

# Effect of Soy Protein Foods on Low-Density Lipoprotein Oxidation and Ex Vivo Sex Hormone Receptor Activity—A Controlled Crossover Trial

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Plant-derived estrogen analogs (phytoestrogens) may confer significant health advantages including cholesterol reduction, antioxidant activity, and possibly a reduced cancer risk. However, the concern has also been raised that phytoestrogens may be endocrine disrupters and major health hazards. We therefore assessed the effects of soy foods as a rich source of isoflavonoid phytoestrogens on LDL oxidation and sex hormone receptor activity. Thirty-one hyperlipidemic subjects underwent two 1-month low-fat metabolic diets in a randomized crossover study. The major differences between the test and control diets were an increase in soy protein foods (33 g/d soy protein) providing 86 mg isoflavones/2,000 kcal/d and a doubling of the soluble fiber intake. Fasting blood samples were obtained at the start and at weeks 2 and 4, with 24-hour urine collections at the end of each phase. Soy foods increased urinary isoflavone excretion on the test diet versus the control ( $3.8 \pm 0.7$  v  $0.0 \pm 0.0$  mg/d,  $P < .001$ ). The test diet decreased both oxidized LDL measured as conjugated dienes in the LDL fraction ( $56 \pm 3$  v  $63 \pm 3$   $\mu$ mol/L,  $P < .001$ ) and the ratio of conjugated dienes to LDL cholesterol ( $15.0 \pm 1.0$  v  $15.7 \pm 0.9$ ,  $P = .032$ ), even in subjects already using vitamin E supplements (400 to 800 mg/d). No significant difference was detected in ex vivo sex hormone activity between urine samples from the test and control periods. In conclusion, consumption of high-isoflavone foods was associated with reduced levels of circulating oxidized LDL even in subjects taking vitamin E, with no evidence of increased urinary estrogenic activity. Soy consumption may reduce cardiovascular disease risk without increasing the risk for hormone-dependent cancers.

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IT HAS BEEN SUGGESTED that soy food consumption may be part of the reason for the low rates of cardiovascular disease and hormone-dependent cancer in the industrialized nations of the Orient.<sup>1</sup> Soy isoflavones have been proposed as the components of soy most responsible for many of the suggested health benefits.<sup>1-4</sup> They have been associated with the cholesterol-lowering action of soy,<sup>4,7</sup> they have antioxidant activity in vitro,<sup>8-12</sup> and, as phytoestrogens, they have been suggested to play a possible role in reducing the risk of breast and prostate cancer by a variety of mechanisms.<sup>1-4,13-17</sup>

On the other hand, there are also those who believe that soy consumption poses potential risks to health<sup>18-24</sup> and that, via excessive phytoestrogen activity, soy consumption may enhance breast tumor growth, cause abnormal sexual development in infants and children, and have adverse effects on the central nervous system.<sup>18-24</sup> Although there have been many studies on soy, many of these issues have not been dealt with specifically. In the meantime, the New Zealand Ministry of Health has issued a warning in relation to thyroid function in infants on soy formula milk substitutes.<sup>25</sup> At the same time, the US Food and Drug Administration (FDA) delayed its decision on a health claim for soy related to a decrease of serum cholesterol and a reduction of cardiovascular disease risk.<sup>26</sup>

We have therefore assessed in hyperlipidemic subjects the antioxidant and sex hormone activities of readily available soy foods fed at a daily dose that would satisfy the proposed FDA requirements for a health claim for cardiovascular disease reduction.<sup>26</sup>

