Isoflavone phytoestrogens consumed in soy decrease F_2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans[1–3]

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ABSTRACT

Background: Oxidative damage to lipids may be involved in the etiology of atherosclerosis, cardiovascular disease in general, and cancer. The soy isoflavone phytoestrogens, genistein and daidzein, and equol (a daidzein metabolite produced by intestinal microflora) are antioxidants in vitro; equol is a particularly good inhibitor of LDL oxidation and membrane lipid peroxidation.

Objective: We sought to investigate the effects of a diet enriched with soy containing isoflavones on in vivo biomarkers of lipid peroxidation and resistance of LDL to oxidation, compared with a diet enriched with soy from which the isoflavones had been extracted.

Design: A randomized, crossover design was used to compare diets enriched with soy that was low or high in isoflavones in 24 subjects. Plasma concentrations of an F_2-isoprostane, 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$), a biomarker of in vivo lipid peroxidation, and resistance of LDL to copper-ion-induced oxidation were determined.

Results: Plasma concentrations of 8-*epi*-$PGF_{2\alpha}$ were significantly lower after the high-isoflavone dietary treatment than after the low-isoflavone dietary treatment (326 ± 32 and 405 ± 50 ng/L, respectively; $P = 0.028$) and the lag time for copper-ion-induced LDL oxidation was longer (48 ± 2.4 and 44 ± 1.9 min, respectively; $P = 0.017$). Lag time for oxidation of unfractionated plasma and plasma concentrations of malondialdehyde, LDL α-tocopherol, polyunsaturated fatty acids, and isoflavonoids did not differ significantly between dietary treatments.

Conclusions: Consumption of soy containing naturally occurring amounts of isoflavone phytoestrogens reduced lipid peroxidation in vivo and increased the resistance of LDL to oxidation. This antioxidant action may be significant with regard to risk of atherosclerosis, cardiovascular disease in general, and cancer. *Am J Clin Nutr* 2000;72:395–400.

KEY WORDS Isoflavone, phytoestrogen, soy, antioxidant, lipid peroxidation, low-density lipoprotein, LDL oxidation, F_2-isoprostanes, cancer prevention, cancer, cardiovascular disease, heart disease, coronary artery disease, atherosclerosis, arteriosclerosis

INTRODUCTION

Dietary phytoestrogens are plant-derived compounds with weak estrogenic and antiestrogenic properties (1, 2). The effects of phytoestrogen consumption on a wide range of biomarkers are currently being investigated to determine the likely importance of phytoestrogens in the prevention of hormone-dependent cancers and cardiovascular disease (3–6).

The isoflavones genistein and daidzein are found predominantly in soybeans and soy products, mostly as their glycones, genistin and daidzin (7). After ingestion, daidzin and genistin are hydrolyzed in the large intestine by the action of bacteria to release genistein and daidzein. Daidzein can be metabolized by the bacteria in the large intestine to form the isoflavan equol (estrogenic) or *O*-desmethylangolensin (nonestrogenic), whereas genistein is metabolized to the nonestrogenic *P*-ethyl phenol (8). Interindividual variation in the ability to metabolize daidzein to equol could therefore influence the potential health-protective effects of soy isoflavones (9).

Genistein, daidzein, and equol have shown antioxidant properties in vitro; the evidence of these properties is particularly strong for equol. The antioxidant effects were predominantly directed against oxidative damage to membrane lipids and lipoprotein particles (10–15) and also against oxidative DNA damage (16). Oxidative damage to LDL was implicated in atherogenesis (17, 18). A recent study showed that resistance of LDL to oxidation increased from baseline values in 6 subjects who consumed 3 soy bars/d for 2 wk (19).

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Much debate has focused on the suitability of various biomarkers of oxidative damage to lipids in vivo. There appears to be increasing agreement that plasma concentrations of F_2-isoprostanes, specific end products of the peroxidation of arachidonic acid residues, may be the best biomarker of lipid peroxidation in the human body (20–25) when they are measured by mass spectrometry. F_2-isoprostane concentrations were found to vary widely in the body fluids of healthy subjects; these variations could result in part from diet (20, 21) but could also stem from other factors, such as endogenous antioxidant defenses and rates of isoprostane metabolism (24). Indeed, some healthy humans appear to have higher rates of lipid peroxidation than do others, even when consuming comparable diets (21, 23, 26), and thus could be at greater risk of diseases involving lipid peroxidation, such as atherosclerosis and cancer.

