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Pasta Naturally Enriched with Isoflavone Aglycons from Soy Germ Reduces Serum Lipids and Improves Markers of Cardiovascular Risk[1]

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Abstract

Most studies of soy and cholesterol have tested foods made from purified soy proteins containing mainly isoflavone glycosides. Fermented soy foods have mainly isoflavone aglycons and account for a high proportion of the soy protein source in Asia, where there is an inverse relationship between soy intake and serum cholesterol. The aim of this study was to compare a novel soy germ pasta, naturally enriched in isoflavone aglycons as a result of the manufacturing process, with conventional pasta for effects on serum lipids and other cardiovascular risk markers. In this randomized, controlled, parallel study design of 62 adults with hypercholesterolemia who consumed a Step II diet that included one 80-g serving/d of pasta, we measured serum lipids, high sensitivity C-reactive protein (hsCRP), urinary isoprostanes, and brachial artery flow-mediated vasodilatation at baseline and after 4 and 8 wk. The pasta delivered 33 mg of isoflavones and negligible soy protein and led to a serum isoflavone concentration of 222 ± 21 nmol/L; 69% of subjects were equol producers. Soy germ pasta reduced serum total and LDL cholesterol by 0.47 ± 0.13 mmol/L ($P = 0.001$) and 0.36 ± 0.10 mmol/L ($P = 0.002$) more than conventional pasta, representing reductions from baseline of 7.3% ($P = 0.001$) and 8.6% ($P = 0.002$), respectively. Arterial stiffness ($P = 0.003$) and hsCRP ($P = 0.03$) decreased and improvements in all the above risk markers were greatest in equol producers. All measures returned to baseline when patients were switched to conventional pasta. In conclusion, pasta naturally enriched with isoflavone aglycons and lacking soy protein had a significant hypocholesterolemic effect beyond a Step II diet and improved other cardiovascular risk markers. J. Nutr. 137: 2270–2278, 2007.

Introduction

Soy has been known for many decades to reduce blood cholesterol (1,2). Following a meta-analysis that summarized the findings from 37 studies (3), the U.S. FDA awarded a health claim to soy protein for reducing the risk of coronary heart disease (4). This pivotal decision led to a surge in the sales of soy foods and increased interest in attempts to better understand the mechanism of the hypocholesterolemic action of soy. The soybean is a complex food that contributes a number of constituents (5) with the potential to affect cholesterol homeostasis either directly or indirectly (6–8). The relative importance of isoflavones vs. soy protein (9) in contributing to the hypocholesterolemic effect of soy foods in patients with hyperlipidemias (3,10,11) remains unclear and is still controversial (12,13). However, the daily intake of soy foods has been clearly shown to be inversely proportional to the fasting serum cholesterol concentration in Japanese adults (14) and is also associated with a reduction in coronary heart disease risk factors in Chinese women (15,16). Despite the continued discrepancies in findings from dietary studies of soy protein and hypercholesterolemia, the cardiovascular benefits of soybeans and soy foods remains well established (17,18).

Most previous investigations aimed at understanding the relative cholesterol-lowering action of soy protein and isoflavones have compared purified soy proteins, such as isolated soy protein (ISP)[6], with alcohol-washed soy protein, casein, or dairy products (3,10,11) as control diets and these latter matrices are considerably different from each other in terms of macro- and

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[6] Abbreviations used: HDL-C, HDL cholesterol; HsCRP, high sensitivity C-reactive protein; ISP, isolated soy protein; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglyceride.

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micronutrient composition (5,19). Results from such studies have yielded highly variable results and much confusion as to the relative merits of soy for cardiovascular health (13). It is debatable whether investigating the effects of foods made from ISP reflects the expected dietary effects of whole soy foods, especially by Asian adults. For example, dietary analysis of the types of soy foods consumed by Asians reveals that ISP, with almost exclusively isoflavone glucosides (20), is rarely a source of soy protein, whereas a high proportion of the isoflavone intake in Asians is in the form of aglycons, because fermented soy foods account for about one-third of the soy protein source (21,22).

In this study, we circumvented some of the above limitations by comparing the effects of a novel pasta that was enriched with isoflavones naturally sourced from the soy germ to those of a conventional soy pasta that lacked isoflavones for its effects on blood lipids in newly diagnosed patients with hypercholesterolemia. Utilization of the soy germ has the advantage that it is naturally milled from the soybean, is not subjected to chemical processing, and is highly concentrated in isoflavones (23). Furthermore, due to a unique interaction between the isoflavones in the soy germ and the semolina wheat, the final product delivered predominantly isoflavones in aglycon form and in this regard, this pasta had the compositional characteristics of a fermented Asian soy food. The resulting soy germ-enriched pasta delivered 33 mg/d of isoflavones, typical of isoflavone intakes by Asians (24), and the study afforded an assessment of the potential role of isoflavones in the absence of substantial amounts of soy protein.

Because isoflavones have been shown in numerous studies to have important effects on a number of surrogate markers of cardiovascular risk, including effects on lipid peroxidation (25,26), inflammation (27), and the vasculature (28–30), we also examined the effects of this isoflavone-enriched pasta on key elements of cardiovascular risk, including body weight, BMI, inflammation, antioxidant status, blood pressure, and endothelial function.

Subjects and Methods

Characteristics of the pasta. Two types of dried pasta were used in this study, a soy germ-enriched pasta and conventional pasta, both manufactured in penne-shaped forms by an Italian pasta manufacturer. The soy germ pasta (Aliveris srl, Perugia, Italy) was made of Durum wheat semolina to which 2% soy germ was blended and the conventional pasta was made only of Durum wheat semolina. Both pastas were indistinguishable from the packaging and had similar sensory attributes. The soy germ-enriched pasta contained 33 mg of total isoflavones per serving (80 g) and provided negligible amounts (0.8 g/serving) of soy protein. The composition of the soy germ was as follows: protein (38.5–41%), carbohydrates (22–28%), fat (14–18%), moisture (4%), fiber (2–4%), saponins (3–5%), lecithin (0.3–0.6%), and tocopherol (0.015–0.025%). The energy value per 100 g of both pastas was similar at 1.49 kJ and the macro-composition consisted of 12 g total protein, 73.5 g carbohydrate, and 1.5 g fat.