## SUBJECTS AND METHODS

Thirty-one hyperlipidemic subjects (19 men and 12 postmenopausal women) completed two 1-month metabolic diet periods separated by at least a 2-week washout period in a randomized crossover study. The study details and data relating to serum lipids, blood pressure, fecal short-chain fatty acids, and fecal bile acids are reported elsewhere.<sup>27</sup> The subjects' mean age was  $56.5 \pm 9.0$  years (range, 31 to 70), with a

body mass index of  $24.6 \pm 2.3$  kg/m<sup>2</sup> (range, 20.8 to 29.1). All subjects had elevated serum low-density lipoprotein (LDL) cholesterol ( $>4.1$  mmol/L) and a triglyceride level less than 4.0 mmol/L at recruitment. None had clinical or biochemical evidence of diabetes or liver or renal disease and none were using hypolipidemic agents, with the exception of one man on lovastatin 20 mg/d throughout the study. One woman was on hormone replacement therapy, 2 women were taking levothyroxine, and 1 man and 1 woman were taking  $\beta$ -blocking agents. Three men and 6 women were using vitamin E supplements (400 to 800 mg/d). Dosage levels of all medications and supplements were held constant for both study periods. Subjects were also instructed to maintain their habitual level of physical activity throughout. Blood samples were obtained after a 12- to 14-hour overnight fast prior to the study and at the end of weeks 2 and 4 of each metabolic phase. Serum was stored at  $-70^{\circ}\text{C}$  prior to analysis. Body weight was measured at the start and at weekly intervals on both metabolic phases. Twenty-four-hour urine collections were made on an outpatient basis at the end of each phase.

The study was approved by the Ethics Committee of the University of Toronto and St. Michael's Hospital. Informed consent was obtained from all subjects and their primary-care physicians.

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## Diets

The metabolic diets were designed in conformity with the National Cholesterol Education Program step 2 dietary principles, but the dietary intake of cholesterol was further reduced. The macronutrient profile of the metabolic diets on each phase is presented in Table 1. The test diet provided 86 mg isoflavones per 2,000 kcal daily, while no isoflavones were detected in the control diet (Table 2).

The test and control metabolic diets followed a 7-day rotating-menu plan. The control diet was a lacto-ovo-vegetarian diet with milk products that were low in fat, consisting of skim milk, 1% dairy fat yogurt, skim-milk cheese, and low-fat cottage cheese. Eggbeaters (Lipton's, Toronto, Ontario, Canada) were used rather than whole eggs to further reduce the cholesterol intake.

In the test diet, 93% of the animal protein was replaced with vegetable protein from soy, other legumes, and cereal foods provided as easy-to-prepare meals or frozen dishes, meat substitutes, and vegetarian "cold cuts." Soluble fiber was increased by the inclusion of oats, barley,

**Table 1. Calculated Macronutrient Intake (mean  $\pm$  SE) on the Test and Control Metabolic Diets (N = 31)**

Nutrient	Control	Test
<b>Energy</b>		
kcal/d	2,519 $\pm$ 86	2,341 $\pm$ 88
MJ/d	10.5 $\pm$ 0.4	9.8 $\pm$ 0.4
<b>Total protein</b>		
g/d	121 $\pm$ 4	118 $\pm$ 4
%	19.2 $\pm$ 0.2	20.2 $\pm$ 0.1
<b>Vegetable protein</b>		
g/d	27 $\pm$ 1	110 $\pm$ 4
%	4.3 $\pm$ 0.1	18.8 $\pm$ 0.1
<b>Soy protein</b>		
g/d	0 $\pm$ 0	33 $\pm$ 4
%	0.0 $\pm$ 0.0	5.7 $\pm$ 0.1
<b>Available carbohydrate</b>		
g/d	343 $\pm$ 12	318 $\pm$ 12
%	54.6 $\pm$ 0.3	54.3 $\pm$ 0.3
<b>Total dietary fiber</b>		
g/d	36 $\pm$ 1	62 $\pm$ 3
g/1,000 kcal	14.3 $\pm$ 0.2	26.3 $\pm$ 0.2
<b>Soluble fiber</b>		
g/d	9 $\pm$ 0	18 $\pm$ 1
g/1,000 kcal	3.4 $\pm$ 0.0	7.6 $\pm$ 0.1
<b>Total fat</b>		
g/d	71 $\pm$ 3	66 $\pm$ 2
%	25.5 $\pm$ 0.2	25.5 $\pm$ 0.2
<b>SFA</b>		
g/d	18 $\pm$ 1	16 $\pm$ 1
%	6.5 $\pm$ 0.0	6.0 $\pm$ 0.1
<b>MUFA</b>		
g/d	24 $\pm$ 1	22 $\pm$ 1
%	8.4 $\pm$ 0.1	8.4 $\pm$ 0.1
<b>PUFA</b>		
g/d	24 $\pm$ 1	25 $\pm$ 1
%	8.7 $\pm$ 0.1	9.6 $\pm$ 0.1
<b>Dietary cholesterol</b>		
mg/d	76 $\pm$ 3	77 $\pm$ 4
mg/1,000 kcal	30.2 $\pm$ 0.4	33.0 $\pm$ 1.4
<b>Alcohol</b>		
g/d	0 $\pm$ 0	0 $\pm$ 0
%	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

