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Coronary vasomotor and blood flow responses to isoflavone-intact soy protein in subjects with coronary heart disease or risk factors for coronary heart disease

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ABSTRACT

Animal data suggest favourable coronary vasomotor actions of isoflavones however the effects of isoflavones on the human coronary circulation are undetermined. We therefore investigated the effects of short-term isoflavone-intact soy protein ingestion on basal coronary arterial tone and stimulated vasoreactivity and blood flow in patients with coronary heart disease (CHD) or risk factors for CHD. Seventy-one subjects were randomised, double-blind, to isoflavone-intact soy protein (active; n=33, mean±SD, aged 58±8 years) or isoflavone-free placebo (n=38, aged 61±8 years) for 5 days prior to coronary angiography. In 25 of these subjects stimulated coronary blood flow was calculated from flow velocity, measured using intracoronary Doppler, and coronary luminal diameter before and after intracoronary adenosine, acetylcholine (ACh) and isosorbide dinitrate (ISDN) infusions. Basal and stimulated coronary artery luminal diameters were measured using quantitative coronary angiography. Serum concentrations of isoflavones genistein, daidzein and equol were increased by active treatment ($p<0.001$, $p<0.001$ and $p=0.03$ respectively). Basal mean luminal diameter was not significantly different between groups (2.9 ± 0.7 vs 2.73 ± 0.44 mm, active vs placebo; $p=0.31$). There was no difference in luminal diameter, flow velocity and volume flow responses to adenosine, ACh or ISDN between groups. Active supplement had no effect on basal coronary artery tone or stimulated coronary vasoreactivity or blood flow compared with placebo. Our results suggest that short-term consumption of isoflavone-intact soy protein is neither harmful nor beneficial to the coronary circulation of humans with CHD or risk factors for CHD. These data are consistent with recent cautions placed on the purported health benefits of plant sterols.

INTRODUCTION

Soy beans, the source of most soy protein consumed in the diet, contain soy protein and other constituents including isoflavones (sometimes termed 'phytoestrogens' due to their similar chemical structure to estradiol and weak binding affinity for estrogen receptor alpha and beta [1;2]). Evidence suggests that isoflavones have health benefits in humans against hormone dependent cancers of the breast and prostate, bone sparing effects, and some favourable effects on cardiovascular disease risk factors such as cholesterol concentrations in hypercholesterolemic subjects [3-6]. The American Heart Association dietary guidelines recommended 'the consumption of soy protein containing isoflavones, along with other heart-healthy diet modifications' in 'high-risk populations with elevated total and low-density lipoprotein (LDL) cholesterol' [7], and the Food and Drug Administration approved labels on foods containing 25g/day soy protein claiming a reduction in heart disease risk [8]. However, a recent American Heart Association dietary advisory committee counsels caution with regard to these recommendations [9].

Our group has demonstrated a relaxing effect of the isoflavone genistein in precontracted coronary arteries [10]. In vivo animal studies indicate a beneficial effect of soy protein with intact isoflavones on coronary reactivity in female but not male atherosclerotic monkeys, and indicate an endothelium-mediated effect [11-13]. Investigation of the vascular effects of soy protein and isoflavones in humans have largely been confined to the peripheral circulation, mostly in healthy postmenopausal women [14-17]. These studies show both positive and negative results using non-standard soy protein or isoflavone preparations and doses. In humans with risk factors for coronary heart disease (CHD) the data are also unclear. One uncontrolled study in hypercholesterolemic non-smoking men showed increases in baseline brachial artery diameter and reactive hyperaemia after 6 weeks of low-fat diet and soy protein substitution [18]. In contrast, a cross-over study in postmenopausal hypercholesterolemic women showed no effect of 25 g soy protein compared with placebo for 6 weeks on lipid profile or brachial artery reactivity [19], and a recent study in hypertensive subjects showed no effect of isoflavone-intact soy protein on arterial function compared with placebo [20].

There are very few published studies investigating the effects of soy protein, with or without intact isoflavones, on the coronary circulation of humans [21]. We therefore investigated the effects of short-term isoflavone-intact soy protein ingestion on basal coronary artery tone and stimulated coronary vasoreactivity and blood flow in patients with CHD or risk factors for CHD undergoing clinically-indicated coronary angiography.