Study design. This 10-wk study was a single-crossover parallel-group, randomized controlled, blinded trial of 62 adult men and women newly diagnosed with hypercholesterolemia and consecutively recruited from outpatient referrals to the Lipid Clinic of the University of Perugia Hospital. Patients with a history of previous coronary heart disease, intestinal disease, or cancer were excluded from the study, as were smokers and anyone consuming >2 drinks of alcohol per day. Patients administered any form of lipid-lowering drugs or blood pressure medication, vegetarians, and anyone knowingly consuming soy foods regularly or taking antioxidant supplements were also excluded. The study was conducted at the University of Perugia School of Medicine (Perugia, Italy) and the study protocol conformed to the ethical guidelines of the Helsinki Declaration of 1975 for research on humans. The patients were counseled regarding the study design and were asked to adhere to the Italian Heart Association Step II diet throughout. This diet included a daily serving of pasta (80 g) and compliance to the study was facilitated by the fact that most Italians consume pasta on a daily basis. Prior to recruitment, all patients had been on a Step I diet for ~4 wk and after enrollment in this study were then placed on the Step II diet for the entire length of the study, including a run-in acclimatization period of 2 wk before the study commenced. At the end of the 2-wk run-in period, blood and urine were collected for baseline measurements of serum lipids and other markers of cardiovascular risk, including high sensitivity C-reactive protein (hsCRP) and urinary isoprostanes. Body weight, BMI, waist circumference, and blood pressure were measured and endothelial function was assessed by change in brachial artery flow-mediated vasodilatation at baseline. The 62 patients were then randomized to 2 groups; 31 patients were assigned to a group that was given 1 daily serving of soy germ-enriched pasta (80 g) for 4 wk and the other 31 patients (control group) consumed conventional pasta for the same period. The patients were unaware of which pasta they were given. After 4 wk, blood and urine were collected and the above tests repeated. After completion of this 4-wk dietary period, patients in the soy germ pasta group were switched to conventional pasta for a further 4 wk to determine the extent, if any, of rebound effect. The control group continued with conventional pasta for a further 4-wk period. After completion of the study, blood was collected and serum lipids were measured. Compliance to the study was assessed from counting any unused pasta returned after completion and from measurements of isoflavone concentrations in the serum from those patients assigned to the soy germ pasta group.

The study sample size was based on the following anticipations of diet-induced changes during the first 4 wk: mean change of total cholesterol (TC) concentrations was expected to be in the order of 5% of a mean baseline value of 6.98 mmol/L (270 mg/dL) in the enriched pasta group [i.e. a difference of ~0.39 mmol/L (15 mg/dL), with a SD of the same magnitude as this difference; no change and the same SD as above were expected in the control group]. Assuming a risk of type I error of 0.05 (2-sided), it was calculated that to attain a power of 0.90 in detecting such a difference between the changes occurring in the 2 groups, we would need to allocate at least 22 subjects in each group.

Anthropometrics. Body weight and height were measured and BMI calculated by standard techniques.

Lipids. Serum TC, triglycerides (TG), and HDL cholesterol (HDL-C) were determined by enzymatic colorimetric method (Dimension Autoanalyser; DADE). LDL cholesterol (LDL-C) was calculated by the Friedewald equation (31). Lipids were expressed as concentration (millimoles per liter) of TC, LDL-C, HDL-C, and TG.

Other markers. Serum hsCRP levels were measured using the Latex-Enhanced CRP assay (Dade Behring High Sensitivity CRP Assay) on a nephelometer. Urinary 8-isoprostane (8-epi-prostaglandin F2α) analysis was measured by previously published methods (32). Serum total and individual isoflavones were measured by GC-MS as described previously (33).

Endothelial function. Brachial arterial blood pressure was measured with a mercury sphygmomanometer after patients sat rested for 10 min or longer. The mean value of 3 measurements was calculated. Endothelial function was measured from brachial artery flow-mediated vasodilatation with B-mode ultrasound imaging of the brachial artery and by assessing the increase in artery diameter during reactive hyperemia (34).

Isoflavone analysis of soy germ pasta. The isoflavone composition of both the soy germ used in the manufacture of the isoflavone-enriched pasta and the final pasta product was determined by reverse-phase HPLC-MS as described previously (20).

Data analysis. Continuous variables were summarized as mean values ± SEM. The significance of baseline comparisons between group means was assessed using the Student's *t* test. Changes that occurred during the study were calculated by subtracting the value determined after 4 or 8 wk from the baseline value. The significance of changes that occurred within each study group was analyzed by a paired *t* test of the hypothesis that the difference had a mean of 0. To compare between groups the changes that occurred in a continuous variable, we used multiple regression analysis to adjust for patients' baseline values and thus control for potential imbalance of that variable between the groups at baseline (35). We built regression equations by including a follow-up value as the dependent variable, the baseline value for that variable, and a binary variable coding for group. The regression coefficient for this group variable was used to estimate the adjusted difference between the mean changes of each group. The same statistical methods were used in the analysis of the 29 patients who consumed isoflavone-enriched pasta and were compared according to equol producer status, the binary variable coding for study group being equol producer status instead of type of pasta. Logarithmic (serum HDL-C and LDL-C, TG, hsCRP, and urinary isoprostane), reciprocal (TC), or square root (flow-mediated dilation) transformations were applied to some variables to achieve homogeneity of variances or approximate normal distributions. Statistical analyses were 2-sided and performed using Stata Statistical Software (version 8.0, Stata). Differences with a *P*-value < 0.05 were considered significant.

Results

Isoflavone analysis of soy germ pasta. The total isoflavone concentration of soy germ quantified by HPLC was 20.6 mg/g expressed as aglycon equivalents, consistent with the manufacturer's certificate of analysis. The soy germ pasta had a final total isoflavone concentration of 0.41 mg isoflavones/g pasta. An 80-g serving of soy germ pasta therefore delivered a total of 33 mg of isoflavones, expressed as aglycons. There was a qualitative difference in the isoflavone composition of the soy germ and the final soy germ-pasta product. A shift in distribution of the relative proportions of conjugated and aglycon forms of isoflavones was striking (**Fig. 1**). Soy germ contained only 2.6% of the total isoflavones in the form of the aglycons daidzein, glycitein, and genistein and the major isoflavones present were β-glycosides, which accounted for 70.4% of the total isoflavones (**Fig. 2**). By contrast, 83.5% of the isoflavones in the soy germ pasta were in the form of aglycons, indicating that hydrolysis of the glycosides had occurred during the manufacture of the pasta. In vitro experiments (data not shown) confirmed that Durham wheat naturally contains β-glucosidase activity that efficiently hydrolyzes the isoflavone conjugates in the soy germ during the process of pasta production to yield a pasta enriched in isoflavones in mainly the aglycon forms (Fig. 1). The relative proportions of the sum of daidzein, glycitein, and genistein and their conjugates in the finished pasta, expressed in aglycon equivalents, was similar to that of the soy germ, with daidzein and glycitein being predominant.

Patient demographics. The 62 hypercholesterolemic patients comprised 37 females and 25 males. After randomization, the age and gender composition of the patients assigned to the 2 groups was similar. In the control group, 2 patients (1 male and 1 female) did not complete the study for personal reasons. Of the 31 patients in the soy germ-pasta group, 22 were female and 9 were male and the age of this group was 58.1 ± 2.2 y. The control group comprised 16 female and 13 male patients aged 52.0 ± 2.4 y.