**Table 2. Isoflavone Content of Study Diets (mean of seven 1-day 2,000-kcal composites)**

Isoflavone (mg/d)	Control	Test
Daidzin	—	16.8 $\pm$ 2.3
Genistin	—	33.7 $\pm$ 4.7
Glycitin	—	3.7 $\pm$ 0.8
Malonyl daidzin	—	11.3 $\pm$ 3.6
Malonyl genistin	—	13.4 $\pm$ 6.0
Malonyl glycitin	—	0.6 $\pm$ 0.6
Daidzein	—	3.3 $\pm$ 0.6
Genistein	—	1.6 $\pm$ 0.8
Glycitein	—	1.5 $\pm$ 0.7
<b>Total daily intake</b>	0.0	86.0 $\pm$ 17.1

and legume dishes as breakfast cereals, soups, and main dishes. The fatty acid profile and dietary cholesterol intake on both diets were balanced by inclusion of butter and whole eggs on the test diet (eg, 1 egg per week on a 2,000-kcal/d diet). Test food items used in this study were all readily available and were obtained from either a supermarket (Too Good To Be True; Loblaw Brands, Toronto, Ontario; and Yves Veggie Cuisine, Vancouver, British Columbia, Canada) or a health food store (MGM Products, Cedar Lake, MI; and Fantastic Foods, Petaluma, CA).

We have reported the details of the dietary and analytical methodology previously.<sup>27</sup> The diets were balanced for fatty acids and plant sterols, since these might alter serum lipids and antioxidant status (Table 3). At each clinic visit, the dietitian assessed compliance using the menus. Subjects were asked to weigh all foods and to check them against the menu plan when eaten. Additional items were noted in a blank column opposite the prescribed diet. These data were used to calculate the dietary intake (Table 1). Body weight was measured at each clinic visit, and the results were used to adjust the total caloric intake. Complete diets were packed at a central location and delivered weekly by courier to each subject's home at a time convenient to them.

## Analyses

Serum stored at  $-70^{\circ}\text{C}$  was analyzed in a single batch for total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol after magnesium chloride precipitation with an automated clinical chemistry analyzer (CH1000; Technicon, Tarrytown, NY) by techniques of the Lipid Research Clinics. The LDL cholesterol level was calculated. These data are reported elsewhere in detail.<sup>27</sup> For direct assessment of LDL oxidation, LDL particles were isolated by precipitation with buffered heparin at their isoelectric point (pH 5.05).<sup>28</sup> The LDL precipitate was centrifuged at  $1,000 \times g$  and resuspended in saline. LDL cholesterol was estimated enzymatically<sup>29</sup> on an aliquot of the saline resuspension using a commercial cholesterol assay kit (Sigma Chemical, St Louis, MO). On a further aliquot, LDL oxidation was estimated as conjugated dienes in LDL fatty acids. Lipids from the resuspended LDL were extracted with chloroform:methanol (2:1), dried under nitrogen, dissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm using a molar extinction coefficient of  $29,500 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$  for conjugated dienes.<sup>30</sup> Oxidized LDL was expressed as total LDL conjugated dienes (micromoles per liter of serum) and as the ratio of conjugated dienes (micromoles) per 1 mmol LDL cholesterol.<sup>30</sup> The coefficient of variation for this assay on 6 replicates was 2.5% for conjugated dienes.