METHODS

Subjects

Subjects aged 30-75 years with clinical indications for coronary angiography, and on the waiting list for the procedure at the Royal Brompton and Harefield hospitals were invited to participate in the study. Female subjects were postmenopausal (follicular stimulating hormone >40 IU/L) and had not taken hormone replacement therapy during the previous 6 months. Subjects with significant bradycardia, asthma, left ventricular hypertrophy or dysfunction, primary valvar heart disease or known 3-vessel coronary disease were excluded. The study complied with the Declaration of Helsinki, the protocol was approved by the local research ethics committee and all subjects gave written informed consent to the full study.

Study design

Subjects were randomised in a double-blind parallel design to twice daily isoflavone-intact soy protein drinks (SUPRO[®] SOY, The Solae Company, PO Box 88940, St Louis, Missouri, USA) or isoflavone-free placebo drinks in addition to an unrestricted diet for 5 days before coronary angiography with the last drink consumed approximately 5 hours before cardiac catheterisation. Blinding was achieved by having the drinks flavoured the same, labelled blindly, and randomised and dispensed by our pharmacy department. Cardiac medication and caffeine-containing beverages were withheld for at least 24 hours prior to cardiac catheterisation.

Firstly, a 10 ml blood sample was taken from the in situ femoral artery sheath for evaluation of plasma isoflavone and lipid concentrations. All subjects then underwent clinically-indicated coronary angiography and these images were used to measure basal coronary artery mean luminal diameter (method described later). Subjects with risk factors for CHD and with at least one non-obstructed major coronary artery proceeded immediately to the intracoronary sub-study.

Intracoronary protocol. Following full heparinisation, a 0.014" Doppler wire (Cardiometrics[®] Inc, Mountain View, California) was positioned in the proximal portion of an unobstructed coronary artery from which continuous traces of average peak blood flow velocity were recorded. Arterial pressure, heart rate (HR) and electrocardiogram were displayed continuously.

Baseline measurements were made then adenosine (30 µg) was given as a bolus intracoronary infusion. This was followed by infusions of increasing concentrations of intracoronary acetylcholine (ACh; 10^{-7} - 10^{-5} M) for 2 minutes each or until peak velocity response. The study protocol was completed with an intracoronary bolus of 300 µg isosorbide dinitrate (ISDN).

Coronary angiograms were performed at baseline, then at peak response to each vasoactive substance. A rest period of at least 1 minute was observed between each infusion to allow all

measured parameters to return to baseline.

Quantitative coronary angiography and calculation of flow

Coronary angiograms were acquired digitally using a real-time digital image acquisition system (Siemens AG, Berlin and Munich, Germany) and analysed off-line using quantitative coronary angiography (QCA; MEDIS, Leiden, The Netherlands). Basal luminal diameter of the entire coronary artery (mean luminal diameter) was measured for all subjects. For the intracoronary protocol angiograms mean luminal diameter and luminal diameter approximately 4 mm distal to the tip of the Doppler wire were measured. The latter measurements were used to quantify volume flow as described previously [22].

Calculation of CFR and coronary resistance

In the intracoronary protocol, adenosine was infused to induce maximal hyperaemic response. Coronary flow reserve (CFR) was then calculated as the quotient of maximal coronary blood flow/baseline coronary blood flow. Coronary resistance was calculated as the quotient of mean arterial pressure (MAP, mmHg) and coronary blood flow (ml/min).

Soy preparation

The isolated soy protein drink (SUPRO[®]SOY) was prepared using water-washing to retain the isoflavones. One 65g powdered beverage or one 316g ready-to-drink beverage was consumed twice daily approximately 12 hours apart. Each SUPRO[®]SOY drink (active) provided 25.8g soy protein containing 71mg isoflavones (aglycone weight). The placebo (control) was made with milk protein isolate and provided 25.7g milk protein with no isoflavones. The mucosal lining of the gut is necessary for the activation of the isoflavones in this form, which takes approximately 3-5 hours. Each beverage (active or control) typically contained 210-240 calories, 1-2 g fat (0g saturated fat), 24-30g carbohydrate (21-26g sugars), 26 g protein (25g from soy or milk), 170-260mg sodium, 480-800mg potassium and 900mg calcium.