BMI. The BMI and waist circumference did not change during the study in the 2 patient groups. BMI was 26.6 ± 0.8 kg/m^2 in both groups at baseline. After 4 and 8 wk, BMI was 26.3 ± 0.8 kg/m^2 and 26.5 ± 0.8 kg/m^2 in the enriched pasta group and 26.3 ± 0.8 kg/m^2 and 26.5 ± 0.8 kg/m^2 in the control group. At both time points, the 2 groups did not differ in the changes from baseline after adjustment for patients' baseline values ($P = 0.72$ and $P = 0.97$, respectively).

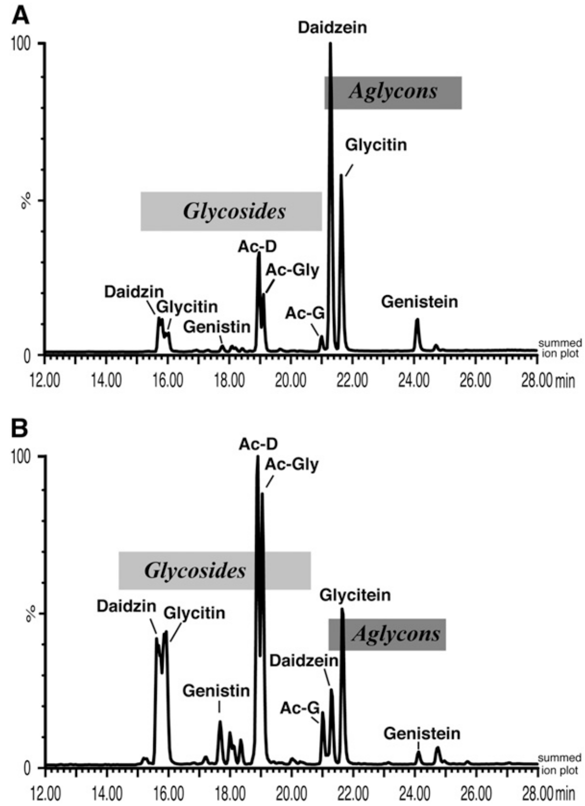

FIGURE 1 Comparison of summed ion current chromatograms from HPLC-MS analysis of soy germ pasta (*A*) and the soy germ (*B*) used to make the pasta, showing evidence for hydrolysis of isoflavone glucosides naturally present in the soy germ to their corresponding aglycons during pasta manufacture.

Serum isoflavones. In 29 of the 31 patients consuming soy germ pasta, compliance was confirmed by the marked increase in serum isoflavone concentrations in 29 of the 31 patients; there was insufficient sera to perform the isoflavone measurement in 2 patients. A diet incorporating soy germ pasta with 33 mg isoflavones boosted fasting serum total isoflavone levels from 42 ± 4 nmol/L (10.5 ± 0.9 μg/L) at baseline in all patients to a steady-state concentration of 222 ± 21 nmol/L (55.5 ± 5.2 μg/L) ($P < 0.001$) (**Fig. 3***A*).

Serum equol concentration ranged from 4.5 to 255 nmol/L (1.1 to 61.9 μg/L) in the isoflavone-enriched pasta group and 20 of 29 patients (69%), an unusually high proportion, were classified as equol producers as defined previously (36,37). The serum equol concentration of the equol producers was 105 ± 17 nmol/L (25.5 ± 4.0 μg/L) compared with 9.5 ± 1.6 nmol/L (2.3 ± 0.4 μg/L) in those patients who did not produce equol after consuming isoflavone-enriched pasta ($P < 0.001$) (Fig. 3*B*).

Serum cholesterol and lipids. At baseline evaluation, patients in the enriched pasta group tended to have lower levels of TC ($P = 0.129$), HDL-C ($P = 0.086$), and TG ($P = 0.132$) than those in the control group (**Table 1**). After 4 wk, serum levels of both TC and LDL-C decreased from baseline levels ($P < 0.001$) in the isoflavone-enriched pasta group, whereas levels did not change

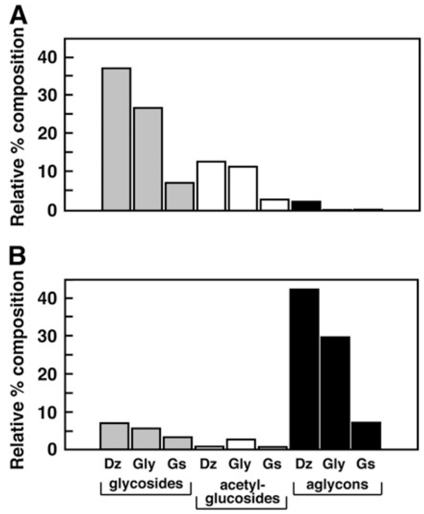

FIGURE 2 Comparison of the relative composition of the different isoflavone forms in soy germ used to manufacture soy germ pasta (*A*) and in the final pasta (*B*) product. Dz, daidzein; Gly, glycitein; Gs, genistein.

in the control group. TC decreased by 0.47 ± 0.13 mmol/L (18 ± 5 mg/dL) more in the isoflavone-enriched pasta group than in the control group ($P = 0.001$) after adjustment for patients' baseline values. Similarly, LDL-C decreased by 0.36 ± 0.1 mmol/L (14 ± 4 mg/dL) more in the isoflavone-enriched pasta group than in the control group ($P = 0.002$).

When the patients in the soy germ pasta group were switched to regular pasta for a further 4 wk, a rebound effect was observed with serum concentrations of TC after 8 wk, showing no significant difference from the baseline values. Likewise, the change that occurred in this and the control group did not differ at that time point after adjustment for patients' baseline values. The same was observed for serum LDL-C, with loss of the improvement gained during isoflavone-enriched pasta feeding when patients were switched to conventional pasta. After 8 wk, the changes that occurred in the soy germ-enriched pasta group and the control group did not differ after adjustment for patients' baseline values.

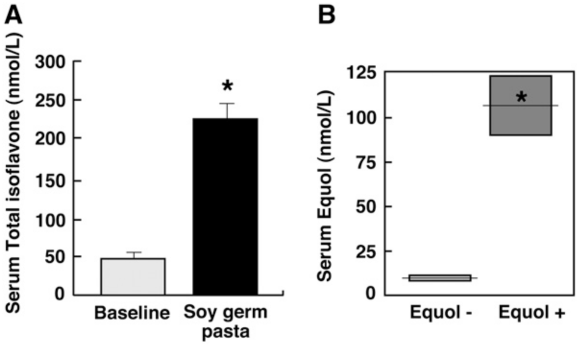

FIGURE 3 Serum total isoflavone concentration in 29 hypercholesterolemic adults at baseline and after consuming soy germ pasta daily for 4 wk (*A*). Values are means ± SEM. *Different from baseline, $P < 0.001$. Serum equol concentration in those patients that were classified as equol producers (Equol +, $n = 20$), or equol nonproducers (Equol −, $n = 9$) (*B*). Values are means and range for each group. *Different from Equol −, $P < 0.001$.

