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A Meal Enriched with Soy Isoflavones Increases Nitric Oxide-Mediated Vasodilation in Healthy Postmenopausal Women[1,2]

Wendy L. Hall,[3]* Nichola L. Formanuik,[3] Duangporn Harnpanich,[3] Monique Cheung,[3] Duncan Talbot,[5] Philip J. Chowienczyk,[4] and Thomas A. B. Sanders[3]

[3]Nutritional Sciences Division, School of Biomedical and Health Sciences, King's College London, London SE1 9NH, UK; [4]Cardiovascular Division, School of Medicine, King's College London, London SE1 7EH, UK; and [5]Unilever Corporate Research, Sharnbrook, Bedfordshire MK44 1LQ, UK

Abstract

Evidence from infusion studies suggests that soy isoflavones influence nitric oxide-dependent vasorelaxation. It is uncertain whether orally consumed isoflavones have similar effects. Healthy postmenopausal women ($n = 22$) consumed 2 low-fat test meals in random order 1 wk apart, with 80 mg isoflavones (ISO) or without isoflavones (CON). Endothelium-dependent vasodilation, assessed by brachial artery flow-mediated dilatation (FMD), was measured in fasting subjects, and 4 and 6 h following the test meal, in addition to blood pressure and pulse wave analysis to derive the peripheral augmentation index (pAIx). Blood samples were taken after fasting, and 5 and 7 h following the test meal for serum isoflavone, plasma 8-isoprostane $F_{2\alpha}$, nitric oxide metabolites (NOx), glucose, and triacylglycerol analysis. Serum genistein and daidzein concentrations (geometric mean, 95% CI) reached 1.49 (1.20–1.84) μmol/L and 0.95 (0.70–1.30) μmol/L, respectively, following ISO (7 h). FMD and plasma NOx concentrations were greater following ISO compared with CON, indicating better postprandial endothelial function. FMD values (%, mean ± SD) were: CON, 5.49 ± 2.32, 4.35 ± 2.32, 4.40 ± 2.26; ISO, 5.38 ± 1.91, 5.08 ± 1.74, 6.11 ± 2.60, at baseline, 4 h, and 6 h, respectively ($P < 0.01$). Plasma NOx concentrations (μmol/L, mean ± SD) were: CON, 20.0 ± 5.1, 16.8 ± 5.1, 23.1 ± 6.0; ISO, 18.6 ± 6.3, 19.5 ± 5.1, 21.3 ± 10.1, at baseline, 5 h, and 7 h, respectively ($P < 0.005$). Treatment did not affect pAIx, blood pressure, or plasma 8-isoprostane $F_{2\alpha}$ concentrations. In conclusion, consuming an isoflavone-enriched low-fat meal acutely increases endothelium-dependent vasodilation in postmenopausal women. Regular consumption of soy isoflavones may protect against endothelial dysfunction. J. Nutr. 138: 1288–1292, 2008.

Introduction

Cardiovascular risk increases with the decline in estrogen production after menopause (1). The estrogenic and potentially cardioprotective properties of isoflavones have been the subject of considerable interest since the early soya-feeding animal studies of the 1960s (2). Epidemiological evidence suggests that soy isoflavone intake is inversely associated with heart disease mortality in Japanese women (3) and intervention trials have demonstrated that the consumption of whole soybean foods and soy protein isolate may improve lipoprotein status (4). However, a recent advisory report from the AHA concluded that the "direct cardiovascular health benefit of soy protein isoflavone supplements is minimal at best" and that isolated soy isoflavones had no effect on blood lipids or blood pressure (5). In contrast, in vitro studies using cultured endothelial cells and animal experiments have demonstrated a wide range of atherosclerosis-related molecular events that are modulated by isoflavones, including oxidative, inflammatory, and nitric oxide (NO)[6] pathways (6,7).

Prolonged activation of vascular mechanisms for protecting against adverse physico-chemical stimuli (inflammatory response, procoagulation, and vasoconstriction) can lead to endothelial dysfunction, an early event in the progression of atherosclerosis. Isoflavones have been suggested to protect the endothelium. For example, habitual dietary isoflavone intakes have been correlated with markers of endothelial function (8). Several studies have found that 6-wk to 12-mo supplementation with isoflavones improves endothelium-dependent vasodilation measurements (9,10), but others have not supported this (11–14). There is, however, persuasive evidence from animal studies that isoflavones can improve endothelial function (15,16). In addition, evidence from acute infusion studies in humans provides

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* To whom correspondence should be addressed. E-mail: wendy.hall@kcl.ac.uk.