Blood analysis methods

Isoflavones genistein, daidzein and equol were isolated and measured from serum using liquid-chromatographic tandem mass spectrometric bioanalytical assays (HFL Ltd, Fordham, Cambridgeshire, UK). Total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride concentrations were measured using a Beckman CX7 analyser. LDL-cholesterol was estimated with the formula of Friedewald.

Statistics

Sample size

As there were no previous studies of isoflavones in the human coronary circulation on which to base our calculations, the sample size calculation for the basal epicardial coronary artery tone endpoint was based on a previous study of 17 β -estradiol published by our laboratory [23], estimating a 12% difference in basal luminal diameter with SD of 15% at 80% power and 5% significance level, giving an estimate of 38 subjects in each group.

The sample size calculation for the intracoronary study was determined by the treatment response of coronary luminal diameter to ACh, using in vivo animal data to estimate the difference between groups [12]. A mean difference of 12% with SD of 7% required 13 subjects in each group, with assumptions of a 5% significance level and 80% power to detect a difference between groups.

Statistical methods

Outcome measures that were normally distributed were compared between groups using a two-sample t-test and are presented as mean \pm SD. The Mann-Whitney test was used to compare between groups when outcomes were not normally distributed and are presented as median (interquartile range). $p < 0.05$ was considered significant.

RESULTS

Subject characteristics

Seventy-one subjects were consented and randomised (Table 1). Thirty-three subjects did not have an coronary artery suitable for the intracoronary protocol (for example due to the extent of coronary atherosclerosis or difficulty placing the coronary catheter in a stable position), 4 did not comply with the study treatment regimen, 1 had severe chest pain on intracoronary contrast injections at diagnostic angiography, 1 had pre-procedural ST-segment changes, 1 had pre-procedural syncope, 2 had taken anti-anginal medication prior to angiography, and 4 had exclusion criteria that were unknown at randomisation.

The remaining 25 subjects proceeded to the intracoronary protocol (Table 2). Thirteen received the active drink and of these 2 subjects had no significant coronary stenosis, 9 had 1-vessel disease (diameter stenosis $\geq 70\%$ in a major epicardial coronary artery) and 2 had 2-vessel disease. In the active group the left anterior descending coronary artery was studied in 1 subject, the left circumflex in 2 subjects and the right coronary artery in 10 subjects. Twelve subjects received placebo and of these 2 subjects had unobstructed coronary arteries, 7 had 1-vessel disease and 3 had 2-vessel disease. In the placebo group the left circumflex coronary artery was

studied in 2 subjects and in the right coronary artery in 10 subjects. There was no significant difference between groups in extent of coronary atherosclerosis ($p=0.81$), artery studied ($p=0.62$), risk factors for CHD, anthropometric measurements or cardiovascular medications (Table 2).

Serum isoflavone concentrations

Genistein (median (95% CI); 340 (247,515) vs 3.9 (1.7, 5.8) ng/mL, $p<0.001$), daidzein (168 (108, 360) vs 1.2 (0.5, 2) ng/mL, $p<0.001$) and equol (0.88 (0.72, 2.41) vs 0.59 (0.35, 0.76) ng/mL, $p=0.03$) concentrations were greater in the active vs control groups. Two subjects who consumed the isoflavone-intact soy protein drink produced significantly greater equol concentrations than the rest of the active group (282.2 and 171.3 ng/mL compared to a mean concentration of 1.2 ng/mL for the rest of the active group). There was no dietary explanation (vegetarianism, herbal supplements) for this variation.

Lipid profile

There was no difference in lipid profile after treatment in either the main study group (active vs placebo; total cholesterol 4.5 ± 0.9 vs 5.1 ± 1 mmol/l, $p=0.06$; HDL-cholesterol 1.2 ± 0.4 vs 1.2 ± 0.4 mmol/l, $p=0.99$; LDL-cholesterol 2.7 ± 1.1 vs 3 ± 0.9 mmol/l, $p=0.17$; triglycerides 1.8 ± 1.5 vs 1.8 ± 1 mmol/l, $p=0.98$) or the intracoronary protocol group (active vs placebo; total cholesterol 5.1 ± 0.9 vs 4.5 ± 1.2 mmol/l, $p=0.19$; HDL-cholesterol 1.3 ± 0.3 vs 1.2 ± 0.5 mmol/l, $p=0.34$; LDL-cholesterol 3.2 ± 0.8 vs 2.7 ± 1.4 mmol/l, $p=0.29$; triglycerides 1.5 ± 0.8 vs 2 ± 2 mmol/l, $p=0.44$).