The aim of the present study was to determine the effects of dietary soy isoflavones on biomarkers of in vivo lipid peroxidation [eg, the F_2-isoprostane 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$)] and resistance of LDL to oxidation in humans. This issue has not been investigated previously in a randomized, controlled, crossover study of a soy-containing food with low or high isoflavone content.

SUBJECTS AND METHODS

Study design

A randomized, controlled, crossover design was used to compare the effects of a textured soy protein high in isoflavones (HI) with a similar product in which most of the soy isoflavones had been removed by alcohol extraction (LI). A mixture of food-grade alcohol (700 g/L or 70%) and water was used in the extraction process, after which no remaining alcohol was detected (limit of detection, 0.1 g/L or 0.01%). The textured soy protein concentrates were supplied by Solbar Hatzor Ltd, Ashdod, Israel. Each treatment period lasted for 17 d and the 2 treatment periods were separated by a 25-d washout period. The subjects were instructed to avoid all other soy products, legumes, and mycoprotein meat-replacement products throughout the study. The subjects received a modest financial payment for their participation in the study. The study protocol was reviewed and approved by the local Human Experimentation Committee at King's College London. All participants gave their written, informed consent.

Subjects

The subjects were healthy adults recruited from the staff and alumni of King's College London and from TNO-BIBRA International. A total of 24 adults (19 women and 5 men) with a mean age of 30 y (range: 19–40 y) and a mean body mass index (BMI; in kg/m^2) of 22.5 ± 3.5 participated in the study. The exclusion criteria included BMI <18 or >30; cigarette smoking; hyperlipidemia; history of cholestatic liver disease, gastrointestinal disease, pancreatitis, diabetes mellitus, or myocardial infarction; use of antihypertensive or lipid-lowering medication; use of antibiotics within the previous 6 mo; and use of vitamin or nutritional supplements. Before a potential subject could enroll, we confirmed that his or her fasting plasma lipid concentrations, body weight, blood cell count, and liver function were within specified limits (these limits included cholesterol <6.5 mmol/L and triacylglycerol <3 mmol/L). Each female subject underwent a pregnancy test (urine method) to confirm that she was not pregnant.

The subjects were asked to make no changes in their diet or lifestyle, including their usual exercise habits, other than those changes necessary for compliance with the study. The subjects were asked to keep 3-d weighed-food records so that their nutrient intakes could be assessed. Nutrient analysis of the food records was carried out with the COMPEAT 4 Program (Nutrition Systems, London).

Dietary intervention

The textured soy proteins (HI and LI) were made into vegetarian burgers and subjects consumed one burger daily during both of the 17-d study periods. Chemical analysis showed that both types of burgers supplied 0.8 MJ, 15 g protein, 9 g fat, and 10 g carbohydrate. On analysis (27), the HI burger was found to contain 21.2 mg (84 μmol) daidzein and 34.8 mg (129 μmol) genistein whereas the LI burger contained 0.9 mg (3.5 μmol) daidzein and 1.0 mg (3.7 μmol) genistein.

Collection and handling of blood samples

Venous blood samples were collected on days 13 and 14 of each treatment period after the subjects had fasted overnight. For measurement of plasma F_2-isoprostane concentrations, blood samples were collected into 4.5-mL evacuated tubes containing sodium citrate (38 g/L); the tubes were kept on ice and indomethicin was added to produce a final concentration of 15 μmol/L. The blood samples were centrifuged at 1500 × *g* for 10 min at 4°C. The plasma was separated and butylated hydroxytoluene (in 960 mL/L ethanol) was added to the plasma to produce a final concentration of 20 μmol/L. The plasma samples were snap-frozen with liquid nitrogen and were stored at −70°C until analyzed. For measurement of plasma malondialdehyde concentrations, blood samples were collected into 10-mL evacuated tubes containing EDTA and were centrifuged at 1500 × *g* for 10 min at 4°C. The plasma was separated, snap-frozen with liquid nitrogen, and stored at −70°C until analyzed.

