Effects of oral soy protein on markers of inflammation in postmenopausal women with mild hypercholesterolemia

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Background Nitric oxide (NO) may protect arteries against atherosclerosis, as suggested by experimental studies. Estrogen therapy enhances the bioactivity of NO in the vasculature of healthy postmenopausal women, but is not acceptable for long-term use by many women. Observational studies have demonstrated beneficial cardiovascular effects of soy protein in premenopausal and postmenopausal women. We examined whether the consumption of isolated soy protein may improve markers of vascular inflammation in postmenopausal women with hypercholesterolemia.

Methods and Results In a randomized, double-blind, placebo-controlled, crossover study, 24 postmenopausal women with hypercholesterolemia received 25 g of soy protein or a placebo daily for 6 weeks, with treatment periods separated by 1 month. Markers of vascular inflammation were measured by enzyme-linked immunosorbent assay methods, including: soluble interleukin-2 receptor (sIL-2r), E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).

There was no effect of soy protein in comparison with placebo on the inflammatory markers: the sIL-2r level was $942.2 \pm 335.3$ pg/mL with soy protein and $868.5 \pm 226.9$ pg/mL with placebo ($P = .311$); E-selectin was $39.6 \pm 16.5$ ng/mL with soy protein and $42.1 \pm 17.6$ ng/mL with placebo ($P = .323$); P-selectin was $157.9 \pm 67.9$ ng/mL with soy protein and $157.5 \pm 47.6$ ng/mL with placebo ($P = .977$); ICAM-1 was $266.0 \pm 81.3$ ng/mL with soy protein and $252.5 \pm 82.7$ ng/mL with placebo ($P = .435$); VCAM-1 was $402.7 \pm 102.1$ ng/mL with soy protein and $416.4 \pm 114.8$ ng/mL with placebo ($P = .53$).

Conclusions Consumption of 25 g of isolated soy protein daily for 6 weeks does not substantially affect markers of vascular inflammation in postmenopausal women with hypercholesterolemia. (Am Heart J 2003;145:e7.)

Nitric oxide (NO), an important regulator of vascular homeostasis, is synthesized continuously in endothelial cells by the constitutive enzyme nitric oxide synthase (NOS III) from the substrate L-arginine. NO plays an important role in vascular inflammation, and thus, affecting NO bioavailability may change vascular inflammation and the progression to atherosclerosis.

Many observational studies have found lower rates of coronary heart disease events in postmenopausal women who take hormone replacement therapy than in women not receiving this therapy. In the Lipid Research Clinics Program Follow Up Study, there was a 66% lower relative risk of death from cardiovascular disease in women who took hormone replacement than in women who did not. In the Nurses' Health Study, 48,470 postmenopausal women were followed for as long as 10 years. There was a 44% lower risk for fatal and nonfatal cardiovascular events in women currently taking estrogen. Estrogen significantly reduced levels of cell adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) relative to respective pretreatment values, with the greatest effect noted with E-selectin. However, during an average follow-up period of 4.1 years of the Heart and Estrogen-
gave written informed consent. Participants were the protein source and iso
of total milk protein (Protein Technologies International), with each treatment period lasting 6 weeks and separated by 1 month in which the patients were off treatment. The only differences between the 2 products that were given for the study and consumed a nitrate-restricted diet 3 days before each visit and measurement. Six women stopped the study in the first few days (4 women dropped out while they were taking the placebo and 2 women dropped out while they were taking soy protein) because of bad taste and nausea caused by the powder. Thus, 24 women completed both phases of the study and served as the source of data for this report.

Methods

Women were randomly assigned dietary treatments of either 25 g of isolated isoflavones (Protein Technologies International, St Louis, Mo) or 25 g of total milk protein (Protein Technologies International), with each treatment period lasting 6 weeks and separated by 1 month in which the patients were off treatment. The only differences between the 2 products were the protein source and isoflavone content. All study participants returned to the hospital for blood drawing and brachial artery reactivity measurements at the end of each treatment period. Subjects were placed on a nitrate-restricted diet (as high as 15 mg/day) for 3 days before each visit to reduce the contribution of dietary nitrates to serum nitrogen oxide levels. The study was approved by the institutional review board of the hospital, and all participants were compliant and took all the samples at each visit. Plasma and serum were separated by centrifugation and stored at −80°C until analysis. SIL-2R, E-selectin, P-selectin, ICAM-1, and VCAM-1 levels were measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, Minn).