Luminal diameter

There was no difference in basal mean luminal diameter between treatment groups (2.9 ± 0.7 vs 2.73 ± 0.44 mm, active vs control respectively; $p=0.31$).

Intracoronary protocol

Luminal diameter

Mean luminal diameter responses to adenosine, ACh (10^{-7} - 10^{-5} M) and ISDN were significantly increased in both treatment groups but there was no difference in response between groups (Figure 1).

Velocity

Blood flow velocity responses significantly increased after each infusion in both treatment groups except after ACh 10^{-7} M in the active group (Figure 1). There was no significant difference

between groups, but a trend towards a significantly lower peak velocity response to ACh 10^{-7} M ($p=0.07$) and ACh 10^{-5} M ($p=0.09$) in subjects taking the active drinks was shown.

Volume flow

Volume blood flow increased significantly compared to baseline after adenosine, ACh 10^{-6} and 10^{-5} M and ISDN in both treatment groups (Figure 1), but there was no difference between groups.

CFR and resistance

There was a trend towards a lower coronary vascular resistance in response to adenosine (0.94 ± 0.33 vs 1.24 ± 0.5 ml/min/mmHg, $p=0.09$) and ISDN (1.12 ± 0.29 vs 1.58 ± 0.7 ml/min/mmHg, $p=0.06$) in the active group compared with control, but no difference in resistance after ACh infusions. CFR was not statistically different between treatment groups (2.79 ± 0.93 vs 2.42 ± 0.77 , $p = 0.3$; active vs control respectively).

Systemic haemodynamics

Baseline systolic blood pressure and diastolic blood pressure were not different between groups (146 ± 18 vs 158 ± 20 mmHg, $p=0.59$ and 79 ± 7 vs 76 ± 5 mmHg, $p=0.25$; active vs placebo). There was no difference in MAP (100 ± 9 vs 97 ± 11 mmHg, $p=0.37$; active vs placebo) and HR (69 ± 11 vs 67 ± 9 bpm, $p=0.73$; active vs placebo) at baseline, and these measurements did not change significantly throughout the procedure.

DISCUSSION

This is the first report to describe invasive in vivo coronary vasomotor and blood flow effects of soy-derived isoflavone consumption in humans. We found that isoflavone-intact soy protein and placebo-treated subjects with CHD or risk factors for CHD had similar basal coronary luminal diameter, and comparable vasodilator and blood flow responses to adenosine, ACh and ISDN. Until now intracoronary studies of soy protein and isoflavones have been confined to animals, and studies in humans investigating vascular function after isoflavone-intact soy protein ingestion have largely been limited to the peripheral circulation, namely the brachial artery. A strength of our study is the direct investigation of coronary vascular effects of isoflavone-intact soy protein in humans. Our results complement those of van der Schouw and colleagues who found no effect of higher dietary intake of phytoestrogens on cardiovascular disease risk [24]. Indeed recent evidence has cast uncertainty on the purported benefits of soy and isoflavone supplementation on cardiovascular health [9].

Effect of variations in isoflavone preparation and ingestion

Vascular responses to isoflavones vary depending on preparation used (isoflavone-intact soy protein vs isolated isoflavones for example), dose of isoflavones, duration of exposure, and existence of risk factors for CVD vs healthy subjects. SUPRO[®]SOY was a commercially-available preparation containing isoflavone-intact soy protein at levels recommended for lipid lowering at the time our study was conceived [7;8]. We favoured this supplement over intracoronary infusions of isoflavones as the combination of soy-protein together with isoflavones had been shown to result in enhanced lipid effects compared to isoflavones alone (summarised in Sacks and colleagues [9]) and in practice therefore isoflavone-intact soy protein would be the supplement of choice. We chose a relatively short treatment duration as pilot data showed that serum isoflavone levels were significantly raised after taking this supplement for 4 days, and that relaxation of precontracted isolated coronary arteries exposed to phytoestrogens occurred within a short time period [10]. It is possible that longer-term treatment may have different effects on the human coronary circulation however primate coronary data would argue against this [11-13]. In the peripheral circulation of healthy postmenopausal women there does appear to be an effect of treatment duration but clear interpretation of a duration effect is complicated by use of non-uniform treatments (isolated isoflavones vs red clover isoflavones vs isoflavone-intact soy protein) [14-17]. Our study population had a clinical indication for undergoing coronary angiography, to investigate chest pain, and all subjects had at least one risk factor for CHD. Whether a similar lack of effect on coronary artery tone and stimulated reactivity would be demonstrated in healthy subjects ingesting isoflavone-intact soy protein is unknown, however data from the peripheral circulation would suggest that there may be a differing effect of isoflavone-intact soy protein on the coronary circulation of healthy subjects [14-17].