After 4 wk, serum HDL-C concentrations did not change significantly from baseline levels in either the isoflavone-enriched pasta group or the control group. Serum TG concentrations during consumption of either type of pasta did not change ($P = 0.301$). Overall, isoflavone-enriched pasta lowered serum TC by 7.3% ($P = 0.001$) and LDL-C by 8.6% ($P = 0.002$) beyond the effect of a 2-wk run-in on a National Cholesterol Education Program Step II diet (**Fig. 4**). This improvement in serum lipids was lost when this group reverted to consuming conventional pasta.

hsCRP. Serum hsCRP concentrations were significantly altered by inclusion of soy germ pasta in the diet (**Fig. 5**). At baseline, the mean serum hsCRP concentration was similar in the 2 study groups and near the upper limit of normal. After 4 wk, serum hsCRP concentrations decreased markedly in the isoflavone-enriched pasta group (−1.2 ± 0.4 mg/L; $P < 0.001$) but did not change in the control group. Serum hsCRP decreased by an estimated 2.2 ± 0.9 mg/L more in the isoflavone-enriched pasta group than in the control group ($P = 0.03$) after adjustment for patients' baseline values. The effect reverted when patients who consumed isoflavone-enriched pasta were switched to conventional pasta for a further 4 wk, so by the end of wk 8, no difference was detectable between the observed changes in the 2 groups ($P = 0.345$).

Urinary isoprostanes. Urinary 8-epi-prostaglandin F2α concentrations, a surrogate marker for antioxidant status, decreased from 58 ± 6 ng/L at baseline to 39 ± 4 ng/L after 4 wk in patients consuming soy germ pasta ($P < 0.001$). Measurements were not performed on the control group.

Endothelial function. At baseline, flow-mediated vasodilation was similar in the 2 study groups (**Fig. 6**). After 4 wk, flow-mediated vasodilation increased 2 ± 0.8% ($P = 0.012$) in the isoflavone-enriched pasta group, whereas there was no change in the control group. Consistent with improved endothelial function after consuming soy germ pasta, flow-mediated vasodilation increased by an estimated 2.3 ± 0.8% more in the enriched pasta group than in the control group ($P = 0.003$) after adjustment for patients' baseline values. Blood pressure did not change in the 2 groups during the study period. After 4 wk and 8 wk, the isoflavone-enriched pasta group and the control group did not differ in the changes from baseline for systolic or diastolic blood pressures after adjustment for patients' baseline values (data not shown).

Associations with equol producer status. Primary data from the patients consuming soy germ-enriched pasta were further analyzed according to equol producer status (**Fig. 7**). The distribution of study variables did not differ between the 2 subgroups at baseline. After 4 wk, equol producers showed reductions ($P < 0.001$) from baseline levels in serum concentrations of both TC and LDL-C. In this patient subgroup, favorable changes from baseline also occurred in hsCRP concentrations ($P = 0.001$), urinary isoprostane excretion ($P = 0.012$), and flow-mediated vasodilation ($P = 0.03$). In equol nonproducers, these variables did not change from baseline, with the exception of urinary isoprostane excretion ($P = 0.038$).

Consistent with favorable effects of isoflavone-enriched pasta occurring in equol producers but not in equol nonproducers after correction for baseline values, LDL-C decreased by an estimated 0.39 ± 0.18 mmol/L (15 ± 7 mg/dL) more in the former than in the latter subgroup ($P = 0.042$) and hsCRP

TABLE 1 Serum lipid concentrations in hypercholesterolemic adults at baseline and the changes after consuming isoflavone-enriched soy germ pasta or conventional pasta for 4 wk, and after consuming conventional pasta for another 4 wk[1]

Group		TC	LDL-C	HDL-C	TG
Isoflavone-enriched	*n*		*mmol/L*		
Pasta group	31				
Baseline		6.76 ± 0.08	4.79 ± 0.10	1.30 ± 0.05	1.33 ± 0.10
Change after 4 wk		−0.49 ± 0.10*	−0.41 ± 0.10*	−0.03 ± 0.03	−0.07 ± 0.07
Change after 8 wk		−0.13 ± 0.08	−0.10 ± 0.08	0 ± 0.03	−0.05 ± 0.08
Control group	29				
Baseline		7.07 ± 0.16	4.81 ± 0.16	1.42 ± 0.05	1.55 ± 0.11
Change after 4 wk		−0.08 ± 0.08	−0.05 ± 0.08	0.03 ± 0.05	−0.05 ± 0.06
Change after 8 wk		−0.08 ± 0.08	−0.03 ± 0.08	0.03 ± 0.03	−0.01 ± 0.06

[1] Values are means ± SEM. *Different from baseline, $P < 0.001$.

decreased by an estimated 0.9 ± 0.5 mg/L ($P = 0.025$). The decreased serum TC tended to be greater in equol producers than in equol nonproducers ($P = 0.103$).

Discussion

We report the results of a randomized blinded parallel single-crossover design study of patients with hypercholesterolemia in whom dietary intervention was adopted as the first strategy for lipid-lowering to reduce cardiovascular risk. All of the patients were on the Italian equivalent of the National Cholesterol Education Program diet prior to and throughout the study period; this diet included 1 serving of pasta per day. Pasta provides an ideal matrix to incorporate soy isoflavones and by using soy germ, an isoflavone-enriched pasta was commercially manufactured that contained 33 mg total isoflavones/serving with negligible soy protein. Soy pastas have been commercially available for some time but these have been made with soy flour, or ISP as an ingredient, and primarily in response to meeting the FDA heart health claim requiring at least 6.25 g of soy protein per serving. To satisfy this requirement, such products need to incorporate ~15% of soy flour, which distorts the sensory properties of the pasta, provides relatively low levels of isoflavones, and results in a product that is easily distinguishable from conventional pasta. By contrast, the soy germ pasta used in this study was unique in being indistinguishable from conventional pasta, thereby making a randomized blinded study possible and furthermore, allowing conventional pasta with similar nutritional qualities to be used as an ideal control. Importantly, the use of this novel food afforded a means of evaluating the hypocholesterolemic effect of isoflavones in the virtual absence of soy protein. Compliance was established from measurement of serum isoflavone concentrations, which showed steady-state serum concentration of 222 ± 21 nmol/L, consistent