For plasma LDL isolation, blood samples were collected into 10-mL evacuated tubes containing EDTA and were centrifuged at 1500 × *g* for 10 min at 4°C. The plasma was separated, snap-frozen with liquid nitrogen, and stored at −70°C until analyzed. In addition, plasma for LDL isolation was stored with 100 g sucrose/L according to the method of Rumsey et al (28). Previously, we showed that there was no significant difference between fresh and frozen plasma with respect to LDL oxidation and the inhibition of LDL oxidation by flavonoids (29). For measurement of plasma oxidation susceptibility, blood samples were collected into 4.5-mL evacuated tubes containing sodium citrate (38 g/L). The blood samples were centrifuged at 1500 × *g* for 10 min at 4°C. The plasma was separated, snap-frozen with liquid nitrogen, and stored at −70°C until analyzed.

Analytic methods

Total (free and esterified) F_2-isoprostanes were isolated by using a solid-phase extraction procedure; concentrations of 8-*epi*-$PGF_{2\alpha}$ were determined by gas chromatography–mass spectrometry according to the method of Nourooz-Zadeh et al (30). Plasma concentrations of malondialdehyde were measured by using the HPLC-based thiobarbituric acid test, whereby 50 μL butylated hydroxytoluene (2 g/L dissolved in ethanol) was added to 0.5 mL of the samples and standards before the thiobarbituric acid reagents were added. This was done to prevent artifacts caused by the amplification of peroxidation during the assay and also to prevent artifacts that could result from variations in sam-

TABLE 1
Dietary intakes of the subjects during the baseline period and the low-isoflavone (LI) and high-isoflavone (HI) treatment periods[1]

	Baseline	LI	HI
Energy (MJ/d)	8.6 ± 0.49	8.2 ± 0.47	8.9 ± 0.58
Fat (g/d)	73.6 ± 3.9	75.6 ± 3.1	89.2 ± 3.5[2]
Carbohydrate (g/d)	253.6 ± 9.3	237.9 ± 6.9	246.5 ± 8.0
Protein (g/d)	78.2 ± 4.0	70.6 ± 3.1	75.6 ± 2.6
Nonstarch polysaccharides (g/d)	16.5 ± 1.6	14.3 ± 1.3	14.7 ± 1.3

[1] $\bar{x}$ ± SE; n = 22.
[2] Significantly different from baseline, $P < 0.05$ (paired t test).

ple lipid content, antioxidant concentration, or both according to the method of Chirico et al (31).

LDL was isolated from plasma by differential ultracentrifugation with a Beckman SW40Ti rotor (Beckman Instruments, Fullerton, CA). This was followed by dialysis under nitrogen for 18 h against 400 volumes of 10-mmol/L phosphate-buffered saline (pH 7.4) according to the method of Redgrave et al (32). LDL protein was measured according to the modified Lowry procedure of Markwell et al (33). LDL oxidation was stimulated by the addition of copper sulfate to produce a final copper ion concentration of 5 μmol/L; the LDL protein concentration was 0.1 g/L. Continuous monitoring of LDL conjugated diene formation was performed according to the method of Esterbauer et al (34). LDL α-tocopherol concentrations were measured with HPLC by using tocopherol acetate as the internal standard according to the method of Thurnham et al (35). The fatty acid composition of LDL was determined by gas chromatography by using flame ionization detection according to the method of Lepage and Roy (36). LDL isoflavonoid concentrations were measured by gas chromatography–mass spectrometry according to the method of Tikkanen et al (19). Plasma concentrations of isoflavonoids were determined by isotope dilution gas-liquid mass spectrometry by using deuterated internal standards according to the method of Adlercreutz et al (37). Oxidation of lipoproteins in unfractionated plasma was stimulated with copper ions by the addition of copper sulfate (to a final copper ion concentration of 100 μmol/L) and was measured by continuous monitoring of conjugated diene formation according to the method of Schnitzer et al (38).

Statistical analysis

We tested for treatment-order effects with repeated-measures analysis of variance (SPSS/PC, version 8.0; SPSS UK Ltd, Chertsey, United Kingdom). Paired comparisons were performed by using a paired t test with a Bonferroni adjustment for multiple comparisons when necessary. Pearson's product-moment correlation coefficients were used to evaluate the data for correlations.

RESULTS

Twenty-four subjects completed the HI treatment and 22 completed the LI treatment. Biomarkers for lipid peroxidation and resistance of LDL to oxidation were measured in only 17–21 samples because of problems with either sample collection or sample isolation; the exact numbers of samples are shown in the relevant tables.