Soy isoflavones and coronary endothelial function

A normally-functioning endothelium is vital for the maintenance of vascular health. The age-related decline in endothelial function predisposes to vascular diseases however evidence suggests that diet assists in the preservation of endothelial function [25]. We observed no effect of isoflavone-intact soy protein consumption on stimulated coronary endothelium-derived nitric oxide however. Results of previous coronary studies, using longer-term soy-isoflavone treatment in primates, suggest that endothelium-dependent responses in the coronary circulation may be gender-related [11-13] although inhibition of atheroma progression appears unrelated to gender [11]. A recent observational study found that higher genistein levels in women referred for

coronary angiography are associated with reduced coronary endothelium-dependent and – independent coronary vascular function [21]. Our study did not include a sufficient number of women to perform a gender-specific analysis so we cannot exclude this as a possible reason for the null result on endothelial function.

Isoflavone concentrations

Dietary isoflavones such as genistein and daidzein are metabolised by intestinal bacteria, conjugated in the liver, circulated in the plasma and excreted in the urine [26]. Daidzein is further converted to equol in approximately one-third of adults depending on gut microflora or/and intestinal conditions [27]. Equol has higher estrogenic potency than daidzein, with higher binding affinity for estrogen receptors and lower binding to sex-hormone binding globulin and albumin [28;29]. Serum concentrations of genistein measured in our study are similar to those reported in a previous pilot study using the same preparation (1.6 μM vs 2.1 μM respectively) [10]. In the present study only 2 subjects receiving the isoflavone-intact soy protein drinks produced significant amounts of serum equol, fewer than the previous report of one-third of the general population [27]. Investigation of coronary vasoreactivity and blood flow responses in active daidzein-equol metabolisers may further clarify the role of soy protein products in the coronary circulation.

Limitations

We acknowledge that pre-treatment data would have helped the interpretation of our data and this is a flaw in our study design. Our protocol was of parallel design as performing cardiac catheterisation before and after treatment raised ethical issues relating to risk/benefit of the additional procedure. We did not control the general diet of subjects enrolled in the study, however measurement of serum isoflavone concentrations confirmed that the subjects in the placebo group did not consume significant amounts of isoflavone-intact soy protein. We feel that consumption of a supplemental isoflavone-intact soy protein drink in addition to an unrestricted diet better mimics the way in which isoflavones would generally be consumed in the diet and therefore enhances the application of our results. We also felt that consuming a supplement would provide more pertinent information than giving isolated isoflavones via intracoronary infusion as we have done in previous intracoronary studies [22;23]. We enrolled 12 and not the required 13 patients into the placebo group meaning that the intracoronary study is slightly underpowered. Whether our results would be replicated in a larger and longer-term study, possibly using non-invasive measures of coronary blood flow is an area for further investigation.

Conclusions

In our study, short-term ingestion of isoflavones together with soy protein does not affect coronary vascular tone or stimulated vasoreactivity and blood flow compared to isoflavone-free placebo. There was no effect on ACh-stimulated coronary luminal diameter and blood flow indicating no effect on endothelium-derived nitric oxide. Our results suggest that consumption of isoflavone-intact soy protein is neither harmful nor beneficial to the coronary circulation of humans with risk factors for, or established CHD. A recent nutritional advisory committee has placed caution on past statements by National organisations asserting that consumption of plant sterols is recommended to combat diseases such as CHD [9], and our results provide further physiological evidence to support this advice.

