Clin. Cardiol. 24, 711–716 (2001)

Soy Protein Diet Significantly Improves Endothelial Function and Lipid Parameters

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Summary

Background: Replacement of animal protein with soy protein in the diet is associated with decreased cholesterol levels. However, the effects of soy protein diet on endothelial function are not well known.

Hypothesis: The aim of the study was to investigate the effects of soy protein diet on plasma lipids and endothelial function parameters assessed by two different methods.

Methods: Twenty hypercholesterolemic, nonsmoker male patients (age 50.1 ± 11.8 years), with a normal body mass index, were included. After calculating their daily requirements, a diet with 25–30% of energy from fats, 10–12% from proteins, and the rest from carbohydrates was instituted. Sixty percent of the animal source proteins of the diet were substituted by soy. The anthropometric measures, lipid parameters, and endothelial functions of the subjects were assessed at baseline and 6 weeks after soy protein diet. Flow-mediated endothelium-dependent dilatation (EDD) and plasma thrombomodulin (TM) levels were evaluated as endothelial function parameters.

Results: After diet, plasma total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, and triglyceride levels decreased significantly ($p<0.001$, $p<0.001$, $p=0.039$, and $p = 0.001$, respectively). The mean plasma TM levels were also significantly reduced with diet ($p = 0.004$). Studies of the brachial artery indicated a borderline dilatation in baseline brachial artery diameter ($p = 0.05$), however the diameter at reactive hyperemia was significantly larger after diet ($p < 0.001$), resulting in a significant improvement of EDD ($p = 0.002$).

Conclusion: Soy protein diet significantly improves plasma lipid profile in patients with hypercholesterolemia. Furthermore, the endothelial function, as judged by two different methods (EDD and plasma TM levels), also improves with soy protein diet.

Key words: soy protein, hypercholesterolemia, endothelial function, thrombomodulin, endothelium-dependent dilatation

Introduction

Endothelial dysfunction is the initial event in atherogenesis preceding plaque formation. The presence of endothelial dysfunction has been associated with coronary risk factor indices including hypercholesterolemia, cigarette smoking, and hypertension. Lipid-lowering drugs, estrogen replacement therapy, and angiotensin-converting enzyme inhibitors have been demonstrated to improve endothelial function.[1–3] Arterial endothelial function can be assessed noninvasively in the brachial artery with high-frequency ultrasound, which measures the endothelium-dependent dilatation (EDD) in response to increased blood flow.[4] Another noninvasive endothelial marker is thrombomodulin (TM), a cell surface glycoprotein located at the luminal surface of vascular endothelium.[5, 6] A soluble form of TM exists in circulating plasma as heterogeneous fragments and appears to be derived from injured endothelial cells.[7] High-serum TM concentrations reflecting endothelial injury have been previously reported in uremic patients during hemodialysis,[8] patients with orthotopic liver transplantation,[9] and in renal transplant recipients.[10]

Replacement of animal protein in the diet with plant protein is associated with a lower risk of coronary artery disease.[11] This effect is thought to reflect the decrease in serum cholesterol concentrations. Over the past 20 years, there have been a number of reports of cholesterol lowering after ingestion of soy protein in humans;[12] however, the effects of soy protein diet on endothelial function are not well known.

This study in part has been presented orally at the XXII Congress of the European Society of Cardiology, Amsterdam, The Netherlands

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Received: October 19, 2000
Accepted: February 22, 2001

The aim of the study was to evaluate the effects of soy protein diet on plasma lipids as well as on endothelial function parameters assessed by two different methods (EDD and plasma soluble TM levels).

Methods

Subjects

Twenty hypercholesterolemic, nonsmoker male patients with a normal body mass index (BMI) were included in the study. Criteria for enrollment included a fasting plasma total cholesterol (T-C) concentration > 230 mg/dl, a fasting plasma low-density lipoprotein cholesterol (LDL-C) concentration > 160 mg/dl despite the use of the National Cholesterol Education Program/American Heart Association (NCEP/AHA) Step 1 diet[13] for at least 6 months. All subjects were carefully screened to exclude those taking cholesterol-lowering medications, consuming alcohol or special diets, and those with chronic illnesses including gastrointestinal problems or diabetes. The anthropometric measures including weight, height, BMI, and skin fold thickness were noted for each patient before and after 6 weeks of soy protein diet.