The dietary intakes of the 22 subjects who completed both treatments are shown in **Table 1**. Energy intakes during the HI and LI treatments were not significantly different from those during the baseline diet. The proportion of energy derived from fat was significantly greater ($P < 0.05$) during the HI treatment (89.2 ± 3.5 g/d or 37.7 ± 1.5% of energy) than during the baseline diet (73.6 ± 3.9 g/d or 32.2 ± 1.7% of energy). The proportion of energy derived from carbohydrate was lower (NS) during the HI treatment (246.5 ± 8.0 g/d or 46.3 ± 1.5% of energy) than during the baseline diet (253.6 ± 9.3 g/d or 49.3 ± 1.8% of energy). However, dietary intakes were not significantly different between the HI and LI dietary treatments.

Plasma concentrations of 8-*epi*-$PGF_{2\alpha}$, malondialdehyde, and isoflavonoids after the HI and LI treatment periods are shown in **Table 2**. Plasma isoflavonoid concentrations were significantly higher after the HI treatment than after the LI treatment. Plasma 8-*epi*-$PGF_{2\alpha}$ concentrations were significantly lower ($P = 0.028$) after the HI treatment than after the LI treatment. Plasma malondialdehyde concentrations did not differ significantly between treatments.

The lag times for oxidation of LDL and unfractionated plasma and the plasma concentrations of LDL α-tocopherol, isoflavonoids, and saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) after the HI and LI treatment periods are shown in **Table 3**. The lag time for copper-ion-stimulated LDL oxidation ex vivo was significantly longer ($P = 0.017$) after the HI treatment than after the LI treatment. Plasma concentrations of LDL α-tocopherol, PUFA, and isoflavonoids did not differ significantly between the 2 treatment periods; for the isoflavonoid analysis, we used only the 6 LDL samples (of a total of 18 samples) that showed the greatest change in lag time between the 2 treatments. The lag time for the copper-ion-stimulated oxidation of unfractionated plasma did not differ significantly between the 2 treatments.

DISCUSSION

The aim of this study was to compare the effects of 2 dietary treatments, soy containing isoflavones and soy from which the

TABLE 2
Plasma 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$), malondialdehyde, and isoflavonoid concentrations after the low-isoflavone (LI) and high-isoflavone (HI) treatment periods[1]

	LI	HI
8-*epi* $PGF_{2\alpha}$ (ng/L)	405 ± 50 [18]	326 ± 32[2] [18]
Malondialdehyde (μmol/L)	0.17 ± 0.013 [18]	0.18 ± 0.014 [18]
Genistein (nmol/L)	28 ± 4 [21]	779 ± 170[3] [23]
Daidzein (nmol/L)	14 ± 2 [21]	317 ± 54[3] [23]
Equol (nmol/L)	2 ± 0.4 [21]	76 ± 30[2] [23]
O-DMA (nmol/L)	1 ± 0.4 [21]	120 ± 35[3] [23]

[1] $\bar{x}$ ± SE. n in brackets. *O*-DMA, *O*-desmethylangolensin.
[2,3] Significantly different from LI (paired t test): [2] $P < 0.05$, [3] $P < 0.01$.

TABLE 3
Lag time for copper-ion-stimulated LDL oxidation and unfractionated plasma oxidation and plasma concentrations of LDL α-tocopherol, fatty acids, and isoflavonoids after the low-isoflavone (LI) and high-isoflavone (HI) treatment periods[1]

	LI	HI
Lag time for LDL oxidation (min)	44 ± 1.9 [18]	48 ± 2.4[2] [18]
α-Tocopherol (μmol/mmol cholesterol)	1.99 ± 0.20 [18]	2.07 ± 0.21 [18]
SFA (μg/mg protein)	650 ± 34 [17]	580 ± 12 [17]
PUFA (μg/mg protein)	890 ± 30[17]	890 ± 18 [17]
MUFA (μg/mg protein)	380 ± 11[17]	390 ± 6 [17]
Genistein (pmol/mg protein)	10 ± 2.9 [6]	10 ± 3.7 [6]
Daidzein (pmol/mg protein)	3 ± 1.0 [6]	2 ± 0.61 [6]
Equol (pmol/mg protein)	ND [6]	0.2 ± 0.2 [6]
O-DMA (pmol/mg protein)	0.2 ± 0.1 [6]	0.6 ± 0.49 [6]
Lag time for plasma oxidation (min)	59 ± 1.5 [21]	60 ± 1.6 [21]