We assessed estrogenic and androgenic activity in an ex vivo human tissue culture system using immunoassays for pS2 and prostate-specific antigen (PSA) produced after stimulation with the test materials.<sup>31-34</sup> BT-474 breast cancer cells were grown to confluence and then subcultured in 24-well microtiter plates. Once they were confluent in

**Table 3. Mean Percentage Fatty Acid Composition and Mean Daily Intake of B-Carotene and Phytosterols From Analysis of the Seven-Day Composites of Test and Control Metabolic Diets**

Parameter	Control	Test
Fatty acid (%)		
SFA		
10:0	0.4	0.3
12:0	0.1	0.0
14:0	2.9	2.6
16:0	15.7	16.2
18:0	5.4	5.6
20:0	0.5	0.5
22:0	0.3	0.7
24:0	0.2	0.3
Total	25.5	26.2
MUFA		
14:1n5	0.2	0.2
16:1n7	0.5	0.6
18:1n9	35.2	32.4
18:1n7	1.6	1.5
20:1n9	0.7	0.6
22:1n9	0.3	0.1
Total	38.6	35.5
PUFA		
18:2n6	32.0	33.5
18:3n6	0.1	0.1
18:3n3	3.8	4.7
Total	35.9	38.3
Beta-carotene ( $\mu\text{g/d}$ )	1,484	1,359
Phytosterols (mg/d)		
Campesterol	87	67
Stigmasterol	14	31
Sitosterol	208	224
Total	310	322
Animal sterols (mg/d)		
Cholesterol	50	64
Total	50	64

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

the wells, the cells were stimulated with urine diluted 1:1,000 or estradiol or dihydrotestosterone (DHT) at  $10^{-7}$  mol/L, the latter two serving as standards. Anhydrous ethanol was used as a negative control. The plates were incubated for 7 days, and estrogenic activity was measured in the supernatant as the pS2 concentration using an immunoradiometric assay (CIS Bio, Gif-Sur-Yvette, Cedex, France). pS2 is an estrogen-regulated protein that is demonstrated in the literature to be a good marker for estrogenic activity.<sup>31-34</sup> Androgenic activity was measured using an enzyme-linked immunosorbent assay for PSA, an androgen-regulated protein.<sup>31-34</sup> The procedure for this assay has been described in detail elsewhere.<sup>31-34</sup> pS2 and PSA concentrations were converted to equivalents of estradiol and DHT, respectively, using pS2 and PSA values obtained in the same assay for the steroid standards. Final values were expressed in picomoles.

Dietary isoflavonoid levels were measured in freeze-dried 24-hour composites of the 7-day test and control diets by high-performance liquid chromatography (HPLC)<sup>25,26</sup> using a 600E multisolvent delivery system with a photodiode array detector monitoring at 200 to 350 nm (Waters, Marlborough, MA) and a YMC-pack ODS-AM 303 column (5  $\mu\text{m}$ , 250 mm  $\times$  4.6 mm ID; YMC, Wilmington, NC) equipped with an AM direct-connect C18 guard column. Appropriate isoflavone standards were analyzed (Table 2). Biochanin A was used as an internal

standard with recovery values of 80% to 100%. Urinary isoflavone levels were measured by HPLC after acid hydrolysis.<sup>36</sup> Chromatographs were obtained from the 3-dimensional array using a photodiode array detector at 258 nm to allow an assessment of the common regions of relatively high absorbance for daidzein, genistein, and the added recovery standard, flavone.