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REFERENCES

1. Miksicek,R.J. (1994) Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J.Steroid Biochem.Mol.Biol.* **49**, 153-160.
2. Kuiper,G.G., Lemmen,J.G., Carlsson,B. et al. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**, 4252-4263.
3. Adlercreutz,H. (2002) Phyto-oestrogens and cancer. *Lancet Oncol.* **3**, 364-373.
4. Anderson,J.W., Johnstone,B.M. and Cook-Newell,M.E. (1995) Meta-analysis of the effects of soy protein intake on serum lipids. *N.Engl.J.Med.* **333**, 276-282.
5. Arjmandi,B.H. (2001) The role of phytoestrogens in the prevention and treatment of osteoporosis in ovarian hormone deficiency. *J.Am.Coll.Nutr.* **20**, 398S-420S.
6. Lissin,L.W. and Cooke,J.P. (2000) Phytoestrogens and cardiovascular health. *J.Am.Coll.Cardiol.* **35**, 1403-1410.
7. Krauss,R.M., Eckel,R.H., Howard,B. et al. (2000) AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation* **102**, 2284-2299.
8. Food and Drug Administration (1999) Food labeling, health claims, soy protein, and coronary heart disease. *Fed.Reg.* **57**, 699-733.
9. Sacks,F.M., Lichtenstein,A., Van Horn,L., Harris,W., Kris-Etherton,P. and Winston,M. (2006) Soy protein, isoflavones, and cardiovascular health. An American Heart Association science advisory for professionals from the nutritional committee. *Circulation* **113**, 1034-1044.
10. Figtree,G.A., Griffiths,H., Lu,Y.-Q., Webb,C.M., MacLeod,K. and Collins,P. (2000) Plant derived estrogens relax coronary arteries in vitro by a calcium antagonistic mechanism.

J.Am.Coll.Cardiol. **35**, 1977-1985.

11. Adams,M.R., Golden,D.L., Williams,J.K., Franke,A.A., Register,T.C. and Kaplan,J.R. (2005) Soy protein containing isoflavones reduces the size of atherosclerotic plaques without affecting coronary artery reactivity in adult male monkeys. *J.Nutr.* **135**, 2852-2856.
12. Honore,E.K., Williams,J.K., Anthony,M.S. and Clarkson,T.B. (1997) Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil.Steril.* **67**, 148-154.
13. Williams,J.K. and Clarkson,T.B. (1998) Dietary soy isoflavones inhibit in-vivo constrictor responses of coronary arteries to collagen-induced platelet activation. *Coron.Artery Dis.* **9**, 759-764.
14. Squadrito,F., Altavilla,D., Morabito,N. et al. (2002) The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. *Atherosclerosis* **163**, 339-347.
15. Squadrito,F., Altavilla,D., Crisafulli,A. et al. (2003) Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. *Am.J Med.* **114**, 470-476.
16. Teede,H.J., Dalais,F.S., Kotsopoulos,D., Liang,Y.L., Davis,S. and McGrath,B.P. (2001) Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J.Clin.Endocrinol.Metab.* **86**, 3053-3060.
17. Teede,H.J., McGrath,B.P., DeSilva,L., Cehun,M., Fassoulakis,A. and Nestel,P.J. (2003) Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. *Arterioscler.Thromb.Vasc.Biol.* **23**, 1066-1071.
18. Yildirim,A., Tokgozoglul,S.L., Oduncu,T. et al. (2001) Soy protein diet significantly

improves endothelial function and lipid parameters. *Clin.Cardiol.* **24**, 711-716.