[6] Abbreviations used: CON, control test meal; FMD, flow-mediated dilatation; ISO, isoflavone test meal; NO, nitric oxide; NOx, nitric oxide metabolite; pAIx, peripheral augmentation index; TAG, triacylglycerol.

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evidence to indicate that isoflavones may be beneficial to the endothelium (17,18). However, studies investigating the acute effects of oral ingestion of isoflavones on endothelial function are lacking. This is an important issue, because in vitro studies suggest that the effects of isoflavones on endothelial cell function are likely to be transient in duration; therefore, conflicting evidence from human dietary intervention may actually be related to timing of the endothelial function measurements. The aim of this study was to investigate whether an acute oral dose of soy isoflavones, consumed as part of a low-fat meal, resulted in changes in NO-dependent vasodilation compared with a control low-fat meal.

Methods

The study was approved by King's College London Research Ethics Committee. All subjects signed an informed consent form.

Subjects

Twenty-seven healthy, nonsmoking postmenopausal women aged 54–70 y were recruited from the community and university. Inclusion criteria were as follows: BMI, 18–36 kg/m^2, blood pressure <160/90 mm Hg, plasma triacylglycerol (TAG) <5.0 mmol/L, and plasma total cholesterol <8.0 mmol/L. Exclusion criteria included: menstruation during the last 12 mo; follicle-stimulating hormone >25 IU/L; current use of estrogen/progesterone, steroid medication (apart from topical corticosteroids), prescribed antiinflammatory or blood-thinning medication; a history of diabetes, myocardial infarction, angina pectoris, stroke, a diagnosis of cancer in the last 5 y (excluding basal cell carcinoma), or thrombosis; a history of excess alcohol intake or substance abuse; alcohol intake exceeding a moderate intake (>224 g/wk); abnormal liver function enzymes, hematology, and fasting glucose; or smoking or recent ex-smoker. Regular consumers of soy foods or soy isoflavone supplements who were unable to avoid consumption of these items for 2 wk before the study and during the study period were also excluded from the study. Consumption of soy foods and supplements during the previous 12 mo were assessed in the screening questionnaire and also by a short FFQ designed specifically for the study. Five subjects dropped out during the study due to inability to attend the metabolic research unit (2 subjects), difficulty obtaining blood samples (1 subject), and family bereavement (2 subjects); 22 subjects completed the study (**Table 1**).

Study protocol

The design of the study was a double-blind, randomized, placebo-controlled crossover. Subjects attended the Department of Clinical Pharmacology, St. Thomas' Hospital, London, on 2 separate occasions and at least 1 wk apart. Subjects were requested to avoid consuming soy foods for 2 wk before their first study visit and throughout the duration of the study period. On the day before each clinical visit, subjects were provided with a standard commercially available low-fat meal containing 8 g fat to be consumed before 2000 h. Subjects were also asked to follow a low-nitrate+nitrite diet the day before each visit to ensure low baseline levels of nitric oxide metabolites (NOx) in their blood for analysis. At the screening session, a list of foods high in nitrate+nitrite (19) was provided in the information booklet and subjects were requested to avoid consuming these food items on the day before the study visits. Subjects were also requested to only drink Buxton mineral water throughout the clinical visit and the day before, as it contained relatively low nitrate levels. Subjects refrained from alcohol, caffeine, and strenuous exercise the day before each visit and did not consume any of their own food or beverages except Buxton mineral water from 2000 until the end of the study day. Subjects arrived at the metabolic research unit between 0800 and 0900. Following a 10-min supine rest, vascular and blood pressure measurements and a fasting blood sample was taken from the antecubital vein in the forearm under venous stasis.

Subjects received a test meal to consume within 5 min, followed by 200 mL Buxton mineral water. Vascular and blood pressure measurements were taken at 4 and 6 h. Subjects rested supine for 10 min before each of these measurements; measurements were repeated in triplicate for pulse wave analysis and blood pressure. Blood samples were taken at 5 and 7 h. After the 5-h blood sample, subjects consumed a low-fat lunch consisting of soup and a bread roll.