#### Statistical Analysis

The results are expressed as the mean  $\pm$  SE. The weight change is expressed as kilograms per month. The percentage treatment differences between the endpoint values for both diets were calculated for each subject, and the data were assessed by Student's *t* test (2-tailed) for paired data. A 2-sample *t* test was used for comparison of treatment effects between subgroups. The absolute difference between treatments was assessed using the General Linear Model procedure and SAS software (PROC GLM/SAS)<sup>37</sup> with the end-of-treatment value as the response variable and the following main effects: diet, sex, treatment order (sequence), diet  $\times$  sex, sex  $\times$  sequence, a random term representing the subject nested within the sex  $\times$  sequence interaction, and the baseline value as a covariate where measured. Pearson correlation coefficients were used to assess the significance of linear associations.<sup>37</sup> A subject group of 30 allowed detection of a 5% treatment difference in conjugated dienes in the LDL fraction (assuming a 10% SD of the effect) and a 28% difference in urinary ex vivo estrogenic activity (assuming a 52% SD of the effect; with  $\alpha = .05$  and  $\beta = 0.8$ ).<sup>37</sup>

## RESULTS

Of 31 subjects, 16 received the test diet first. The diets were well accepted and compliance was good. On the test diet, subjects consumed  $95\% \pm 7\%$  of the calories provided. The respective control figure was  $96\% \pm 6\%$ . The test diet provided 86 mg isoflavones per 2,000-kcal diet daily, with no isoflavones detectable in the control diet (Table 2). There was a significant weight gain over the 1 month of the control diet ( $0.2 \pm 0.1$  kg,  $P = .043$ ) and a nonsignificant weight loss over the 1 month of the test diet ( $0.1 \pm 0.2$  kg,  $P = .484$ ). The treatment difference approached significance ( $0.3 \pm 0.2$  kg,  $P = .069$ ).

#### Urinary Isoflavone Excretion

The 24-hour urinary volume on the test diet ( $1.84 \pm 0.17$  L) and control diet ( $2.01 \pm 0.14$  L) was similar. On the test diet,  $0.8 \pm 0.2$  mg/d genistein and  $3.0 \pm 0.6$  mg/d daidzein were excreted in the urine. On the control diet, no isoflavonoids were excreted in the urine except for very low levels of daidzein in one woman (0.1 mg/d) who had the highest levels on the test diet (5.3 mg/d).

#### Blood Lipids and Oxidized LDL

There were no significant differences in pretreatment values for blood lipids between the test and control diets (Table 4). Mean blood lipid concentrations, in general, were lower on the test diet compared with the control for weeks 2 and 4. These data are reported in detail elsewhere.<sup>27</sup> Using the mean of weeks 2 and 4, the percentage difference assessed by paired *t* test between the two dietary treatments demonstrated lower mean test values for oxidized LDL assessed as conjugated dienes ( $-10.8\% \pm 2.9\%$ ,  $P < .001$ ). The percentage treatment differences at week 4 were not significantly different from those found at week 2. The significance of the effect of diet on serum

Table 4. Body Weight and Serum and Urinary Data (mean  $\pm$  SE) on Test and Control Metabolic Periods (N = 31)