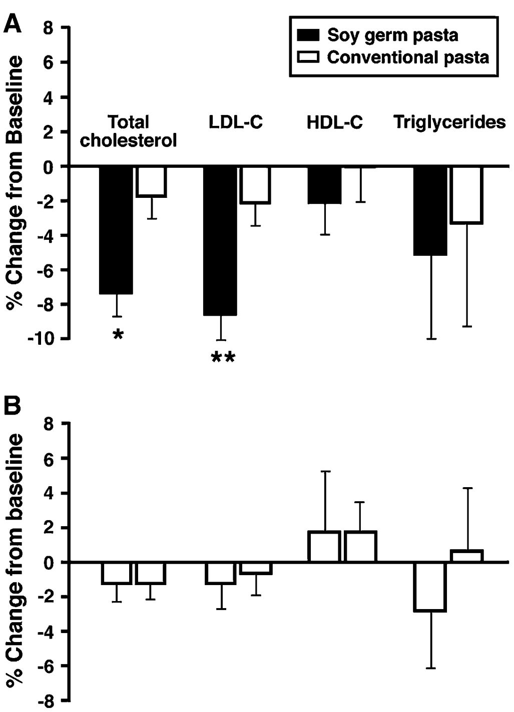

FIGURE 4 The percent change in serum lipid concentrations in hypercholesterolemic adults after consuming pasta enriched with isoflavones from soy germ (*A*; $n = 31$) or conventional pasta (*B*; $n = 29$) daily for 4 wk. The changes from baseline are also shown after single-crossover of the soy germ group to conventional pasta for a further 4 wk whereas the control group continued to consume conventional pasta (*B*). Values are means ± SEM. Asterisks indicate different from baseline: $*P = 0.001$ and $**P = 0.002$. For each variable in B, 4-wk data are on the left and 8-wk data on the right.

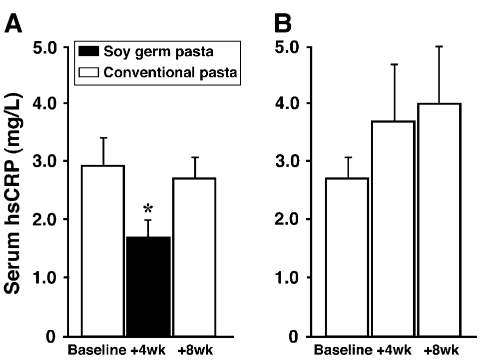

FIGURE 5 Serum hsCRP concentrations in hypercholesterolemic adults at baseline, after consuming soy germ pasta enriched with isoflavones (*A*; $n = 31$) or conventional pasta (*B*; $n = 29$) daily for 4 wk (+4 wk) and after both groups consumed conventional pasta for a further 4 wk (+8 wk). Values are means ± SEM. * Different from baseline, $P < 0.001$.

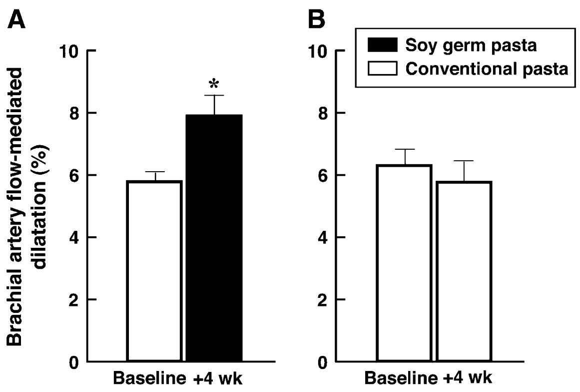

FIGURE 6 Brachial artery flow-mediated dilatation assessed in hypercholesterolemic adults at baseline and after consuming pasta enriched with isoflavones from soy germ (*A*; $n = 31$) or conventional pasta (*B*; $n = 29$) daily for 4 wk. Values are means ± SEM. *Different from baseline, $P < 0.001$.

with the known pharmacokinetics of isoflavones (33,38). For those patients given conventional pasta, compliance was judged by the number of packages of pasta returned at the end of the study and was facilitated by the fact that most Italians consume pasta daily.

After randomization of the patients, the baseline serum TC, HDL-C, and TG concentrations were slightly higher, although not significantly so, in patients assigned to the control group compared with those in the soy germ pasta group. Any cholesterol-lowering effect of the pasta diets should therefore have been more achievable in the control group. Despite this, patients who consumed conventional pasta experienced no change from baseline in serum TC and LDL-C during the entire 8-wk period (Fig. 4). By contrast, isoflavone-enriched soy germ pasta led to significant reductions from baseline in mean serum TC and LDL-C beyond that observed from the Step II diet alone (Fig. 4) and the extent of change after 4 wk was highly significant when compared with patients assigned to conventional pasta. Sex differences or differences between pre- and postmenopausal women in the observed effects were not examined, because the sample size lacked sufficient statistical power. Our thought that this effect was a function of the presence of isoflavones in the pasta rather than the food matrix was supported by the rebound effect observed when the soy germ pasta group was switched to regular pasta for a further 4 wk. After 8 wk, the serum TC and LDL-C concentrations in this patient group returned to near baseline levels and the relative percent change in serum lipids was not significant, and similar to that of the control group. The effect of both pastas on HDL-C and TG was minimal, due perhaps to the fact that these lipids were not abnormal and also because of the large inter-individual variabilities. This may be expected based on meta-analyses of soy and cholesterol showing HDL-C is largely unaffected by soy protein, or at best marginally increased, whereas TG are typically lowered 7–10% in hypertriglyceridemia (3,10,11).

One explanation for the profound effects of this novel food on serum lipids may be related to an interaction between the food matrix and the isoflavones. Soy germ contains primarily the glucoside conjugates of daidzein and glycitein, whereas the proportion of aglycons is typically <3% (23). However, due to the natural occurrence of β-glucosidases in semolina wheat (39), hydrolysis of the isoflavone glucosides occurred, leading to a pasta product that contained primarily aglycon forms of isoflavones (Figs. 1 and 2). In this regard, the food used in our study differs from all previous dietary intervention studies of soy and cholesterol. In the original meta-analysis on soy and cholesterol (3), the majority of the studies used products containing almost exclusively isoflavone glucosides (20) and none included a fermented soy food or a food in which the aglycon content was high. Furthermore, isoflavone supplement studies that have shown variable effects on serum lipids (28,40–43) also deliver primarily the glucoside conjugates. Dietary analysis of soy foods consumed by Japanese adults revealed that fermented soy foods comprise a relatively high proportion of the total soy protein source, whereas ISP is rarely consumed. Based on typical dietary intakes of isoflavones by Asians, which range from 20 to 50 mg/d (24), and from the known composition of isoflavones in different soy foods, we estimate that 20–30 mg/d of isoflavone aglycons are consumed by Japanese adults from fermented soy foods, similar to the daily intake of isoflavone aglycons from the isoflavone-enriched pasta.