[1] $\bar{x}$ ± SE. *n* in brackets. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; *O*-DMA, *O*-desmethylangolensin; ND, not detected.
[2] Significantly different from low-isoflavone, $P < 0.05$ (paired *t* test).

isoflavones had been extracted, when these treatments were added to the usual diets of healthy adults. We examined the effects of these 2 different dietary treatments on biomarkers of both lipid peroxidation in the human body and resistance of LDL to oxidation. We found substantially higher plasma isoflavonoid concentrations after the HI treatment than after the LI treatment.

Plasma 8-*epi*-$PGF_{2\alpha}$ concentrations were significantly lower ($P = 0.028$) after the HI treatment than after the LI treatment, which suggests that the soy isoflavones consumed and their metabolites were protecting against lipid peroxidation in vivo. Measurements of plasma F_2-isoprostane concentrations, currently the biomarker of choice for assessing lipid peroxidation in the human body (22–25), were not reported in other published studies of soy consumption. However, in heavy smokers, plasma F_2-isoprostane concentrations were ≈2-fold higher than in age- and sex-matched control subjects (23). In the present study, we found no detectable differences between the HI and LI treatments in another index of lipid peroxidation, plasma malondialdehyde concentration measured by the HPLC–thiobarbituric acid test. However, the values measured were close to the limit of detection (0.075 μmol/L) and this technique is probably best suited for use in clinical conditions such as hyperlipidemia where there is increased lipid peroxidation and thus elevated malondialdehyde production (31).

A recent study in 6 subjects who consumed 3 soy bars/d for 2 wk found a significantly longer lag time for LDL oxidation after soy consumption than at baseline (19). This agrees with the findings of the present study, in which the lag time for LDL oxidation was significantly greater after the HI treatment than after the LI treatment. The conjugated diene traces that we obtained were essentially the same as those in the study by Tikkanen et al (19). We did not observe any significant changes in the propagation rate after the HI treatment compared with the LI treatment, which also agrees with the study by Tikkanen et al (19). The amounts of isoflavones consumed in the 2 studies were almost identical: 35 mg genistein and 21 mg daidzein in the HI treatment period of the present study and 36 mg genistein and 21 mg daidzein in the study by Tikkanen et al (19). The increase in the lag time for LDL oxidation was greater in the study by Tikkanen et al (19) (mean increase: 20 min; $P < 0.02$) than in the present study (mean increase: 4 min; $P = 0.017$). However, these results represent a difference in lag time for LDL oxidation of ≈8% between the 2 treatments in the present study compared with 15% in the study by Tikkanen et al (19), indicating that the findings of the 2 studies are more similar than they may appear initially.

Measuring lipoprotein oxidation in unfractionated plasma may be of greater physiologic relevance than measuring resistance of isolated LDL to oxidation because plasma contains water-soluble antioxidants. In the present study, it was paradoxical that there was no significant difference in resistance of plasma to oxidation between the HI and LI treatments, yet isolated LDL showed increased oxidation resistance after the HI treatment. It is possible that any protective effect of the isoflavonoids against plasma oxidation was masked by the protective effects of water-soluble antioxidants, such as ascorbate and urate, that were present in plasma but not in the isolated LDL system. Indeed, the lag time of the plasma was significantly longer than that of the isolated LDL. Furthermore, although inhibition of lipoprotein oxidation in whole plasma was reported when isoflavonoids were added to plasma in vitro (10), most of the concentrations used (0.1, 1, and 10 μmol/L) were greater than those found in plasma in the present study.

Overall in the present study, the subjects' dietary intakes did not differ significantly between the 2 treatment periods. This suggests that dietary changes, such as changes in fat intake, and subsequent effects on LDL composition are unlikely to have contributed to the effects on resistance of LDL to oxidation observed here. Indeed, there were no significant differences in the concentrations of LDL α-tocopherol and PUFA between the 2 treatment periods, indicating that the protective effect of the soy isoflavones against LDL oxidation was not a result of changes such as greater antioxidant protection by α-tocopherol or decreased PUFA content and thus reduced susceptibility to oxidative damage. The subjects were asked to make no changes in their diets or lifestyles (including exercise habits) other than those necessary for compliance with the study treatments. The study of Shern-Brewer et al (39) showed that the susceptibility of LDL to oxidation was greater in men who engaged in short-term aerobic exercise than in sedentary control subjects, but was lower (compared with control subjects) in men who exercised aerobically over the long term.

