and undertook all of the *in vitro* gut contractility studies and was the principal and corresponding author of all papers (1-8).

Richard J. Head assisted in the experimental design to paper 1 and assisted in the manuscript draft.

Mahinda Y. Abeywardena was involved in study design for papers 1, 5, 6, and 7 and assisted in the drafting of papers.

Edward J. McMurchie was involved with study design for papers 1 and 3 and assisted in the manuscript draft.

Anthony R. Bird was involved with study design for papers 2, 4, and 8 and assisted in drafting of the manuscripts.

David L. Topping was involved with study design for papers 2, 4, 7 and 8, and assisted in the drafting of the manuscripts.

Anisa Jahangiri was involved with study design of paper 3 and assisted in the manuscript draft.

Michael J. Adams was involved with study design for papers 5, 6 and 7, and assisted in manuscript draft and undertook fatty acid analysis for 3, 5, 6, 7 and 8.

Julie A. Dallimore was involved with study design for papers 5, 6, and 7, and assisted in manuscript draft.

Paul F. Rogers assisted with muscarinic receptor binding studies for paper 6 and assisted with drafting of the manuscript.

Michael A. Conlon was involved with study design for paper 8 and assisted in the manuscript draft.

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I agree with the statements made above concerning the contributions of the authors to the papers involved with this thesis.

1. **Glen S. Patten**.....

2. **Richard J. Head**.....

3. **Mahinda Y. Abeywardena**.....

4. **Edward J. McMurchie**.....

5. **Anthony R. Bird**.....

6. **David L. Topping**.....

7. **Anisa Jahangiri**..... .....

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10. **Paul F. Rogers**.....

11. **Michael A. Conlon**.....

ABBREVIATIONS

AA – Arachidonic acid
ALA – Alpha linolenic acid
Ang II – Angiotensin II
ATP- Adenosine triphosphate
B_{max} – Maximal amount of binding that would occur
Ca^{2+} - Calcium ion
$[Ca^{2+}]_i$ – Intracellular calcium ion concentration
CCh - Carbachol
CCK- Cholecystokinin
CD - Crohn's disease
Cdk2 - Cyclin-dependent kinase-2
CHN – CSIRO Human Nutrition
CLA - Conjugated linoleic acid
COX - Cyclooxygenase
CSIRO – Commonwealth Scientific and Industrial Research Organisation
CVD – Cardiovascular disease
$\Delta\Psi m$ - Mitochondrial transmembrane potential
DHA – Docosahexaenoic acid
DPA – Docosapentaenoic acid
EC - Enterochromaffin cell
EC_{50} – Effective concentration at which half the maximal biological effect is achieved
EDHF – Endothelium derived hyperpolarising factor
EPA – Eicosapentaenoic acid
FA(s) – Fatty acid(s)
FO - Fish oil
GIT – Gastrointestinal tract
GLA – Gamma linolenic acid
HAMS –High amylose maize starch
5-HEPE - 5-Hydroxyeicosapentaenoic acid
5-HETE – 5-Hydroxyeicosatetraenoic acid
5-HPETE – 5-Hydroperoxyeicosatetraenoic acid
5-HT – 5-Hydroxytryptamine, serotonin
IC_{50} – Inhibitory concentration at which 50% of the biological effect occurs
ICAM-1 – Intercellular adhesion molecule-1
IBD – Inflammatory bowel disease
I_{cat} - Non-selective cation current
IκB - Inhibitory binding protein-κB
IL-1 – Interleukin-1
8-*iso*-PGE_2 – 8-isoprostaglandin E_2
K_d – The concentration at which 50% of maximal binding has occurred
LA – Linoleic acid
LC – Long chain
LDL – Low density lipoprotein
LOX - Lipoxygenase
LT - Leukotriene
NF-κB - Nuclear factor κB
PDGF – Platelet derived growth factor
NO - Nitric oxide

iNOS – Nitric oxide synthetase (inducible)
OO - Olive oil
PD1 - Protectin D1
PDGF – Platelet derived growth factor
5-PEPE - 5-Per(oxy)eicosapentaenoic acid
PG - Prostaglandin
PGE_2 – Prostaglandin E_2
$PGF_{2\alpha}$ - Prostaglandin $F_{2\alpha}$
PGH_2 – Prostaglandin H_2
PGI_2 – Prostaglandin I_2
PHGG – Partially hydrolysed guar gum
PKC - Protein kinase C
PL - Phospholipid
PMN – Polymorphonuclear leukocytes
PPARγ - Peroxisome proliferator-activated receptor gamma
PS - Phosphatidylserine
PUFA(s) – Polyunsaturated fatty acid(s)
PYY – Peptide YY
ROS – Reactive oxygen species
rhIL-11 – Human recombinant IL-11
RS – Resistant starch
SCFA(s) – Short chain fatty acid(s)
SD – Sprague -Dawley
SD – Standard deviation
SEM – Standard error of the mean
SF – Saturated fat
SFA – Saturated fatty acid
SHR – Spontaneously hypertensive rat
SMC – Smooth muscle cell
SO - Sunflower (or safflower oil)
SREBP-1c - Sterol receptor element binding protein-1c
SR - Sarcoplasmic reticulum
TAG - Triacylglyceride
Th – T helper cell
TNF_α - Tumour necrosis factor-alpha
TTX - Tetrodotoxin
TXA_2 – Thromboxane A_2
UC - Ulcerative colitis
VCAM-1 – Vascular cell adhesion molecule-1
VSMC – Vascular smooth muscle cell
WKY – Wistar-Kyoto