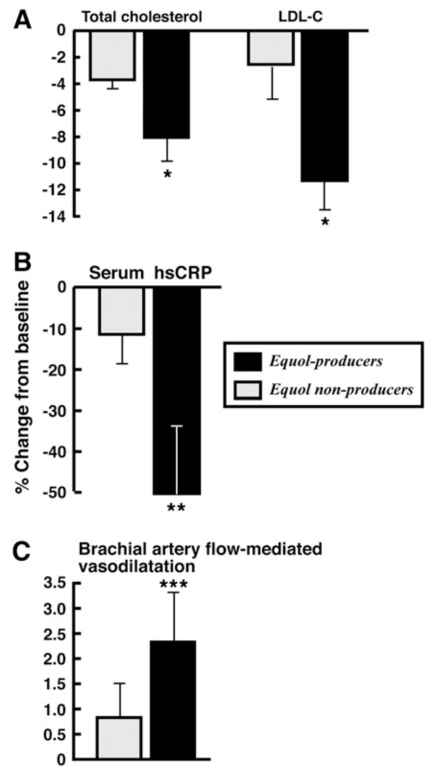

FIGURE 7 Percent change from baseline in serum TC and LDL-C concentrations (*A*), hsCRP concentrations (*B*), and brachial artery flow-mediated vasodilatation (*C*) for 29 hypercholesterolemic adults consuming isoflavone-enriched soy germ pasta for 4 wk and classified as equol producers ($n = 20$) or equol nonproducers ($n = 9$). Asterisks indicate different from nonproducers: $^*P < 0.001$, $^{**}P = 0.002$, $^{***}P = 0.03$.

The probable advantage of delivering isoflavones in the aglycon form is that they are more rapidly absorbed than the isoflavone glucosides (33,44,45) that require hydrolysis for bioavailability (46). Furthermore, the predominance of daidzein in the soy germ pasta, coupled with a possible prebiotic effect of a hard wheat food matrix, could facilitate the intestinal biotransformation of daidzein to equol (47,48), suggested to be a key factor in the efficacy of soy (36). In this regard, 69% of the hypercholesterolemic patients consuming soy germ pasta were equol producers. This is much higher than the expected 20–30%

of equol producers typically observed in Western populations consuming a mixed diet (36,37) but is comparable to the frequency of equol producers in Japanese adults (45,49,50). When the lipid data were analyzed according to equol producer status, the equol producers showed significant reductions from baseline in serum TC and LDL-C compared with those who did not produce equol (Fig. 7). This finding lends support to the theory that equol may be a key metabolite in the clinical efficacy of soy (36).

Independent of cholesterol, cardiovascular risk is also associated with other surrogate markers and in this respect we examined the effect of the soy germ pasta on a number of key elements of cardiovascular risk. Atherosclerosis leading to cardiovascular disease is now well recognized as a chronic inflammatory process (51). CRP is a nonspecific marker of inflammation that is often elevated in patients with atherosclerosis and cardiovascular disease (52,53). Several clinical studies have measured a number of inflammatory markers, including serum CRP levels in response to dietary intervention with soy protein products containing isoflavones (26,54–58). The findings have been inconsistent, although the consensus is that unlike estrogen therapy, which increases CRP levels (59,60), the presence of isoflavones either reduces or has no effect on CRP. In our study, serum hsCRP concentrations at baseline in both groups of patients were close to the upper limit of the normal range and did not differ between the groups. Soy germ pasta reduced serum hsCRP concentration by 42% after 4 wk ($P < 0.001$) and this improvement was abolished when these patients switched to conventional pasta for a further 4 wk. By contrast, serum hsCRP did not change in patients assigned to conventional pasta. The difference in hsCRP levels between the soy germ pasta and conventional pasta groups was highly significant. Consistent with earlier studies, this observation confirms the opposing action of isoflavones compared with estrogens. Consistent with the findings for cholesterol, the reduction in serum hsCRP was greater ($P = 0.001$) in equol producers than equol nonproducers.

Isoflavones have antioxidant properties (61), with equol reportedly possessing the highest in vitro antioxidant activity (62,63). Several clinical studies (25,26,64,65), although not all (66–68), in which soy isoflavones have been used reported significant reductions in ex vivo lipid peroxidation. We did not determine the extent of lipid peroxidation but did measure urinary isoprostanes as a surrogate marker of antioxidant status in the patients assigned to the soy germ pasta group and found significantly decreased urinary isoprostanes after 4 wk. However, because we did not measurement urinary isoprostanes in patients consuming conventional pasta, it is not possible to know if this decrease was influenced by the presence of isoflavones in the pasta or would have occurred independent of isoflavones.

Numerous lines of evidence indicate that isoflavones exert effects in the vasculature. Prompted by the early observation that a soy diet fed to monkeys improved vascular reactivity, Clarkson et al. (70) showed that acute administration of genistein has potent vasodilatory effects (69) similar to estrogen. The potency of genistein (aglycon) was subsequently confirmed in human studies where it produced an NO-dependent vasodilatation in men and women, comparable to that observed with agents such as acetylcholine. Oral administration of 54 mg/d pure genistein for 1 y led to an improvement in brachial artery flow-mediated dilatation in healthy postmenopausal women with a mean improvement of 5.5% (71). Dietary intervention studies have yielded mixed results regarding the effects of soy isoflavones on endothelial function (30,43,58,66,72–75). However, the inconsistency among the findings may be because these studies used different forms of isoflavones, mostly isoflavone glycosides; some studies used soy protein, whereas some were without soy protein and provided either as supplements or as components of the diet. Other than the 1 study of pure genistein (71), no previous study to our knowledge has examined the potential of isoflavones delivered as aglycons. In our study, endothelial function was measured by the change in brachial artery flow-mediated vasodilatation (34), considered a gold-standard measure for arterial reactivity. Isoflavone-enriched pasta increased brachial arterial flow-mediated dilatation by 2.3 ± 0.8% ($P = 0.003$) over just a 4-wk period. By contrast, no change was observed with conventional pasta. The mechanism by which isoflavones improve endothelial function has been postulated to be through a number of possible channels. Genistein, the isoflavone most studied, may act through gene expression to alter endothelial-dependent vasodilatation (29,71), or its effects could be through endothelial-independent actions such as calcium channel antagonism, which has been shown in rabbit aorta (76). It appears not to act through estrogen receptor α, but whether estrogen receptor β, which is found in abundance in the vasculature (77), plays a role is uncertain. The soy germ pasta delivered mainly daidzein, rather than genistein, resulting in a high proportion of equol producers. Interestingly, equol was recently shown to increase endothelial NO synthase gene expression (78), activate endothelial NO synthase, and increase NO production in human aorta and umbilical vein endothelial cells (79). We speculate that the high proportion of equol producers in the soy germ pasta group may explain the vasodilatory effects of the isoflavone-enriched pasta.

Despite the improved endothelial function from consuming soy germ pasta, we did not observe an effect on blood pressure. Several studies have alluded to small improvements in blood pressure with isoflavone intake (80–82) and epidemiological data of Chinese women show an inverse relationship between long-term soy food intake and blood pressure, which is strongest in older subjects (16). It is possible that the duration of dietary intervention with soy germ pasta may have been too short to discern beneficial effects on blood pressure.

Body weight, BMI, and waist circumference were unaffected by daily consumption of pasta with or without isoflavones for 8 consecutive weeks. There was a slight reduction in BMI in both groups, but this was not significant, an observation of relevance given that concerns over weight gain has been one of the main reasons for a marked reduction in commercial sales of pasta in recent years.