The greater resistance of LDL to oxidation observed in the present study and in a previous one (19) suggests that the soy isoflavones consumed, their metabolites in plasma, or both are associated with the LDL particles in vivo and thus can act as antioxidants when the LDL is challenged with copper-ion-stimulated oxidation ex vivo. However, Tikkanen et al (19) found that isoflavones and their metabolites were present only in very small amounts in the LDL, and in the present study, no significant amounts of isoflavonoids were found associated with the LDL particles after isolation. Furthermore, although it was shown that esterified isoflavones can be incorporated into LDL ex vivo, resulting in greater oxidation resistance (40), it has not yet been shown that isoflavones can be esterified to LDL in vivo.

Further explanations for the observed protective effect of isoflavones (*see* also 19) include the possibility that dietary soy isoflavones may alter LDL particle size, which is thought to influence resistance to oxidation, and the possibility that plasma isoflavonoids may protect the LDL in vivo by acting as antioxidants to reduce formation of the preformed lipid hydroperoxides.

These hydroperoxides enhance the initiation rate of oxidation, which in turn influences the length of the lag time (41), although we did not measure initial concentrations of hydroperoxides in the LDL in the present study. Thus, the presence of isoflavonoids in the LDL particles ex vivo would not be essential for them to exert a protective effect.

Although the resistance of isolated plasma LDL to oxidation is often assumed to be a good indicator of atherogenic risk, the results of 2 studies do not support this assumption (42, 43). The ability of antioxidants to protect isolated LDL against copper-ion-induced oxidation ex vivo was not a good predictor of the antiatherogenic efficacy of antioxidants in Watanabe heritable hyperlipidemic (WHHL) rabbits. Both probucol and an antioxidant combination containing vitamin E increased the lag time for LDL oxidation, but only probucol was associated with a greatly reduced aortic atherosclerotic lesion formation compared with that in the control group (42). In the second study, only probucol and not its metabolite bisphenol enhanced resistance of LDL to oxidation. Although both compounds strongly inhibited aortic accumulation of lipid hydroperoxides, only probucol had a moderately significant effect on the extent of lesion formation in WHHL rabbits (43). Probucol appears to reduce atherogenesis by mechanisms not shared by all antioxidants, and the observed dissociation of atherogenesis from aortic accumulation of hydroperoxides in WHHL rabbits adds to the complexity of the assessment of antioxidants as antiatherogenic agents (43). Furthermore, the biological significance of a 4-min increase in mean lag time for LDL oxidation, as observed in the present study, is unclear. The importance of such an increase in the context of a developing atherosclerotic lesion is currently not known, especially because antioxidant regimens that prevent atherosclerosis by inhibiting LDL oxidation increase the lag time by significantly more than this (43). Therefore, further studies are needed.

In the present study, total plasma cholesterol and apolipoprotein B concentrations were unchanged but HDL and apolipoprotein A-I concentrations increased significantly after the HI treatment; these data were reported previously (44). This increase in HDL concentration could protect LDL against oxidation in vivo because the lipids in HDL are preferentially oxidized before those in LDL (45). The earliest stages of HDL oxidation are accompanied by the oxidation of specific methionine residues in apolipoprotein A-I and apolipoprotein A-II (46). HDL also has antioxidant activity, which is probably mediated by the esterase paraoxonase when associated with HDL in plasma (47–49); this could produce greater resistance of LDL to oxidation ex vivo. Other antiatherogenic mechanisms of HDL include its role in reverse cholesterol transport (50) and its ability to preferentially transport oxidized cholesteryl esters to the liver for excretion into bile (51).

The results of the present study indicate that soy isoflavonoids appear to protect against oxidative damage to lipids and lipoprotein particles in humans, although paradoxically, plasma oxidation was not affected. The antioxidant action observed may contribute to the ability of soy isoflavonoids to protect against the oxidative lipid damage implicated in the development of cardiovascular disease and cancer.

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