Properties of the Diet

Before the initiation of the soy protein diet, all subjects completed a 3-day (including 1 weekend day) diary at home. The diaries were reviewed with a dietitian to determine the subject's energy intake, eating habits, and to check the compliance with the previous diet recommendations. The food taken was noted in the forms of animal/plant protein, saturated/monounsaturated/polyunsaturated fat, as well as milligram cholesterol consumption.

Daily physical activity of the subject was also noted on 3 consecutive days. Before the soy diet was initiated, the subjects' daily energy requirements were calculated depending on daily physical activity and the basal metabolic rate calculated with the Harris-Benedict formula (66.5 + (13.75 × body mass) + (5.03 × height) − (6.75 × age)). In this diet, 25–30% of energy was from fats, 10–12% from proteins, and the rest from carbohydrates. Sixty percent of the animal source proteins of the diet were replaced by soy protein, so that the soy protein constituted 30% of the total protein intake. The soy products were provided in the form of soy flour, soybeans, and soy bean sprouts.

Blood Samples and Laboratory Determinations

After at least 12 h of fasting, blood samples were drawn from a large antecubital vein without interruption of venous flow before inclusion and at Week 6 of the soy protein diet. The concentrations of plasma T-C, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were measured with enzymatic colorimetric tests (Boehringer Mannheim kits, Mannheim, Germany; Hitachi autoanalyzer). Plasma levels of apolipoprotein A1, apolipoprotein B, and lipoprotein (a) were measured with the rate nephelometric method using Beckman Immage Immunochemistry systems (Beckman Coulter, Inc. Fullerton, Calif., USA). Plasma thrombomodulin levels were measured by two-site enzyme-linked immunosorbent assay (ELISA) with two monoclonal antihuman TM antibodies (ELISA, Asserachrom Thrombomodulin, Diagnostica Stago, France). The intra- and interassay coefficients of variation for TM measurement were 5.2 and 6.5%, respectively.

In all tests, the laboratory technicians were blinded to the clinical status of the patient.

Brachial Artery Studies

Brachial artery ultrasound studies were performed in a quiet, temperature-controlled room with the subjects in the resting, supine position after having abstained from alcohol, caffeine, tobacco, and food for 12 h before the study. Studies were performed using a Toshiba SS 250-A (Japan) ultrasound machine and a high resolution (7.5 MHz) linear array transducer by a single, highly skilled sonographer. The right brachial artery was imaged at a location of 3–7 cm above the antecubital crease with the probe positioned at an angle of 70°. After obtaining adequate images, the arm was marked and kept in constant position for the rest of the study. First, baseline brachial artery diameter was measured. Then, a blood pressure cuff was inflated on the proximal portion of the arm to 250–300 mmHg and kept for 5 min. Blood flow was increased through the brachial artery after deflation of the cuff. Brachial artery diameter was measured after deflation in the first 15 s during reactive hyperemia. Flow-mediated EDD was calculated as the % increase in diameter at reactive hyperemia compared with baseline diameter.

Statistics

The Statistical Package for the Social Science (SPSS 9.1 version for Windows, SPSS Inc., Chicago, Ill., USA) was employed for statistical analysis. Student's paired *t*-test was used for within-group comparisons of the parameters at baseline with that at 6 weeks. Values were expressed as mean ± standard deviation (SD) or percentages as appropriate. Relations between variables were analyzed using Pearson correlation analysis. Percentages of change (versus baseline) in concentrations (Conc) of a parameter were calculated as $[(Conc_{6week} - Conc_{baseline})/Conc_{baseline}] \times 100\%$. Statistical significance was set at $p < 0.05$.