Study foods

The isoflavone test meal (ISO) consisted of 60 g half-sugar orange spread mixed with 200 mg of soybean isoflavone dry extract 40% (Solgen 40, Solbar Plant Extracts), which provided 80 mg isoflavones; the control test meal (CON) consisted of 60 g half-sugar orange spread. Both orange spreads were served on 2 slices of toasted white bread (**Table 2**). The ISO and CON test meals were identical in appearance. The study investigators were also blinded to the identity of the test meals, as the isoflavone-enriched and control spreads had been prepared in advance and given a code by a 3rd party. The code was not broken until the end of the study. Following the 5-h blood sample, subjects were given a lunch consisting of a tin of low-fat chicken and tomato soup and a whole-meal bread roll (no spread) containing 1182 kJ, 4.2 g fat, 46.0 g carbohydrate, and 14.8 g protein, consumed with 200 mL Buxton mineral water. Subjects were allowed to sip Buxton mineral water as desired throughout the day, but they were dissuaded from drinking large volumes at once.

Anthropometric measurements

Height and weight were recorded without shoes in light clothing. Waist circumference was measured at the level of the umbilicus and hip circumference was measured at the level of the femoral trochanter.

Assessment of endothelial function: flow-mediated dilatation

Endothelial function was assessed by measuring flow-mediated dilatation (FMD) of the brachial artery according to current guidelines (20).

TABLE 1 Characteristics of healthy postmenopausal subjects who completed the study[1]

Variable	
Age, *y*	62 ± 5.4
BMI, *kg/m^2*	25.6 ± 4.5
Systolic blood pressure, *mm Hg*	123.5 ± 16.1
Diastolic blood pressure, *mm Hg*	76.0 ± 7.8
Waist:hip ratio	0.80 ± 0.1
Body fat, *%*	34.8 ± 8.1
Plasma glucose, *mmol/L*	5.2 ± 0.4
Plasma total cholesterol, *mmol/L*	5.63 ± 0.8
Plasma HDL cholesterol, *mmol/L*	1.95 ± 0.6
Plasma LDL cholesterol, *mmol/L*	3.23 ± 0.6
Plasma total:HDL cholesterol	3.1 ± 0.8
Plasma TAG, *mmol/L*	1.02 ± 0.4
Follicle-stimulating hormone, *IU/L*	61.2 ± 24.2

[1] Values are means ± SD, $n = 22$.

TABLE 2 Nutrient composition of the test meals per serving

	ISO	CON
Energy, *kJ*	1206	
Fat, *g*	1.8	
Carbohydrate, *g*	59.2	
Protein, *g*	8.2	
Soy isoflavone extract, *mg*	200.0	0
Isoflavones	80.8	0
Genistein + genistin,	46.3	0
Daidzein + daidzin,	32.0	0
Glycitein + glycitin,	2.5	0

All measurements were made by the same observer who was blind to the allocation of treatment. A high resolution ultrasound (Siemens Accuson CV70) system with 7–10 mH linear array transducer, positioned by a stereotactic manipulator, was used to scan the brachial artery in a longitudinal section 2–15 cm above the elbow. After optimal positioning of the transducer, a baseline scan is recorded. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the arterial segment being scanned) to a pressure of 250 mm Hg for 5 min, followed by release. Another scan commenced 10 s before release of the cuff and continued for 3 min after cuff deflation. FMD is expressed as the percentage increase in brachial artery diameter from baseline to maximal dilation, which occurs 30–90 s after release of the cuff. After 10 min to allow vessel recovery, another resting scan was taken. Images were coded and recorded on videotape, then digitized for subsequent blinded analysis using automated edge detection software (Brachial Analyser, Medical Imaging Applications).

Assessment of arterial function: pulse wave analysis

Measurements were performed with subjects in a supine position in a quiet, temperature-controlled (22–24°C) environment after at least 15 min of rest. Blood pressure was recorded using an automated sphygmomanometer (Omron 70CP). The measurements were made by a single observer who was unaware of the treatment allocation. A micromanometer-tipped probe coupled to a SphygmoCor device (SphygmoCor VW apparatus with Sphygmocor analysis software; SphygmoCor version 7.01 AtCor Medical) was used to noninvasively record the pulse pressure wave at the radial artery by applanation tonometry. After 20 sequential waveforms had been acquired, the integral software was used to generate a mean peripheral waveform. Three successive recordings were obtained and measurements were repeated when the waveform(s) did not pass the automatic quality controls specified by the Sphygmocor software. The peripheral pressure waveform was used to determine the peripheral augmentation index (pAIx), which is primarily determined by the intensity and timing of reflected pressure waves, thereby providing a measure of both arterial stiffness and vasodilation of small muscular arteries/arterioles (21). The central augmentation index (which is derived from pAIx using a transfer function) is markedly responsive to administration of vasodilatory and vasoconstricting drugs (21) and therefore any acute changes in NO production following the test meals were expected to be evident from pAIx measurements.