19. Blum,A., Lang,N., Vigder,F. et al. (2003) Effects of soy protein on endothelium-dependent vasodilatation and lipid profile in postmenopausal women with mild hypercholesterolemia. *Clin.Invest.Med.* **26**, 20-26.
20. Teede,H.J., Giannopoulos,D., Dalais,F.S., Hodgson,J. and McGrath,B.P. (2006) Randomised, controlled, cross-over trial of soy protein with isoflavones on blood pressure and arterial function in hypertensive subjects. *J.Am.Coll.Nutr.* **25**, 533-540.
21. Pepine,C.J., von Mering,G.O., Kerensky,R.A. et al. (2007) Phytoestrogens and coronary microvascular function in women with suspected myocardial ischemia: a report from the Women's Ischemia Syndrome Evaluation (WISE) Study. *J Womens Health* **16**, 481-488.
22. Webb,C.M., McNeill,J.G., Hayward,C.S., de Ziegler,D. and Collins,P. (1999) Effects of testosterone on coronary vasomotor regulation in men with coronary artery disease. *Circulation* **100**, 1690-1696.
23. Webb,C.M., Ghatei,M., McNeill,J.G. and Collins,P. (2000) 17 β -Estradiol decreases endothelin-1 levels in the coronary circulation of postmenopausal women with coronary artery disease. *Circulation* **102**, 1617-1622.
24. van der Schouw,Y.T., Kreijkamp-Kaspers,S., Peeters,P.H., Keinan-Boker,L., Rimm,E.B. and Grobbee,D.E. (2005) Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in Western women. *Circulation* **111**, 465-471.
25. Brown,A.A. and Hu,F.B. (2001) Dietary modulation of endothelial function: implications for cardiovascular disease. *Am.J.Clin.Nutr.* **73** (4), 673-686.
26. Cassidy,A. (2003) Potential risks and benefits of phytoestrogen-rich diets. *Int.J.Vitam.Nutr.Res.* **73**, 120-126.
27. Setchell,K.D., Brown,N.M., Desai,P. et al. (2001) Bioavailability of pure isoflavones in

healthy humans and analysis of commercial soy isoflavone supplements. *J.Nutr.* **131**, 1362S-1375S.

28. Nagel,S.C., vom Saal,F.S. and Welshons,W.V. (1998) The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. *Proc.Soc.Exp.Biol.Med.* **217**, 300-309.
29. Shutt,D.A. and Cox,R.I. (1972) Steroid and phyto-oestrogen binding to sheep uterine receptors in vitro. *J.Endocrinol.* **52**, 299-310.

Stage 2(a) POST-PRINT

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Figure 1 Coronary artery change in mean luminal diameter, blood flow velocity and volume blood flow after infusions of adenosine (AD), ACh 10^{-7} - 10^{-5} M (A7, A6, A5) and ISDN in subjects taking isoflavone-intact soy protein (black) or placebo (grey). ** $p \leq 0.01$, *** $p \leq 0.001$ Active vs respective baseline; † $p \leq 0.05$, †† $p \leq 0.01$, ††† $p \leq 0.001$ control vs respective baseline. There was no significant difference in response between the two groups.

Stage 2(a) POST-PRINT

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Table 1 Subject characteristics

Values are mean±SD or number. M, male; F, female; MI, myocardial infarction; BMI, body mass index.

Characteristic	Active (n=33)	Placebo (n=38)	p
Age (years)	58±8	61±8	0.051
Gender (M/F)	31/2	31/7	0.12
Previous MI	10	10	0.69
Hypercholesterolaemia	21	26	0.51
Hypertension	18	18	0.7
Diabetes mellitus	4	4	0.83
Cigarette smoking status			0.69
current	4	6	
ex	19	17	
never	10	6	
unknown	0	2	
CHD			0.81
Unobstructed	8	6	
1 vessel disease	9	10	
2 vessel disease	9	13	
3 vessel disease	7	9	
Height (cm)	175±6	174±9	0.62
Weight (kg)	90±15	85±14	0.3
BMI (kg/m ²)	29±4	28±3	0.46

Table 2 Subject characteristics intracoronary flow protocol

Values are mean±SD or number. M, male; F, female; MI, myocardial infarction; BMI, body mass index; ACE, angiotensin converting enzyme; K, potassium; ISMN, isosorbide mononitrate.

Characteristic	Active (n=13)	Placebo (n=12)	p
Age (years)	58±6	59±7	0.66
Gender (M/F)	13/0	9/3	0.13
Previous MI	1	3	0.11
Hypercholesterolaemia	6	5	0.85
Hypertension	6	6	0.54
Diabetes mellitus	3	1	0.32
Cigarette smoking status			0.34
current	3	2	
ex	8	5	
never	2	5	
Height (cm)	175±7	174±7	0.9
Weight (kg)	90±15	84±12	0.2
BMI (kg/m ²)	29±4	28±4	0.4
Cardiovascular medications			
ACE inhibitor	2	3	0.57
Statin	6	5	0.83
β-blocker	6	8	0.32
Calcium antagonist	7	4	0.32
K channel activator	1	3	0.26
ISMN	5	4	0.8

