# CARDIOVASCULAR AND MENTAL HEALTH BENEFITS OF SOY CONSUMPTION: ROLE OF SOY ISOFLAVONES

**Alicia A Thorp**

**B Med Pharm Biotech (Hons)**

**A thesis submitted for the degree of Doctor of Philosophy**

**Discipline of Physiology**

**University of Adelaide**

**South Australia**

**May 2008**

## TABLE OF CONTENTS

# ABSTRACT

Regular soy consumption has been shown to reduce cardiovascular (CV) risk through plasma cholesterol reduction. According to the current health claim, this benefit is attributed to soy protein (SP). Dietary intervention trials indicate that isoflavones (ISO), weak phytoestrogens in soy, may also contribute by offering additional vascular and metabolic protection. Equol, a metabolite of the ISO daidzein (DAZ) with greater estrogenic potency, may be an important mediator of such effects.

This thesis examines effects of soy, in particular, ISO consumption on CV risk factors and the potential for ISOs to enhance cognition, possibly through improvements of circulatory function. Two crossover design intervention trials were undertaken: a food-based intervention, investigating differential effects of SP and ISO on plasma lipids and other risk factors for CVD, and an ISO supplementation trial, examining effects on cognition and vascular function. Both addressed whether benefits were dependent on equol production.

In the first trial, 91 subjects with untreated mild hypercholesterolemia were randomised to consume each of the following three diets in random order for sequential 6 week periods: (S) soy foods containing 24 g of SP and 75-90 mg ISO per day, (SD) soy/dairy foods containing 12 g SP, 12 g dairy protein (DP) and 75-90 mg ISO per day or (D) dairy foods containing 24 g DP only per day. At the end of each diet period, blood lipids, flow-mediated dilatation (FMD) of the brachial artery, blood pressure, arterial compliance and anthropometric measures were assessed. Compared with the control diet (D), there was a small but significant reduction in total cholesterol on the S diet only (2.8 + 1.1%, $P<0.05$), which could be accounted for by a decrease in saturated fat intake. FMD was found to be significantly improved when SD and S diet data were nested ($P=0.03$). Plasma triglycerides (TG) improved on both the SD and S diets compared with D ($P<0.01$). Other lipid, metabolic and vascular parameters did not differ between diets. There were no differences in outcomes between equol (n=30) and non equol producers (n=61).

In a subsequent 12 week double-blind supplementation trial, 34 healthy males were randomised to take 4 capsules providing 120mg ISO per day or a matching placebo for 6 weeks, after which they crossed over to the alternate supplement. FMD and cognitive assessments relating to measures of

memory and executive function were performed at the beginning and end of each treatment phase. Spatial working memory, a test in which females consistently perform better than males, was significantly improved by ISO supplementation ($P<0.02$). However, other measures of cognition and FMD were unaffected and there were no differences between equol (n=8) and non-equol producers (n=26).

These interventions indicate that ISOs offer specific health benefits, independent of equol production. ISO supplementation can enhance specific cognitive processes which appear dependent on estrogen activation. Additionally, soy foods containing ISOs improved FMD and TG but were unable to improve LDL cholesterol, even in equol producers. Thus dietary ISOs may reduce CV risk but the validity of the current health claim for SP is questioned.

# DECLARATION

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 my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

*Signed: Ms Alicia Thorp*

*(Wednesday 28th May, 2008)*

# ACKNOWLEDGEMENTS

I would like to acknowledge the Australian Research Council, So Natural Foods and Soy Health Pty Ltd who provided financial assistance and food products/supplements to conduct research for my PhD.

To my principal supervisor Professor Peter Howe, thank you allowing me the opportunity to work with you over the last five years. It's been a genuine privilege to be able to utilise your extensive skills and expertise in the area of nutrition research. The knowledge and advice you shared with me during the numerous hours spent in your counsel is sincerely appreciated.

To my co-supervisors, Associate Professor Jon Buckley and Dr Alison Coates thank you for your enthusiasm, patience and friendship over the years. I have learnt a great deal from you both and am extremely appreciative how approachable and supportive you have been as supervisors.