Blood sample processing and analysis

A 10-mL EDTA tube, 4-mL fluoride oxalate tube, 3- × 4.5-mL sodium citrate tubes, and a 6-mL tube with no added preservative (for serum) were used for the collection of blood samples at 0 (baseline), 5, and 7 h.

Nitric oxide metabolites. NO reacts with oxygen and is metabolized to nitrate and nitrite (NOx). A 3-mL sample of blood was collected into EDTA tubes for the analysis of plasma NOx. Samples were centrifuged at 1600 × *g*; 10 min at 4°C. Plasma samples were stored at −80°C before analysis. Defrosted samples underwent centrifugal filtration to remove proteins >10 kDa (Amicon Ultra Centrifugal Filter devices, Millipore) and total NOx concentration was analyzed using a Nitric Oxide Quantitation kit (Active Motif).

Isoprostanes. Blood (3 × 4.5 mL) for analysis of isoprostanes was collected into ice-chilled sodium citrate tubes and was analyzed by GC-MS as previously described (22).

Plasma TAG and glucose. Blood was collected into EDTA tubes (3 mL) for the analysis of plasma TAG and into fluoride oxalate tubes (4 mL) for analysis of plasma glucose. We determined plasma TAG and glucose concentrations using enzymatic assays on an ILab 650 biochemical analyzer (Instrumentation Laboratories).

Serum isoflavones. A 6-mL blood sample was taken into a serum tube and allowed to clot for 10 min before centrifuging at 1600 × *g*; 10 min, 4°C. Serum was stored at −80°C until analysis. Serum daidzein, genistein, and equol (equol results not reported here) were analyzed using time-resolved fluorescence immunoassays (DELFIA, Perkin-Elmer Life Sciences) (23). Daidzein was measured directly from serum, whereas genistein and equol required prior extraction of the serum sample. Mean extraction efficiency was calculated from the assayed extracted and unextracted daidzein values and used to adjust the extracted genistein values (mean recovery 20.1%). Therefore, all reported serum genistein concentrations have been adjusted for extraction efficiency and all daidzein concentrations are reported from unextracted serum.

Statistical analysis

Data were analyzed using GraphPad Prism version 5.01 software (GraphPad Software). Values in the text are means ± SD unless otherwise indicated. Postprandial differences in means or mean changes from baseline were analyzed using 2-way repeated-measures ANOVA, with treatment and time as within-subject factors. Data were log-transformed where necessary. Post hoc analysis using Bonferroni *t* tests was conducted when main effects were identified by the 2-way ANOVA. Values of $P \leq 0.05$ were considered significant.

Results

Serum genistein concentrations (geometric mean, 95% CI) reached 1.49 (1.20–1.84) μmol/L following ISO and 0.06 (0.05–0.08) μmol/L following CON ($P < 0.0001$) at 7 h. At 7 h, serum daidzein concentrations (geometric mean, 95% CI) reached 0.95 (0.70–1.30) μmol/L following ISO and 0.07 (0.06–0.08) μmol/L following CON ($P < 0.0001$) (**Fig. 1**). FMD was greater following ISO than CON at 6 h after the meal ($P < 0.05$) (**Table 3**). Brachial artery diameter before hyperemia did not differ between treatments or time points (CON: baseline, 3.16 ± 0.48; 4 h, 3.19 ± 0.51; 6 h, 3.20 ± 0.48 mm; ISO: baseline, 3.23 ± 0.45; 4 h, 3.15 ± 0.44; 6 h, 3.13 ± 0.43 mm), although there was a significant treatment × time interaction ($P < 0.01$); post hoc analysis did not reveal any specific pairwise treatment differences for each time point. PAIx and systolic and diastolic blood pressure did not differ between test meals but decreased at 6 h after both meals (time effect for all, $P < 0.0001$) (Table 3). Plasma 8-isoprostane $F_{2\alpha}$ concentration did not change significantly over time or differ between meals (Table 3). Plasma NOx differed between treatments over time ($P = 0.0028$) (Table 3); post hoc analysis of the raw data did not reveal any significant pairwise comparisons, but post hoc analysis of the change from baseline (CON: 5 h, −3.20 ± 3.89; 7 h, 3.11 ± 5.29 μmol/L; ISO: 5 h, 0.95 ± 4.96; 7 h, 2.74 ± 4.35 μmol/L) showed that