Parameter	Control		Test		Mean Treatment Difference	P*
	Baseline (wk 0)	Mean Treatment (mean of wk 2 and 4)	Baseline (wk 0)	Mean Treatment (mean of wk 2 and 4)		
Body weight (kg)†	67.9 $\pm$ 2.0	68.1 $\pm$ 2.0	68.2 $\pm$ 2.0	68.0 $\pm$ 1.9	-0.1 $\pm$ 0.3	.895
Serum data						
Cholesterol (mmol/L)						
Total	6.42 $\pm$ 0.17	6.16 $\pm$ 0.13	6.48 $\pm$ 0.16	5.78 $\pm$ 0.14	-0.38 $\pm$ 0.07	<.001
LDL	4.37 $\pm$ 0.15	4.13 $\pm$ 0.10	4.40 $\pm$ 0.15	3.85 $\pm$ 0.12	-0.27 $\pm$ 0.07	<.001
HDL	1.24 $\pm$ 0.06	1.17 $\pm$ 0.05	1.26 $\pm$ 0.06	1.17 $\pm$ 0.05	-0.01 $\pm$ 0.02	.915
Triglycerides (mmol/L)	1.78 $\pm$ 0.14	1.94 $\pm$ 0.15	1.79 $\pm$ 0.13	1.67 $\pm$ 0.12	-0.26 $\pm$ 0.08	.068
Oxidized LDL						
Conjugated dienes (pmol/L)	62 $\pm$ 3	63 $\pm$ 3	67 $\pm$ 3	56 $\pm$ 3	-7 $\pm$ 2	<.001
Conjugated dienes:LDL	14.6 $\pm$ 0.8	15.7 $\pm$ 0.9	15.7 $\pm$ 0.8	15.0 $\pm$ 1.0	-0.7 $\pm$ 0.4	.032
Urine data†						
Output (L/d)		2.01 $\pm$ 0.14		1.84 $\pm$ 0.17	-0.16 $\pm$ 0.14	.258
Creatinine (mmol/d)		9.83 $\pm$ 0.56		9.80 $\pm$ 0.55	-0.03 $\pm$ 0.44	.941
Sex hormone equivalent (pmol/d)						
Total estrogen equivalents		45.13 $\pm$ 5.20		45.10 $\pm$ 7.20	-0.03 $\pm$ 4.92	.996
Total androgen equivalents		1.02 $\pm$ 0.53		0.61 $\pm$ 0.27	-0.41 $\pm$ 0.56	.472
Sex hormone equivalents, creatinine-corrected						
Estrogen		5.32 $\pm$ 0.88		4.63 $\pm$ 0.60	-0.69 $\pm$ 0.60	.259
Androgen		0.10 $\pm$ 0.06		0.06 $\pm$ 0.03	-0.04 $\pm$ 0.06	.536

NOTE. To convert cholesterol and triglycerides to mg/dL, multiply by 38.67 and 88.57, respectively. These values have been reported previously.<sup>27</sup>

\*Significance of the diet effect using the General Linear Model in SAS.<sup>37</sup>

†Mean treatment data are week 4 values.

lipids was confirmed by the General Linear Model procedure (conjugated dienes,  $P < .001$ ; Table 4). In addition to the absolute concentration of oxidized LDL, the ratio of conjugated dienes to cholesterol in the LDL fraction was also reduced, but to a lesser extent ( $15.0 \pm 1.0$  v  $15.7 \pm 0.9$ ,  $P = .032$ ).

There were no treatment differences between the sexes in the response to diet or between the 9 subjects using vitamin E supplements and the rest of the group, although the mean values for conjugated dienes in the LDL fraction and the ratio of conjugated dienes to LDL cholesterol were lower at all points during the study in the subjects using vitamin E (Fig 1).

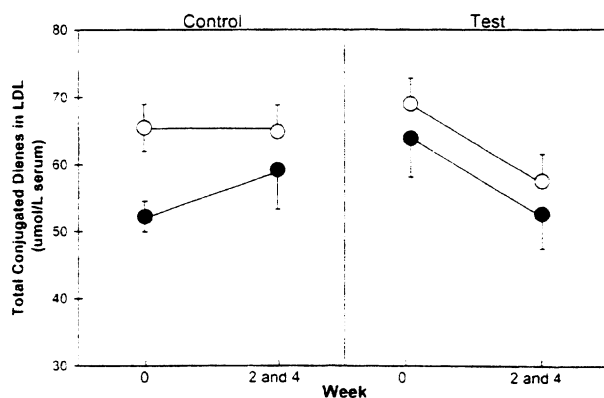


Fig 1. Total conjugated dienes in the LDL fraction ( $\mu\text{mol/L}$ ) on the test and control diets in subjects with vitamin E supplementation (●, n = 9) or without (○, n = 21).

#### Ex Vivo Sex Hormone Receptor Activity

No significant difference was found between treatments in urinary sex hormone receptor activity either before or after creatinine adjustment, although the mean values on the test diet tended to be lower than the control diet (Fig 2). On the control diet, urinary estrogen equivalents were higher for women than men after creatinine correction ( $7.5 \pm 2.0$  v  $3.9 \pm 0.6$ ,  $P = .045$ ). This difference between the sexes was no longer significant on the test, due to a reduction in estrogen levels in women (Fig 2).