In conclusion, in this study of newly diagnosed hypercholesterolemic adults, we have shown that an isoflavone-enriched soy germ pasta delivering levels of isoflavones consistent with those consumed in Asian countries (24) but in the virtual absence of soy protein has important beneficial effects beyond that of a Step II diet on serum TC and LDL-C, serum hsCRP, urinary 8-isoprostanes, and brachial artery flow-mediated vasodilatation. Such effects are important in reducing the long-term risk of cardiovascular disease. These changes rebounded to baseline values when patients reverted to pasta that did not contain isoflavones and no lipid-lowering effects were observed in patients consuming conventional pasta lacking isoflavones. The improvement in serum lipids and other cardiovascular risk markers was greatest in patients who were equol producers, suggesting a possible role for equol in the mechanism of action. It is proposed that the effectiveness of this pasta is accounted for by the unique chemical composition of the isoflavones, which by interaction with β-glucosidases naturally present in Durum wheat results in a predominance of isoflavone aglycons and a food product more characteristic of an Asian fermented soy food. We speculate the predominance of aglycon forms of isoflavones and the possible prebiotic effect of semolina wheat explains the high proportion of equol producers and the overall clinical effectiveness of this novel food.

Literature Cited

1. Carroll KK, Kurowska EM. Soy consumption and cholesterol reduction: review of animal and human studies. J Nutr. 1995;125:S594–7.
2. Sirtori CR, Lovati MR, Manzoni C, Monetti M, Pazzucconi F, Gatti E. Soy and cholesterol reduction: clinical experience. J Nutr. 1995;125: S598–605.
3. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med. 1995; 333:276–82.
4. Food labeling: health claims: soy protein and coronary heart disease. Food and Drug Administration, HHS: final rule: soy protein and coronary heart disease. Fed Reg 1999;64:57700–33.
5. Fang N, Yu S, Badger TM. Comprehensive phytochemical profile of soy protein isolate. J Agric Food Chem. 2004;52:4012–20.
6. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr. 1995;125:S606–11.
7. Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: potential mechanisms. Am J Clin Nutr. 1998;68: S1390–3.
8. Ricketts ML, Moore DD, Banz WJ, Mezei O, Shay NF. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem. 2005;16: 321–30.
9. Crouse JR III, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med. 1999;159:2070–6.
10. Zhuo XG, Melby MK, Watanabe S. Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. J Nutr. 2004;134:2395–400.
11. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr. 2005;81:397–408.
12. Sirtori CR, Arnoldi A, Johnson SK. Phytoestrogens: end of a tale? Ann Med. 2005;37:423–38.
13. 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.
14. Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr. 1998;128:209–13.
15. Ho S, Woo J, Leung S, Sham A, Lam T, Janus E. Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. J Nutr. 2000;130:2590–3.
16. Yang G, Shu XO, Jin F, Zhang X, Li HL, Li Q, Gao YT, Zheng W. Longitudinal study of soy food intake and blood pressure among middle-aged and elderly Chinese women. Am J Clin Nutr. 2005;81: 1012–7.
17. Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soybean intake. Am J Clin Nutr. 1999;70: S464–74.
18. Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. Am J Clin Nutr. 1999;70:S439–50.
19. Gianazza E, Eberini I, Arnoldi A, Wait R, Sirtori CR. A proteomic investigation of isolated soy proteins with variable effects in experimental and clinical studies. J Nutr. 2003;133:9–14.
20. Setchell KDR, Cole SJ. Variations in isoflavone levels in soy foods and soy protein isolates and issues related to isoflavone databases and food labeling. J Agric Food Chem. 2003;51:4146–55.
21. Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, Lin Y, Nakayama T, Wada M, Ohno Y. Dietary intake and sources of isoflavones among Japanese. Nutr Cancer. 1999;33:139–45.
22. Somekawa Y, Chiguchi M, Ishibashi T, Aso T. Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstet Gynecol. 2001;97:109–15.
23. Murphy PA, Barua K, Hauck CC. Solvent extraction selection in the determination of isoflavones in soy foods. J Chromatogr B. 2002;777: 129–38.
24. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutr Cancer. 2006;55:1–12.
25. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci USA. 1998;95:3106–10.
26. Jenkins DJA, Kendall CWC, Jackson C-J, Connelly PW, Parker T, Faulkner D, Vidgen E, Cunnane SC, Leiter LA, et al. Effects of high-and low-isoflavone soy foods on blood lipids, oxidized LDL, homocystine and blood pressure in hyperlipidemic men and women. Am J Clin Nutr. 2002;76:365–72.
27. Sadowska-Krowicka H, Mannick EE, Oliver PD, Sandoval M, Zhang XJ, Eloby-Childess S, Clark DA, Miller MJ. Genistein and gut inflammation: role of nitric oxide. Proc Soc Exp Biol Med. 1998;217: 351–7.
28. Nestel PJ, Yamashita T, Sasahara T, Pomeroy S, Dart A, Komesaroff P, Owen A, Abbey M. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol. 1997;17:3392–8.
29. 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.
30. Lissin LW, Oka R, Lakshmi S, Cooke JP. Isoflavones improve vascular reactivity in post-menopausal women with hypercholesterolemia. Vasc Med. 2004;9:26–30.
31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
32. Maclouf J, Grassi J, Pradelles P. Development of enzyme-immunoassay techniques for the measurement of eicosanoids. In: Walden TL, Hughes HN, editors. International Conference on Prostaglandin and Lipid Metabolism in Radiation Injury. New York: Plenum Press; 1987. p. 355–64.
33. Setchell KDR, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe B, 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.
34. Celermajer DS, Sorensen KE, Gooch VM, Spielgelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340:1111–5.
35. Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. BMJ. 2001;323:1123–4.
36. Setchell KDR, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol: a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002;132:3577–84.
37. Setchell KDR, Cole SJ. Method of defining equol-producer status and its frequency among vegetarians. J Nutr. 2006;136:2188–93.
38. Setchell KDR, Brown NM, Desai PB, Zimmer-Nechemias L, Wolfe B, Jakate AS, Creutzinger V, Heubi JE. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J Nutr. 2003;133: 1027–35.
39. Nikus J, Jonsson LMV. Tissue localization of β-glucosidase in rye, maize and wheat seedlings. Physiol Plant. 1999;107:373–8.
40. Hodgson JM, Puddey IB, Beilin LJ, Mori TA, Croft KD. Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. J Nutr. 1998; 128:728–32.
41. Nikander E, Tiitinen A, Laitinen K, Tikkanen M, Ylikorkala O. Effects of isolated isoflavonoids on lipids, lipoproteins, insulin sensitivity, and ghrelin in postmenopausal women. J Clin Endocrinol Metab. 2004;89: 3567–72.
42. Uesugi T, Fukui Y, Yamori Y. Beneficial effects of soybean isoflavone supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: a four-week study. J Am Coll Nutr. 2002;21: 97–102.
43. Petri Nahas E, Nahás Neto J, De Luca L, Traiman P, Pontes A, Dalben I. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. Maturitas. 2004;48:372–80.

44. Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glycosides in humans. J Nutr. 2000;130:1695–9.
45. Watanabe S, Yamaguchi M, Sobue T, Takahashi T, Miura T, Arai Y, Mazur W, Wahala K, Adlercreutz H. Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J Nutr. 1998;128:1710–5.
46. Setchell KDR, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe B, Kirschner AS, Heubi JE. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr. 2002;76:447–53.
47. Axelson M, Sjovall J, Gustafsson BE, Setchell KDR. Soya: a dietary source of the non-steroidal oestrogen equol in man and animals. J Endocrinol. 1984;102:49–56.
48. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. Am J Clin Nutr. 1984;40:569–78.
49. Arai Y, Uehara M, Sato Y, Kimira M, Eboshida A, Adlercreutz H, Watanabe S. Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. J Epidemiol. 2000;10:127–35.
50. Akaza H, Miyanaga N, Takahima N, Naito S, Hirao Y, Tsukamoto T, Fujioka T, Mori M, Kim WJ, et al. Comparisons of percent equol producers between prostate cancer patients and controls: case-controlled studies of isoflavones in Japanese, Korean and American residents. Jpn J Clin Oncol. 2004;34:86–9.
51. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med. 1999;340:115–26.
52. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342:836–43.
53. Wilson AM, Ryan MC, Boyle AJ. The novel role of C-reactive protein in cardiovascular disease: risk marker or pathogen. Int J Cardiol. 2006;106:291–7.
54. Nikander E, Metsa-Heikkila M, Tiitinen A, Ylikorkala O. Evidence of a lack of effect of a phytoestrogen regimen on the levels of C-reactive protein, E-selectin, and nitrate in postmenopausal women. J Clin Endocrinol Metab. 2003;88:5180–5.
55. Hilpert KF, Kris-Etherton PM, West SG. Lipid response to a low-fat diet with or without soy is modified by C-reactive protein status in moderately hypercholesterolemic adults. J Nutr. 2005;135:1075–9.
56. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F. The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and C-reactive protein level in postmenopausal women. Acta Obstet Gynecol Scand. 2005;84:474–7.
57. Yildiz MF, Kumru S, Godekmerdan A, Kutlu S. Effects of raloxifene, hormone therapy, and soy isoflavone on serum high-sensitive C-reactive protein in postmenopausal women. Int J Gynaecol Obstet. 2005;90:128–33.
58. Hall WL, Vafeiadou K, Hallund J, Bugel S, Koebnick C, Reimann M, Ferrari M, Branca F, Talbot D, et al. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr. 2005;82:1260–8.
59. Cushman M, Legault C, Barrett-Connor E, Stefanick ML, Kessler C, Judd HL, Sakkinen PA, Tracy RP. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. Circulation. 1999;100:717–22.
60. Manning PJ, Sutherland WH, Allum AR, de Jong SA, Jones SD. Effect of hormone replacement therapy on inflammation-sensitive proteins in post-menopausal women with Type 2 diabetes. Diabet Med. 2002;19:847–52.
61. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res. 1995;22:375–83.
62. Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res. 1997;26:63–70.
63. Arora A, Nair MG, Strasburg GM. Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. Arch Biochem Biophys. 1998;356:133–41.
64. Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, Sanders TA. Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr. 2000;72:395–400.
65. Jenkins DJA, Kendall CWC, Garsetti M, Rosenberg-Zang RS, Jackson C-J, Agarwal S, Rao AV, Diamandis EP, Parker T, et al. Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity: a controlled crossover trial. Metabolism. 2000;49:537–43.
66. Steinberg FM, Guthrie NL, Villablanca AC, Kumar K, Murray MJ. Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. Am J Clin Nutr. 2003;78:123–30.
67. Vega-Lopez S, Yeum KJ, Lecker JL, Ausman LM, Johnson EJ, Devaraj S, Jialal I, Lichtenstein AH. Plasma antioxidant capacity in response to diets high in soy or animal protein with or without isoflavones. Am J Clin Nutr. 2005;81:43–9.
68. Engelman HM, Alekel DL, Hanson LN, Kanthasamy AG, Reddy MB. Blood lipid and oxidative stress responses to soy protein with isoflavones and phytic acid in postmenopausal women. Am J Clin Nutr. 2005;81:590–6.
69. Honore EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril. 1997;67:148–54.
70. Gilligan DM, Quyyumi AA, Cannon RO III. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. Circulation. 1994;89:2545–51.
71. 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.
72. Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. J Clin Endocrinol Metab. 2001;86:3053–60.
73. Hermansen K, Hansen B, Jacobsen R, Clausen P, Dalgaard M, Dinesen B, Holst JJ, Pedersen E, Astrup A. Effects of soy supplementation on blood lipids and arterial function in hypercholesterolaemic subjects. Eur J Clin Nutr. 2005;59:843–50.
74. Colacurci N, Chiantera A, Fornaro F, de Novellis V, Manzella D, Arciello A, Chiantera V, Improta L, Paolisso G. Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause. 2005;12:299–307.
75. Kreijkamp-Kaspers S, Kok L, Bots ML, Grobbee DE, Lampe JW, van der Schouw YT. Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women. Am J Clin Nutr. 2005;81:189–95.
76. Figtree GA, Griffiths H, Lu Y-Q, Webb CM, Macleod K, Collins P. Plant-derived estrogens relax coronary arteries in vitro by a calcium antagonistic mechanism. J Am Coll Cardiol. 2000;35:1977–85.
77. Lindner V, Kim SK, Karas RH, Kuiper GG, Gustafsson JA, Mendelsohn ME. Increased expression of estrogen receptor-beta mRNA in male blood vessels after vascular injury. Circ Res. 1998;83:224–9.
78. Mahn K, Borras C, Knock GA, Taylor P, Khan IY, Sugden D, Poston L, Ward JP, Sharpe RM, et al. Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. FASEB J. 2005;19:1755–7.
79. Joy S, Siow RC, Rowlands DJ, Becker M, Wyatt AW, Aaronson PI, Coen C, Jacob R, Kallo I, et al. The isoflavone equol mediates rapid vascular relaxation: Ca2+-independent activation of eNOS/Hsp90 involving ERK1/2 and Akt phosphorylation in human endothelial cells. J Biol Chem. 2006;281:27335–45.
80. Washburn S, Burke GL, Morgan T, Anthony M. Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. Menopause. 1999;6:7–13.
81. Rivas M, Garay RP, Escanero JF, Cia P, Alda JO. Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. J Nutr. 2002;132:1900–2.
82. He J, Gu D, Wu X, Chen J, Duan X, Whelton PK. Effect of soybean protein on blood pressure: a randomized, controlled trial. Ann Intern Med. 2005;143:1–9.