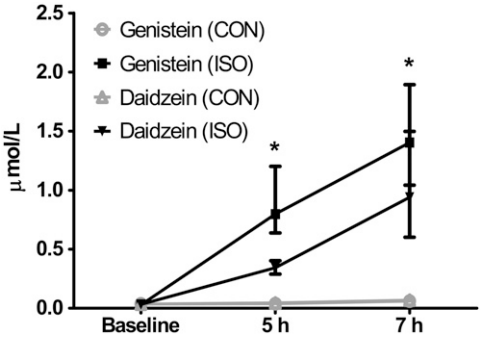

FIGURE 1 Serum genistein and daidzein concentrations in healthy postmenopausal women following consumption of ISO and CON test meals on 2 separate occasions. Values are medians ± interquartile range, $n = 22$. Treatment effect, $P < 0.0001$; treatment × time, $P < 0.0001$; time effect, $P < 0.0001$ for both serum genistein and daidzein, 2-way repeated measures ANOVA on log-transformed data. *Difference between treatments at that time point for both genistein and daidzein, $P < 0.0001$.

TABLE 3 Effects of ISO and CON test meals on FMD, pAIx, systolic and diastolic blood pressure, plasma 8-isoprostane $F_{2\alpha}$, and NOx in healthy postmenopausal women[1]

	Baseline	4 or 5 h	6 or 7 h
FMD, %[2]			
CON	5.49 ± 2.32[a]	4.35 ± 2.32[b]	4.40 ± 2.26[b]
ISO	5.38 ± 1.91	5.08 ± 1.74	6.11 ± 2.60
ISO − CON	−0.11 (−1.73 to 1.51)	0.73 (−0.89 to 2.35)	1.71 (0.09 to 3.32)*
pAIx, %			
CON	87.4 ± 12.0[a]	87.9 ± 12.2[a]	80.6 ± 13.8[b]
ISO	88.1 ± 12.3[a]	89.0 ± 15.0[a]	81.1 ± 13.9[b]
ISO − CON	0.73 (−8.96 to 10.41)	1.15 (−8.53 to 10.8)	0.48 (−9.20 to 10.17)
Systolic blood pressure, *mm Hg*			
CON	118.2 ± 15.4[a]	117.9 ± 14.9[a]	112.2 ± 14.2[b]
ISO	117.3 ± 12.8	118.5 ± 14.0[a]	112.8 ± 11.1[b]
ISO − CON	−0.87 (−10.95 to 9.21)	0.67 (−9.41 to 10.76)	0.58 (−9.51 to 10.66)
Diastolic blood pressure, *mm Hg*			
CON	67.6 ± 6.6[a]	68.1 ± 8.2[a]	63.2 ± 6.9[b]
ISO	67.7 ± 6.9[a]	67.8 ± 7.3[a]	63.5 ± 6.3[b]
ISO − CON	0.14 (−5.01 to 5.30)	−0.39 (−5.55 to 4.76)	0.27 (−4.88 to 5.43)
NOx, $\mu mol/L$[2]			
CON	20.0 ± 5.1	16.8 ± 5.1[a]	23.1 ± 6.0[b]
ISO	18.6 ± 6.3	19.5 ± 5.1	21.3 ± 4.3
ISO − CON	−1.46 (−5.38 to 2.46)	2.69 (−1.23 to 6.61)	−1.82 (−5.74 to 2.10)
Plasma 8-isoprostane $F_{2\alpha}$, *pmol/L*			
CON	202.5 ± 56.7	203.7 ± 52.5	200.3 ± 56.1
ISO	208.8 ± 60.9	209.5 ± 63.8	193.2 ± 58.4
ISO − CON	6.1 (−36.2 to 48.5)	5.8 (−36.6 to 48.1)	−7.1 (−49.4 to 35.2)

[1] Values are means ± SD and mean difference between treatments with 95% CI, $n = 22$. Vascular measurements were taken between 4 and 5 h and 6 and 7 h; blood samples were taken at 5 and 7 h. Means in a row without a common letter differ, $P < 0.05$.
[2] Treatment × time interaction, $P < 0.01$. *Difference between treatments at that time point, $P < 0.05$.

plasma NOx concentrations were lower at 5 h following CON compared with no change following ISO ($P < 0.01$). Plasma TAG and glucose changed over time following both meals ($P < 0.01$), but test meals did not differ in the changes (data not shown).