#### DISCUSSION

Our data indicate that readily available soy foods providing modest levels of soy protein reduce the indices of oxidized LDL without increasing urinary estrogen activity. This study supports previous reports that soy isoflavone consumption may protect LDL cholesterol from copper-mediated oxidative damage in vitro.<sup>9-10</sup> However, in previous studies, no direct measurements were reported on unmodified LDL.<sup>9-10</sup> This study therefore demonstrates an additional cardioprotective effect of soy in reducing oxidized LDL cholesterol levels at a dose of soy protein that reduces serum cholesterol and would qualify for an FDA cardiovascular disease risk reduction health claim. These effects were achieved with no increase in urinary estrogen activity. Our data therefore do not support prior concerns about the possible increased breast cancer risk in women or altered sexual development in children consuming soy at these levels.<sup>18-21</sup>

Oxidized LDL is more readily taken up by the macrophages of the scavenger system in the arterial wall and may contribute to plaque formation.<sup>18-39</sup> The consumption of antioxidant flavo-

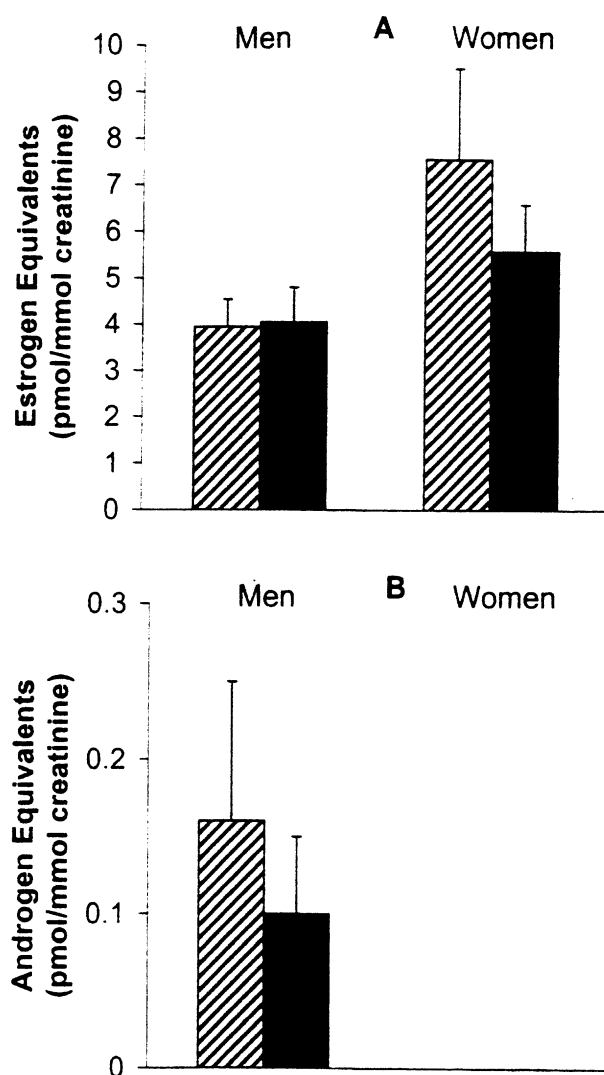


Fig 2. Creatinine-corrected urinary estrogen (A) and androgen (B) equivalents (pmol/mmol) in men ( $n = 19$ ) and postmenopausal women ( $n = 12$ ) on test (■) and control (▨) diets.

noids in tea, fruit and vegetables, lycopene in tomato products, and vitamin E as a supplement have all been associated with a reduced risk of coronary heart disease.<sup>40-43</sup> Vitamin E and lycopene have powerful antioxidant properties, reducing LDL oxidation and oxidative damage to plasma proteins.<sup>42,43</sup>