Laboratory assays

Blood samples for laboratory assays were obtained between 12 and 1 pm, after patients had a light breakfast, and the samples were immediately coded so that investigators performing laboratory assays would be blinded to subject identity or study sequence. Plasma and serum were separated by centrifugation and stored at −80°C until analysis. IL-2R, E-selectin, P-selectin, ICAM-1, and VCAM-1 levels were measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, Minn).

Results

Thirty postmenopausal women with hypercholesterolemia (age 55 ± 5 years) who had not taken hormone replacement, antioxidant vitamins, or lipid-lowering therapies in the preceding 2 months and who had low-density lipoprotein (LDL) cholesterol levels >130 mg/dL were enrolled in the study. All had plasma 17β-estradiol levels <50 pg/mL and follicle stimulating hormone levels >50 pg/mL. No subject had hypertension or diabetes mellitus or was a current cigarette smoker. The baseline lipid profile levels were, total cholesterol 270.45 ± 31.87 mg/dL, LDL cholesterol 178.5 ± 28.5 mg/dL, high-density lipoprotein (HDL) cholesterol 60.42 ± 17.78 mg/dL, and triglyceride 133.2 ± 49.55 mg/dL. Treatment with all nonsteroidal anti-inflammatory agents (including aspirin) was discontinued during the study. Twenty-four study participants were compliant and took all the sachets that were given for the study and consumed a nitrate-restricted diet 3 days before each visit and measurement. Six women stopped the study in the first few days (4 women dropped out while they were taking the placebo and 2 women dropped out while they were taking soy protein) because of bad taste and nausea caused by the powder. Thus, 24 women completed both phases of the study and served as the source of data for this report.

Effects of soy protein on vascular inflammatory markers

There was no effect of soy protein in comparison with the placebo on the vascular inflammatory markers that were examined. The cell adhesion molecules (CAMs) that were studied are NO-dependent. The sIL-2r level was 942.2 ± 50 pg/mL with soy protein and 868.5 ± 226.9 pg/mL with placebo (P = .311); the E-selectin level was 39.6 ± 16.5 ng/mL with soy protein and 42.1 ± 17.6 ng/mL with placebo (P = .323); the P-selectin level was 157.9 ± 67.9 ng/mL with soy protein and 157.5 ± 47.6 ng/mL with placebo (P = .977); the ICAM-1 level was 266.0 ± 81.3 ng/mL on soy protein and 252.5 ± 82.7 ng/mL with placebo (P = .435); the VCAM-1 level was 402.7 ± 102.1 ng/mL with soy protein and 416.4 ± 114.8 ng/mL with placebo (P = .53) (Table I).
Effects of soy protein on cholesterol level

Soy protein and the placebo (milk protein) both decreased the total cholesterol level: from 270.45 ± 31.87 mg/dL to 241.52 ± 35.99 mg/dL and 239 ± 32.08 mg/dL, respectively (P < .001). Both soy protein and the placebo decreased the LDL cholesterol level: from 178.5 ± 28.50 mg/dL to 143.23 ± 29.79 mg/dL and 138.0 ± 28.77 mg/dL, respectively (P < .0001). There was no statistically significant difference between total- and LDL-cholesterol levels after 6 weeks of soy protein or placebo. High-density lipoprotein (HDL) cholesterol levels were not statistically changed with soy protein or the placebo: from 60.41 ± 17.78 mg/dL to 59.31 ± 12.99 mg/dL and 61.81 ± 15.13 mg/L, respectively (P = not significant).