Discussion

The aim of the study was to investigate whether consumption of isoflavones could increase endothelium-dependent vasodilation during the postprandial period. To our knowledge, there have been no published studies until now considering the acute effects of an isoflavone-enriched meal on endothelial function. We hypothesized that the maximal vasodilatory effect of isoflavone consumption would occur at peak blood isoflavone concentrations and this would explain why the results of chronic dietary intervention studies on isoflavones and endothelial function were inconclusive when considered together, because endothelial function measurements were probably not made at the time of peak circulating isoflavone levels in many of these studies. Our results showed that FMD increased 6 h following ISO compared with CON. This is a relatively large dose of isoflavones compared with daily dietary intakes reported previously for East Asian countries such as China or Japan (30–50 mg/d) but not so large that it could be considered unphysiological (24). The increase in endothelium-dependent vasodilation is supported by greater plasma NOx concentrations following ISO compared with CON, suggesting that circulating isoflavones stimulated increased endothelial NO production.

The similarity in pAIx between treatments suggests that consumption of isoflavones specifically increases shear stress-induced NO production. This may be important under certain physiological conditions, such as augmentation of NO production during physical activity. Both pAIx and blood pressure significantly decreased 6 h following both test meals, which was also 2 h after the standard lunch and at the time when plasma glucose concentrations were elevated (>6 mmol/L). The vasodilatory effects of insulin may have mediated this decrease in pAIx and blood pressure. The fact that 8-isoprostane $F_{2\alpha}$ concentrations were unaffected by either test meal suggested that reduced oxidative stress may not have played an important role in the observed effects on endothelium-dependent vasodilation, although antioxidant activity cannot be completely excluded as a possible mechanism without further investigation.

There is conflicting evidence in the literature as to whether chronic consumption of isolated soy isoflavones can modulate endothelial function (4). Because the isoflavone-induced postprandial endothelium-dependent vasodilatory effects shown in this study were apparent within 6 h of ingestion, the mechanism is likely to be linked to activation of nongenomic endothelial NO synthase-related cell signaling pathways. The isoflavone extract contained both genistein/genistin and daidzein/daidzin and therefore it is not clear whether the acute effects on endothelium-dependent vasodilation are induced by 1 of these isoflavones or both. Acute endothelium-dependent vasodilation occurred during brachial artery infusion of genistein at plasma concentrations of ~2 μmol/L in healthy men and premenopausal women, whereas daidzein had no effect (17). This suggests that daidzein may be ineffective in inducing NO-dependent vasodilation, although circulating genistein and daidzein would have been circulating in their conjugated forms in the present study and it cannot be assumed that isoflavone aglycones and conjugated

forms would have equivalent effects on the vasculature. Interestingly, the circulating genistein:daidzein ratio found at peak concentrations was 1.50:1, almost the same ratio of genistein/genistin:daidzein/daidzein provided in the soy isoflavone extract (1.45:1). This suggests that genistein provided from this extract was equal in bioavailability to daidzein, in contrast to previous findings (25); however, the study was not designed to test isoflavone bioavailability, and more frequent time points are required to confirm time to peak concentrations for each form of isoflavone.

In conclusion, these findings demonstrate that consumption of isoflavones has an acute effect on endothelial function in postmenopausal women. This suggests that postmenopausal women consuming isoflavones at intervals throughout the day (thereby continuously maintaining elevated circulating levels of isoflavones) may benefit from an improvement in their endothelial function. Maintaining a regular dietary intake of isoflavones over a number of years could potentially retard atherosclerosis development in postmenopausal women through the antiinflammatory and vasodilatory actions of NO, but large, robustly designed, randomized, controlled dietary intervention trials are required to confirm this.