In the present study, the effect of soy in reducing the oxidized LDL concentration was observed even in subjects on vitamin E supplementation, in whom the level of LDL conjugated dienes was already reduced (Fig 1). The soy antioxidant effect appeared to be additive to that of vitamin E. In this respect, it is of interest that studies of subjects with established cardiovascular disease who were fed soy as part of the intervention demonstrated angiographic improvement in coronary arterial diameter compared with control subjects.<sup>44</sup>

Previous studies suggesting a protective effect of soy isoflavonoids on LDL oxidation assessed the susceptibility of LDL to copper- or endothelial cell-induced oxidation *in vitro*.<sup>9-11,45</sup> However, caution may be needed in the interpretation of such

data, due to the lack of direct evidence that copper-induced oxidizability is relevant to oxidative resistance *in vivo*.<sup>9</sup> We believe our data provide this evidence, since we measured conjugated dienes directly in the LDL fraction from serum obtained from our subjects without *in vitro* incubation with copper or other free-radical-generating systems. The conjugated dienes therefore represent the steady-state level of circulating oxidized LDL.

LDL concentrations were reduced on the soy-containing diets.<sup>27</sup> Both the amino acid composition of soy proteins and their isoflavone content have been suggested to be responsible for the cholesterol-lowering action of soy,<sup>5,6,46-48</sup> although not all studies have reported an isoflavone effect.<sup>49,50</sup> Soy contains many other potentially active components, including saponins, plant sterols, and polyunsaturated fatty acids, all of which may contribute to cholesterol reduction. In the present study, the lipids and sterols were separated from the soy protein isolate used in the majority of the soy products consumed. Soy fatty acids and sterols were therefore unlikely to play a part in the lipid changes,<sup>27</sup> as confirmed by the similarity of test and control values derived from the analysis of 24-hour dietary composites.

Comparatively few studies have focused on the lipid-lowering and antioxidant properties of other vegetable proteins. However, yeast protein (quorn) also appears to be hypocholesterolemic,<sup>51</sup> while gluten in high-fiber bread has been associated with reduced serum triglyceride.<sup>52</sup> In rabbits, gluten has been shown to be protective in atherogenic diets.<sup>53</sup>

It is well recognized that plant-derived sex hormone analogs may have important physiological effects.<sup>54,55</sup> However, despite the high plasma levels which may be achieved, compared with endogenous hormones, isoflavones have relatively low potency. Of even greater importance, they may act both as potential agonists and antagonists.<sup>1</sup> Evidence for their blocking action includes studies in which soy isoflavones fed to young women resulted in significant lengthening of the menstrual cycle.<sup>54</sup> In our study, the ratio of dietary isoflavones to soy protein of 2.6 mg/g was associated with a relatively low urinary output of genistein and daidzein<sup>56</sup> and a tendency, on soy, for a reduction in *ex vivo* sex hormone activity in the urine. These data indicate that major hormonal changes may not be found at modest levels of soy protein intake that reduce the risk factors for cardiovascular disease.<sup>27</sup> Therefore, in relation to breast cancer,<sup>20,57</sup> the concerns about unwanted increases in estrogenic activity at moderate levels of soy intake appear unwarranted. Conversely, the tendency for sex hormone activity to be depressed on soy diets is in line with current strategies to treat and possibly prevent hormone-dependent cancers with sex hormone-blocking agents.<sup>58,59</sup>

We conclude that a moderate intake of soy foods reduces the concentration of oxidized LDL cholesterol, possibly due to the increased consumption of isoflavonoids associated with soy protein. These changes were achieved without a significant alteration in urinary *ex vivo* hormone activity, which has been a major concern for those who predict potentially harmful effects from soy food consumption. Currently, soy consumption is advocated based on its cholesterol-lowering ability alone.<sup>5</sup> However, in view of the apparent success of antioxidant agents

in preventing experimental arteriosclerosis<sup>38</sup> and the effect of dietary antioxidants in reducing the risk of cardiovascular disease in cohort studies.<sup>40,41</sup> the antioxidant effect of soy may add to its potential value in coronary heart disease risk reduction with no apparent risk of adverse effects.

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