

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

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## 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 ( $2.8 \pm 1.1\%$ ,  $P < 0.05$ ), 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 28<sup>th</sup> May, 2008)*





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## GLOSSARY OF ABBREVIATIONS

5-DHT	=	5 $\alpha$ -dihydrotestosterone
AMP	=	adenylate cyclase
ANPA	=	atrial natriuretic peptide receptor A
ANOVA	=	analysis of variance
$\beta$ -conglycinin	=	7S globulin
BDNF	=	brain derived neurotrophic factor
BMI	=	body mass index
BP	=	blood pressure
Ca <sup>2+</sup>	=	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
CO <sub>2</sub>	=	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
ER $\alpha$	=	estrogen receptor alpha subtype
ER $\beta$	=	estrogen receptor beta subtype
ERE	=	estrogen response element
ERK	=	extracellular signal-regulated kinase
ERR- $\alpha$ 1	=	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	=	glycitol
Glycinin	=	11S globulin
H <sub>2</sub> O <sub>2</sub>	=	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 <sup>+</sup>	=	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- $\alpha$	=	nuclear liver X receptor
MAP	=	mean arterial pressure
MAPK	=	mitogen-activated protein kinase
mDNA	=	messenger DNA
MeOH	=	methanol
MnSOD	=	manganese superoxide dismutase
Na <sup>2+</sup>	=	sodium
NADPH	=	nicotinamide adenine dinucleotide phosphate
NGF	=	nerve growth factor
NO	=	nitric oxide
NTG	=	nitrotriglycerate
O <sup>-</sup>	=	superoxide
O-DMA	=	O-desmethylangolensin
PET	=	photo emission tomography
PGI <sub>2</sub>	=	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- $\alpha$	=	tumour necrosis factor $\alpha$
TVI	=	total vascular impedance
vLDL	=	very low density lipoprotein
VSMC	=	vascular smooth muscle cell
WHR	=	waist hip ratio
WT	=	wild type

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## 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 24<sup>th</sup>, 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