THESIS TITLE:

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

Candidate: Mr Glen Stephen Patten BSc (Hons)

To be submitted for: PhD by prior publication

Academic Organisation Unit:

Faculty of Sciences, School of Molecular Biosciences, Discipline of Physiology, The University of Adelaide

And

CSIRO Human Nutrition

- August 2007 -
# TABLE OF CONTENTS

Title page......................................................................................................................1

Table of contents..........................................................................................................2

Abstract..........................................................................................................................4

Declaration....................................................................................................................7

Acknowledgments.........................................................................................................8

List of Publications and Abstracts Contributing to this Thesis.................................9

Statement on jointly authored papers and authors’ contributions.........................11

Abbreviations...............................................................................................................13

Literature Review..........................................................................................................15

1.1. Introduction and historical background..............................................................15
1.2. Background to experimentation relating to this thesis.....................................17
1.3. Hypothesis...........................................................................................................18
1.4. Experimental aims..............................................................................................19
1.5. Definition of fatty acids.....................................................................................19
1.6. Eicosanoid synthesis from membrane phospholipid pool...............................22
1.7. Effects of n-3 PUFA on contractility of normal and ischaemic heart muscle.........................24
1.8. Effects of n-3 PUFA on contractility of VSMC and blood flow.......................27
1.9. Effects of n-3 PUFA on contractility of gastrointestinal tissue.....................35
1.10. Definition of fibre and resistant starch..............................................................37
1.11. Effects of dietary fibre on contractility of the gastrointestinal tract...............38
1.12. Conclusions........................................................................................................40

Experimental Studies Related to this Thesis............................................................44

2.1. Methodology........................................................................................................44
2.2. Dietary fibre feeding trial in newborn guinea pigs...........................................44
2.3. Fish oil feeding trial in newborn guinea pigs.....................................................46
2.4. Fish oil feeding trial in Sprague-Dawley rats.....................................................48
2.5. Dietary saturated fat feeding trial in WKY rats and SHR..................................49
2.6. Fish oil, SF and canola oil dietary feeding trial in SHR and WKY rats...........51
2.7. Dietary fish oil dose effects in WKY rats............................................................54
2.8. Interactive effects of resistant starch and fish oil in young Sprague-Dawley rats........................................56
2.9. Summary of major experimental findings relating to this thesis...................58
2.10. Future studies.....................................................................................................62

Bibliography...............................................................................................................68
List of Author’s Full Papers, Reviews & Chapters in Books………………………………84
Attachment of full publications relating to thesis………………………………………88
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-prostaglandin (PG)E₂-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 PGE₂, PGF₂α, 8-iso-PGE₂ and U-46619 in ileum but not colon, without changes in sensitivity (EC₅₀), 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 (PGE₂ and PGF₂α) 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.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works’

………………………………………………………………………………

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


Abstracts


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.

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

8. Michael J. Adams

9. Julie A. Dallimore

10. Paul F. Rogers

11. Michael A. Conlon
ABBREVIATIONS

AA – Arachidonic acid
ALA – Alpha linolenic acid
Ang II – Angiotensin II
ATP- Adenosine triphosphate
Bmax – Maximal amount of binding that would occur
Ca2+ - Calcium ion
[Ca2+]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
ΔΨm - Mitochondrial transmembrane potential
DHA – Docosahexaenoic acid
DPA – Docosapentaenoic acid
EC - Enterochromaffin cell
EC50 – 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
IC50 – Inhibitory concentration at which 50% of the biological effect occurs
ICAM-1 – Intercellular adhesion molecule-1
IBD – Inflammatory bowel disease
Icat - Non-selective cation current
IkB - Inhibitory binding protein-κB
IL-1 – Interleukin-1
8-iso-PGE2 – 8-isoprostaglandin E2
Kd – 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
PGE₂ – Prostaglandin E₂
PGF₂α - Prostaglandin F₂α
PGH₂ – Prostaglandin H₂
PGI₂ – Prostaglandin I₂
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
TNFα - Tumour necrosis factor-alpha
TTX - Tetrodotoxin
TXA₂ – Thromboxane A₂
UC - Ulcerative colitis
VCAM-1 – Vascular cell adhesion molecule-1
VSMC – Vascular smooth muscle cell
WKY – Wistar-Kyoto