Results

Twenty hyperlipidemic male subjects with a mean age of 50.1 ± 11.8 years (range 30–72) were included in the study. The daily energy consumption, calculated based on daily requirements, was 1844 kcal/day before the soy protein diet was

TABLE I Mean daily intake of nutrients before and during the soy protein diet

	Before diet	After diet
Energy (kcal/day)	1844.0 ± 355.4	1845.5 ± 344.1
Total Protein (g/day)	68.2 ± 12.2	68.5 ± 12.5
Animal source protein	30.2 ± 6.2	16.4 ± 5.5 [b]
Plant source protein (excluding soy)	36.4 ± 8.9	32.2 ± 10.7 [a]
Soy protein	—	19.9 ± 2.2
Total fat (g/day)	51.6 ± 13.6	52.3 ± 12.8
Saturated	20.8 ± 8.6	11.5 ± 4.4 [b]
Monounsaturated	21.3 ± 5.9	21.7 ± 5.5
Polyunsaturated	9.4 ± 3.3	19.4 ± 4.7 [b]
Carbohydrates (g/day)	277.5 ± 56.1	279.5 ± 56.8
Cholesterol (mg/day)	160.0 ± 48.5	94.6 ± 24.4 [b]
Dietary fiber (g/day)	5.6 ± 0.9	5.8 ± 0.9

[a] $p < 0.05$.
[b] $p < 0.001$ (compared with prediet value).

TABLE II Physical characteristics and plasma lipid parameters of study subjects before and after 6 weeks of soy protein diet

	Before diet	After diet	p Value
Weight (kg)	74.6 ± 5.2	74.0 ± 5.1	0.065
BMI (kg/m^2)	25.3 ± 1.3	25.1 ± 1.3	0.03
Waist/hip ratio	0.96 ± 0.07	0.96 ± 0.07	0.273
Skin fold thickness (mm)	10.6 ± 1.1	10.4 ± 0.9	0.073
T-C (mg/dl)	261.8 ± 32.7	221.1 ± 33.0	< 0.001
Triglyceride (mg/dl)	252.5 ± 98.5	201.3 ± 58.2	0.001
HDL (mg/dl)	41.3 ± 7.8	41.0 ± 6.1	0.824
LDL (mg/dl)	174.3 ± 28.7	138.3 ± 30.6	< 0.001
T-C/HDL	6.5 ± 1.2	5.5 ± 1.1	< 0.001
Apolipoprotein AI (mg/dl)	130.9 ± 34.2	130.3 ± 20.4	0.937
Apolipoprotein B (mg/dl)	149.0 ± 41.7	134.5 ± 32.2	0.039
Lipoprotein (a) (mg/dl)	23.4 ± 28.6	22.2 ± 28.0	0.492

Abbreviations: BMI = body mass index, T-C = total cholesterol, HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol.

instituted and did not change during the diet (1845 kcal/day, $p > 0.05$). The details of the characteristics of food consumption before and during the soy protein diet are summarized in Table I. Total protein, fat, carbohydrate, and dietary fiber consumptions remained stable during the study diet; however, the protein from animal sources was significantly lower ($p < 0.001$). There was a slight decrease in the plant source protein if the soy protein was excluded from analysis ($p < 0.05$). However, during the study diet, mean soy protein intake was 19.9 ± 2.2 g/day, and as a total plant source protein was significantly higher during the soy protein diet ($p < 0.001$). Saturated fat consumption was significantly lower and, accordingly, polyunsaturated fat consumption was significantly higher during the diet (both $p < 0.001$). The cholesterol intake with the previous diet was < 200 mg/day (on average 160 mg/day); as suggested by the NCEP/AHA Step 1 diet, that was decreased to 94 mg/day during soy consumption ($p < 0.001$).

Physical characteristics and lipid parameters of the subjects at enrollment and after 6 weeks of soy protein diet are summarized in Table II. Weight, waist/hip ratio, and skin fold thickness of the subjects did not change with the soy protein diet, however there was a slight but significant decrease in BMI (25.3 ± 1.3 kg/m^2 vs. 25.1 ± 1.3 kg/m^2, $p = 0.03$).

After 6 weeks of diet, plasma T-C, LDL-C, and triglyceride levels decreased significantly ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively). A similar improvement was noted in T-C/HDL-C and apolipoprotein B levels ($p < 0.001$ and $p = 0.039$). The HDL-C, apolipoprotein A, and lipoprotein (a) levels were not affected with the soy protein diet ($p < 0.05$). The mean changes were as follows: T-C −15%, LDL-C −20%, and TG −14%. When the baseline T-C levels were divided into quartiles (< 237, 237–254, 254–271, and > 271 mg/dl), the most significant decrease was noted in patients with the highest baseline cholesterol levels (−15%, −12%, −14%, and −21%, respectively).