Discussion

The intake of soy protein (25 g daily for 6 weeks) did not affect markers of vascular inflammation in 24 postmenopausal women with hypercholesterolemia, compared with a placebo (milk protein). We focused on isolated soy protein containing naturally occurring isoflavones because of observational, epidemiologic, population-based studies that have demonstrated cardiac protection and antiatherogenic effects resulting from soybean isoflavones. “Dietary estrogens” are absorbed from the intestinal tract, transported to the liver, and undergo an enterohepatic circulation. The mechanisms through which isoflavones may exert the aforementioned effects seem to depend on their estrogen agonist/antagonist properties. Soy protein in the amount that we used (25 grams daily) has been shown to reduce total and LDL cholesterol levels in subjects with hypercholesterolemia. On the basis of these data, we reasoned that soy protein could have the same effects as estrogens on vascular inflammation in postmenopausal women. However, our results did not demonstrate anti-inflammatory effects. Our results could support the data presented in the HERS trial, with no demonstrated beneficial effects of estrogens given to postmenopausal women. Several groups have reported increases in NO synthase activity in endothelial cell cultures after incubation with physiologic concentrations of estradiol, an effect blocked by estrogen receptor inhibitors. Increased NO bioactivity from estrogen administration may improve other important homeostatic properties of endothelium, such as inhibition of activation of proinflammatory genes. NO has been found to inhibit the activation of an important proinflammatory nuclear transcription factor, NFκB. In the presence of reduced cytosolic NO or increased cytosolic oxidant stress, NFκB is activated by dissociation from its inhibitor subunit (IκB) after IκB phosphorylation in the cytosol. The heterodimer then translocates to the nucleus, where it binds to promo-

| Table I. Effects of soy protein on markers of inflammation |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | **After soy protein** | **After placebo** | **P value** |
| **slr-2R (pg/ml)**             | 942.22 ± 335.32  | 868.54 ± 226.96  | .311          |
| **E-selectin (ng/ml)**         | 39.62 ± 16.53   | 42.08 ± 17.59   | .323          |
| **P-selectin (ng/ml)**         | 157.91 ± 67.90  | 157.52 ± 47.68  | .977          |
| **ICAM-1 (ng/ml)**             | 266.04 ± 81.27  | 252.50 ± 82.73  | .435          |
| **VCAM-1 (ng/ml)**             | 402.70 ± 102.09 | 416.45 ± 114.81 | .53           |

slr-2R, Soluble interleukin-2 receptor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

tor regions of several proinflammatory genes, with transcription and synthesis of protein mediators of inflammation, including cytokines, chemokines, and cell adhesion molecules.

The pathophysiologic relevance of soluble cell adhesion molecules measured in human sera has been suggested by their localization in atherosclerotic plaques, their higher levels in patients with atherosclerosis compared with control subjects, and their association with increased risk of myocardial infarction in apparently healthy subjects. Serum concentrations of E-selectin, ICAM-1, and VCAM-1 were reported to be higher in postmenopausal women with coronary artery disease who were not taking hormone therapy than in postmenopausal women with coronary artery disease who were taking hormone therapy at the time of cardiac catheterization. It has been reported that estrogen significantly reduced levels of the cell adhesion molecules E-selectin, ICAM-1, and VCAM-1 compared with respective pretreatment values, with the greatest effect noted with E-selectin, the cell adhesion molecule specific to the activated endothelium. It has been demonstrated that raloxifene (a selective estrogen receptor modulator) also significantly reduced levels of E-selectin and ICAM-1 relative to placebo values, but had no effect on VCAM-1.

Study limitations

A possible explanation for failure to demonstrate an effect of soy protein is that the dose used in our study (25 g daily for 6 weeks) was insufficient. However, we did not want to use higher doses, because we wanted to prevent any possible adverse effects, and several studies (including this study) have reported an effect on the lipid profile with that amount.

Levels of asymmetric dimethylarginine were not measured in the plasma of our participants. Thus, it is possible that had levels of asymmetric dimethylarginine been found to be low in our patients, improvement in markers of vascular inflammation with soy protein would have been expected.

The sample size was small, and with a sample size of 24 in a crossover design study and continuous out-
comes, power calculations can determine the minimal detectable effect with 80% power. This effect is expressed as a standardized effect size (Cohen’s d), so it can be extrapolated across te various outcomes. A much larger study would have found differences of this magnitude to be statistically significant.

Summary
We found that soy protein did not affect markers of vascular inflammation in postmenopausal women with mild hypercholesterolemia. Further and longer studies with different doses of soy protein should be conducted in a double-blind, placebo-controlled manner and in different populations.

References