To those researchers I collaborated with throughout my candidature; Associate Professor Barbara Meyer, Dr Trevor Mori, Dr Jonathon Hodgson and Dr Natalie Sinn. Thank you for all your advice, the technical assistance you provided in order for me to conduct my research and for helping critique my manuscript and thesis drafts. I am especially grateful to Roger King for teaching me how to operate the HPLC system and perform isoflavone analysis. Your time and support was truly invaluable!

To my fellow NPRC postgraduates and my amazing circle of friends, thank you for all your uplifting words, keeping me grounded and never letting me lose site of my ultimate goal.

And last but not least to my beautiful family, thank you for all your love and encouragement. I can honestly say I would not have been able to complete my PhD if it had not been for your tremendous support over the last few years.

# GLOSSARY OF ABBREVIATIONS

| | | |
|---|---|---|
| 5-DHT | = | 5α-dihydrotestosterone |
| AMP | = | adenylate cyclase |
| ANPA | = | atrial natriuretic peptide receptor A |
| ANOVA | = | analysis of variance |
| β-conglycinin | = | 7S globulin |
| BDNF | = | brain derived neurotrophic factor |
| BMI | = | body mass index |
| BP | = | blood pressure |
| Ca2+ | = | calcium |
| cAMP | = | cyclic adenylate cyclase |
| CCK | = | cholecystokinin |
| cDNA | = | complimentary DNA |
| cGMP | = | cyclic guanosine monophosphate |
| ChAT | = | choline acetyl transferase |
| CHD | = | coronary heart disease |
| COX | = | cyclo-oxygenase |
| CO2 | = | carbon dioxide |
| CPT-1 | = | carnitine- palmitoyl transferase 1 |
| CV | = | cardiovascular |
| CVD | = | cardiovascular disease |
| D | = | dairy diet |
| DAZ | = | daidzein |
| DBP | = | diastolic blood pressure |
| DP | = | dairy protein |
| GEN | = | genistein |
| EC50 | = | transcriptional potency |

| | | |
|---|---|---|
| ECD | = | electrochemical detection |
| ECE-1 | = | endothelin converting enzyme-1 |
| EDHF | = | endothelium derived hyperpolarizing factor |
| EDTA | = | ethylene diamine tetra acetic acid |
| eNOS | = | endothelial nitric oxide synthase |
| ER | = | estrogen receptor |
| ERα | = | estrogen receptor alpha subtype |
| ERβ | = | estrogen receptor beta subtype |
| ERE | = | estrogen response element |
| ERK | = | extracellular signal-regulated kinase |
| ERR-α1 | = | estrogen related receptor alpha 1 |
| ET-1 | = | endothelin |
| FATP | = | fatty acid transport protein |
| FDA | = | food and drug administration |
| FMD | = | flow mediated dilatation |
| fMRI | = | functional magnetic resonance imaging |
| GLY | = | glycitin |
| Glycinin | = | 11S globulin |
| H2O2 | = | hydrogen peroxide |
| HAEC | = | human aortic endothelial cell |
| HCl | = | hydrochloric acid |
| HDL-C | = | high density lipoprotein cholesterol |
| HMG-CoA | = | hydroxymethyl glutaryl- CoA |
| HPLC | = | high performance liquid chromatography |
| HRT | = | hormone replacement therapy |
| HREC | = | human research ethics committee |
| ISO | = | soy isoflavone |

| | | |
|---|---|---|
| K+ | = | potassium |
| LAEI | = | large artery elasticity index |
| LBD | = | ligand binding domain |
| LDL-C | = | low density lipoprotein cholesterol |
| LDL-R | = | low density lipoprotein receptor |
| LPH | = | lactase phlorizin hydrolase enzyme |
| LXR-α | = | nuclear liver X receptor |
| MAP | = | mean arterial pressure |
| MAPK | = | mitogen-activated protein kinase |
| mDNA | = | messenger DNA |
| MeOH | = | methanol |
| MnSOD | = | manganese superoxide dismutase |
| Na2+ | = | sodium |
| NADPH | = | nicotinamide adenine dinucleotide phosphate |
| NGF | = | nerve growth factor |
| NO | = | nitric oxide |
| NTG | = | nitrotriglycerate |
| O- | = | superoxide |
| O-DMA | = | O-desmethylangolensin |
| PET | = | photo emission tomography |
| PGI2 | = | prostacyclin |
| PPAR | = | peroxisome proliferator activator receptor |
| PPRE | = | peroxisome proliferator hormone response element |
| PKG | = | cGMP- dependent protein kinase |
| PTK | = | protein tyrosine kinase |
| PWV | = | pulse wave velocity |
| RAVLT | = | rey's auditory verbal learning task |