TABLE III Studies of the brachial artery before and after 6 weeks of soy protein diet

	Before diet	After diet	p Value
Baseline diameter (mm)	4.3 ± 0.5	4.5 ± 0.4	0.05
Diameter at reactive hyperemia (mm)	4.7 ± 0.5	5.0 ± 0.4	< 0.001
EDD (%)	8.2 ± 0.6	12.6 ± 0.6	0.002

Abbreviation: EDD = endothelium-dependent dilatation.

Studies of the brachial artery before and after the soy protein diet are shown in Table III. There was a borderline dilatation in baseline brachial artery diameter ($p = 0.05$); however, the diameter at reactive hyperemia was significantly larger after the diet ($p < 0.001$). The EDD was also significantly improved with the soy protein diet ($p = 0.002$).

The mean plasma TM levels before the soy protein diet were 49 ± 22 ng/dl, which were decreased to 44 ± 17 ng/dl after diet ($p = 0.004$) (Fig. 1).

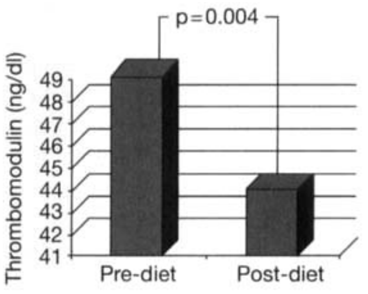

FIG. 1 Plasma thrombomodulin levels before and after soy protein diet.

Discussion

Soy protein has been shown to be hypocholesterolemic in animals.[14–16] However, the studies of soy protein diet in human subjects have revealed inconsistent results, some showing a significant hypocholesterolemic effect of the soy protein,[17–21] whereas others report an insignificant effect on hypercholesterolemic subjects.[22–24] This variation can be attributed to the differences in study design. Various soy products such as soy flour, soy protein concentrate, textured soy protein, isolated soy protein, and soymilk have been used in different studies, resulting in ingestion of different quantities of soy protein by the subjects. Furthermore, in some studies, the effect of soy protein could not be distinguished from the hypocholesterolemic effect of the simultaneous reduction in fat and cholesterol intakes associated with the diet. The diet consumed by the patients throughout the study was consistent with the recommendations of the NCEP/AHA Step 1 for lowering plasma cholesterol concentrations, with the cholesterol intake limited to < 200 mg/dl. The text diet given to study subjects included 30% of total protein as soy protein, an average 20 g/day. The rest of the composition of the diet including total protein, fat, carbohydrate, and energy was similar whether or not soy was added. The fiber content of both diets was also similar (≌ 6g/day). The substitution of soy protein for animal protein in the diet resulted in significant decreases in the animal source protein and saturated fat consumption with similar total protein intake. Compliance with the diet was checked with daily phone calls and weekly visits by the dietitian, and the subjects were reminded to consume all foods in the recommended amounts to minimize changes in body weight.

A meta-analysis of 38 clinical trials indicated that soy protein ingestion was associated with significant reductions in T-C and LDL-C concentrations of −9.3 and −12.9%, respectively.[12] These decreases were greater in patients with higher baseline cholesterol levels. High-density lipoprotein cholesterol showed a 2.4% increase and TG decreased 10.5%; however, none of these parameters was statistically significant. In these trials, the average intake of soy was 47 g/day, and the extent of reduction was dependent upon the baseline cholesterol levels. In subjects with moderate hypercholesterolemia (259–333 mg/dl), the decrease in T-C was 7.4%, whereas subjects with severe hypercholesterolemia (> 335 mg/dl) achieved a decline of 19.6%. In our study, the decreases in T-C and LDL-C were on average −15 and −20%, respectively, which is higher than those in many of the studies reported so far. However, the soy protein intake in this study was associated with low cholesterol and saturated fat consumption. Furthermore, subjects included in the study were all hypercholesterolemic, a criterion increasing the hypocholesterolemic effect of soy diet. The hypocholesterolemic effect of soy protein also has been shown to be minimal or negligible in normocholesterolemic subjects.[25] We also found the hypocholesterolemic effect of the soy diet to be higher in the patients with higher baseline T-C levels.