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Literature Cited

1. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med. 1976;85:447–52.
2. Howard AN, Gresham GA, Jones D, Jennings IW. The prevention of rabbit atherosclerosis by soya bean meal. J Atheroscler Res. 1965;5: 330–7.
3. Nagata C. Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan. Int J Epidemiol. 2000;29:832–6.
4. Cassidy A, Albertazzi P, Lise Nielsen I, Hall W, Williamson G, Tetens I, Atkins S, Cross H, Manios Y, et al. Critical review of health effects of soyabean phyto-oestrogens in post-menopausal women. Proc Nutr Soc. 2006;65:76–92.
5. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. Circulation. 2006;113:1034–44.
6. Cassidy A, de Pascual Teresa S, Rimbach G. Molecular mechanisms by which dietary isoflavones potentially prevent atherosclerosis. Expert Rev Mol Med. 2003;5:1–15.
7. Mann GE, Rowlands DJ, Li FY, de Winter P, Siow RC. Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression. Cardiovasc Res. 2007;75: 261–74.
8. van der Schouw YT, Pijpe A, Lebrun CE, Bots ML, Peeters PH, van Staveren WA, Lamberts SW, Grobbee DE. Higher usual dietary intake of phytoestrogens is associated with lower aortic stiffness in postmenopausal women. Arterioscler Thromb Vasc Biol. 2002;22:1316–22.
9. Clerici C, Setchell KD, Battezzati PM, Pirro M, Giuliano V, Asciutti S, Castellani D, Nardi E, Sabatino G, et al. Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. J Nutr. 2007;137:2270–8.
10. Squadrito F, Altavilla D, Crisafulli A, Saitta A, Cucinotta D, Morabito N, D'Anna R, Corrado F, Ruggeri P, et al. Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. Am J Med. 2003;114:470–6.
11. Hallund J, Bugel S, Tholstrup T, Ferrari M, Talbot D, Hall WL, Reimann M, Williams CM, Wiinberg N. Soy isoflavone enriched cereal bars affect markers of endothelial function in postmenopausal women. Br J Nutr. In press 2006.
12. Lissin LW, Oka R, Lakshmi S, Cooke JP. Isoflavones improve vascular reactivity in post-menopausal women with hypercholesterolemia. Vasc Med. 2004;9:26–30.
13. Simons LA, von Konigsmark M, Simons J, Celermajer DS. Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. Am J Cardiol. 2000;85:1297–301.
14. Teede HJ, McGrath BP, DeSilva L, Cehun M, Fassoulakis A, Nestel PJ. Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. Arterioscler Thromb Vasc Biol. 2003;23: 1066–71.
15. Sobey CG, Weiler JM, Boujaoude M, Woodman OL. Effect of short-term phytoestrogen treatment in male rats on nitric oxide-mediated responses of carotid and cerebral arteries: comparison with 17beta-estradiol. J Pharmacol Exp Ther. 2004;310:135–40.
16. Squadrito F, Altavilla D, Squadrito G, Saitta A, Cucinotta D, Minutoli L, Deodato B, Ferlito M, Campo GM, et al. Genistein supplementation and estrogen replacement therapy improve endothelial dysfunction induced by ovariectomy in rats. Cardiovasc Res. 2000;45:454–62.
17. Walker HA, Dean TS, Sanders TA, Jackson G, Ritter JM, Chowienczyk PJ. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17beta-estradiol. Circulation. 2001;103:258–62.
18. Chin-Dusting JP, Boak L, Husband A, Nestel PJ. The isoflavone metabolite dehydroequol produces vasodilatation in human resistance arteries via a nitric oxide-dependent mechanism. Atherosclerosis. 2004; 176:45–8.
19. Meah MN, Harrison N, Davies A. Nitrate and nitrite in foods and the diet. Food Addit Contam. 1994;11:519–32.
20. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002; 39:257–65.
21. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. Hypertension. 2001;37:1429–33.
22. Hall WL, Sanders KA, Sanders TA, Chowienczyk PJ. A high-fat meal enriched with eicosapentaenoic acid reduces postprandial arterial stiffness measured by digital volume pulse analysis in healthy men. J Nutr. 2008;138:287–91.
23. Talbot DC, Ogborne RM, Dadd T, Adlercreutz H, Barnard G, Bugel S, Kohen F, Marlin S, Piron J, et al. Monoclonal antibody-based time-resolved fluorescence immunoassays for daidzein, genistein, and equol in blood and urine: application to the Isoheart intervention study. Clin Chem. 2007;53:748–56.
24. Hall WL, Rimbach G, Williams CM. Isoflavones and endothelial function. Nutr Res Rev. 2005;18:130–44.
25. Setchell KD, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, Kirschner AS, Cassidy A, Heubi JE. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J Nutr. 2001;131:S1362–75.