| | | |
|---|---|---|
| RBA% | = | relative binding affinity as a percentage compared to estradiol |
| RIA | = | radio immunoassay |
| ROS | = | reactive oxygen species |
| RXR | = | retinoid X receptor |
| S | = | soy diet |
| SD | = | combination soy and dairy diet |
| SAC | = | systemic arterial compliance |
| SAEI | = | small artery elasticity index |
| SBP | = | systolic blood pressure |
| SERMs | = | selective estrogen receptor modulators |
| SHBG | = | sex hormone binding globulin |
| SMC | = | smooth muscle cell |
| smRLC | = | smooth muscle myosin regulatory light chains |
| SP | = | soy protein |
| SREBP | = | sterol regulatory element binding protein |
| SVR | = | systemic vascular resistance |
| TChol | = | total cholesterol |
| TG | = | triglyceride |
| TNF-α | = | tumour necrosis factor α |
| TVI | = | total vascular impedance |
| vLDL | = | very low density lipoprotein |
| VSMC | = | vascular smooth muscle cell |
| WHR | = | waist hip ratio |
| WT | = | wild type |

# LIST OF FIGURES

# LIST OF TABLES

# PUBLICATIONS

## Publications arising from PhD Thesis

### *Papers- In Press*

Thorp AA, Howe PRC, Mori TA, Coates AM, Buckley JD, Hodgson J, Mansour J, Meyer BJ. Soy food consumption does not lower LDL-cholesterol in either equol or non-equol producers. American Journal of Clinical Nutrition (accepted for publication on April 24th, 2008)

### *Abstracts and Conference Presentations*

Thorp A, Sinn N, Buckley J, Coates A & Howe P. Soy Isoflavone Supplementation Improves Spatial Working Memory in Healthy Males. Nutrition Societies of New Zealand and Australia Annual Scientific Meeting. Asia Pac J Clin Nutr 2007; 16 (Suppl 3): S34.

Thorp A, Buckley J, Coates A, Mori T, Hodgson J, Mansour J, Howe P and Meyer B. Importance of soy protein and isoflavone intake for protection against heart disease. NSA Annual Scientific Meeting. Asia Pac J Clin Nutr 2006;15(Suppl 3): S47.

***Awarded Best Student Oral Presentation; Nutrition Society of Australia (NSA), 30th Annual Scientific Meeting, 29 November 2006 Sydney***

Thorp A, Morris A, Buckley J, Mori T, Hodgson J, Meyer B & Howe P. Lack of effect of soy diets on LDL cholesterol. ASMR SA Divn. Annual Scientific Meeting, 2006; pg 57 (Poster Presentation)

Thorp A, Morris A, Buckley J, Mori T, Hodgson J, Meyer B & Howe P. Lipid lowering effects of foods containing soy protein and isoflavones. ASMR SA Divn. Annual Scientific Meeting, 2005; abstr. O20

***Awarded Healthy Aging Research Cluster (HARC) and Australian Society of Medical Research (ASMR) Healthy Ageing Research Prize for best oral presentation; ASMR Annual Scientific Meeting, 15 June, 2005.***

**Additional Publications during PhD Candidature**

***Papers- Published***

Buckley JD, Thorp AA, Murphy KJ, Howe PRC. Dose-Dependent Inhibition of the Post-Prandial Glycaemic Response to a Standard Carbohydrate Meal following Incorporation of Alpha-Cyclodextrin. Ann Nutr Metab 2006; 50:108-114

***Abstract-Published***

Parker B, Thorp A, Denichilo M, Rowney M, Coates A, Buckley J, Howe P. Effect of dairy based replacement meals on food intake and appetite in lean and obese subjects. Nutrition Societies of New Zealand and Australia Annual Scientific Meeting. Asia Pac J Clin Nutr 2006; 15 (Suppl 3): S128