The exact mechanism of the hypocholesterolemic effect of the soy protein diet is not known, but several mechanisms have been proposed. The soy protein diet has been shown to increase fecal excretion of bile acids.[26] Changes in hepatic metabolism include increase in cholesterol biosynthesis and LDL receptor activity.[27] Soy protein increases thyroxine, free thyroxine index, and, in some cases, thyroid-stimulating hormone levels and initiates a hyperthyroid state.[28] Both increases and decreases have been noted in the concentrations of insulin and glucagon, whereas the insulin: glucagon ratio generally decreases upon feeding soy protein,[28, 29] which leads to a decrease in lipogenesis.

Although the energy intake was adjusted to preserve body weight, slight weight loss after the soy protein diet was observed in our study. This effect might be related to the hyperthyroid state induced by soy diet. Weight loss can decrease serum T-C and increase HDL-C, but a considerable amount of weight must be lost (10–15%) to produce noticeable effects, and this is unlikely in our study.

What component of the soy protein-containing products fed is causing these changes in blood lipids? Anderson *et al.*[12] speculated the phytoestrogens to be responsible for the hypocholesterolemic effect of the soy diet. Phytoestrogens are naturally occurring, plant-based diphenolic compounds that are similar to estradiol in structure and function. The most common and best studied phytoestrogen is the class of isoflavones, which include genistein and daidzein as the active components. These agents have selective estrogenic actions that are dependent on the affinity of binding to the estrogen receptors. The expression of estrogen receptor β in vascular and other nonreproductive tissue has been proposed to be one of the mechanisms by which isoflavones exert direct effects on the arterial system.[30] Isoflavones are present in whole soybeans and in various soy products, but their concentration is related to the processing technique used to prepare the product. Ethanol extraction removes isoflavones. Several studies have suggested that the alcohol-extractable component of soy protein other than the amino acid composition lowers plasma cholesterol and contributes to its antiatherosclerotic effects.[31]

The data related to the effects of soy protein on endothelial markers are limited. Genistein, the major isoflavone present in soy, has been shown to be capable of inhibiting the expression of certain adhesion molecules, namely intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1), on human endothelial cells cocultured with monocytes.[32] Resveratrol is another potentially important phytoestrogen, present in grapes. It has been shown to bind to human estrogen receptors, activate estrogen-regulated genes, cause proliferation of estrogen-dependent breast cancer cell lines, and inhibit the expression of VCAM-1 and ICAM-1 in human endothelial cells.[33] In the present study, the plasma levels of TM were significantly decreased with soy protein consumption. This reduction was thought to be related to the combined effect of the improvement in the lipid profile and the nonlipid effects of soy protein consumption.

Preclinical studies suggest that vascular reactivity may be favorably influenced by phytoestrogens. In vitro studies of isolated vessels have examined the mechanisms of phytoestrogen-induced vasodilation.[34] Primates manifest an improvement in endothelium-mediated vasodilation when treated with phytoestrogens. Postmenopausal monkeys on a phytoestrogen-rich diet for 6 months exhibited normal coronary artery vasodilation in response to locally administered acetylcholine, whereas a vasocontrictive response was seen in male animals as well as female monkeys with a low intake of phytoestrogens.[35] The intake of soy isoflavones was also shown to improve systemic arterial compliance in menopausal and perimenopausal women.[36] Studies of the brachial artery in response to hyperemia-induced vasodilation have previously been demonstrated to be a reliable method of noninvasive evaluation of endothelial function.[4] Endothelial function assessed by this method has been shown to be impaired in several conditions, including hypercholesterolemia and coronary artery disease, after high fat consumption and to be improved with hypolipidemic therapy and estrogen replacement.[1,2,37–40] To the best of our knowledge, this is the first study investigating the effects of a soy protein diet on EDD of the brachial artery. Our data indicated that the brachial artery diameter during reactive hyperemia and EDD significantly improved with soy protein consumption. This effect was again thought to be related to the combination of both lipid lowering of the diet and the direct effects of soy components (isoflavones) themselves.

Conclusions

In conclusion, it is possible to decrease the cholesterol content of the diet significantly while the protein remains the same using a soy diet. The soy protein diet significantly decreases plasma T-C, LDL-C, TG, and apolipoprotein B levels. Endothelial function as judged by two different methods, EDD and plasma TM levels, also improves with soy diet. This improvement may be due to cholesterol lowering as well as a specific effect the soy diet has on the endothelium. However, there are still many unanswered questions related to the effects of soy on cardiovascular system.

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