Effects of Soy Protein Diet on Endothelial Functions and Lipid Parameters

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SOYA PROTEİN DİYETİNİN ENDOTEL FONKSİYONLARI VE LİPİD PARAMETRELERİNE ETKİSİ

ÖZET

Diyetteki hayvansal kaynaklı proteinin soya proteini ile yer değiştirmesinin kolesterol düzeylerini düşürdüğü gösterilmiştir. Ancak soya diyetinin endotel fonksiyonlarına etkisi net olarak bilinmemektedir. Bu çalışmanın amacı soya proteininin plazma lipidlerine ve iki farklı yöntemle incelenen endotel fonksiyonlarına olan etkisinin değerlendirilmesidir.

Metod: Çalışmaya hiperkolesterolemik, sigara kullanmayan ve beden kitle indeksi normal olan 20 erkek hasta (yaş 50±12) dahil edildi. Günlük enerji gereksinimi hesaplanan hastalara total kalorinin %25-30'u yağlardan, %10-12'si proteinlerden ve kalanı karbonhidratlardan oluşan bir diyet uygulanmaya başlandı. Diyetteki hayvansal kaynaklı proteinin %60'ının yerine soya proteini önerildi. Hastaların antropometrik ölçümleri, lipid parametreleri ve endotel fonksiyonları diyet öncesi ve diyetin 6. haftasında değerlendirildi. Endotel fonksiyon parametreleri olarak endotele bağımlı dilatasyon (EBD) ve plazma trombomodulin (TM) düzeyleri incelendi.

Diyet sonrası plazma total kolesterol, düşük dansiteli lipoprotein kolesterol, apolipoprotein B ve trigliserid düzeylerinde anlamlı azalma saptandı (p<0.001, p<0.001, p=0.039 ve p=0.001 sırasıyla). Diyet sonrası plazma TM düzeyleri de anlamlı olarak azaldı (p=0.004). Brakiyal arter ultrason incelemelerinde bazal brakiyal arter çapında sınırda bir dilatasyon izlenirken (p=0.05), reaktif hiperemi sırasındaki brakiyal arter çapının diyet sonrası anlamlı olarak genişlediği (p<0.001) ve endotele bağımlı dilatasyonun düzeldiği saptandı (p=0.002). Sonuç olarak, hiperkolesterolemik hastalarda soya diyetinin plazma lipid profilinine olumlu etkileri vardır. Buna ilaveten iki farklı yöntemle ölçülen (EBD ve plazma TM düzeyleri) endotel fonksiyonlarını da olumlu yönde etkilenmektedir.

Anahtar kelimeler: *Soya proteini, hiperkolesterolemi, endotel fonksiyonları, trombomodulin.*

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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 non-invasively 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]. 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.

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

METHODS

Subjects

Twenty hypercholesterolemic, non-smoker 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 >230mg/dl, a fasting plasma low-density lipoprotein cholesterol (LDL-C) concentration >160mg/dl despite the use of NCEP/AHA diet [13] for at least 6 months. All subjects were carefully screened to exclude those taking cholesterol-lowering medications, consuming alcohol or special diets, 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 soy protein diet all subjects completed a three-day (including one 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 (NCEP/AHA) recommendations. The food taken was noted in forms of animal/plant protein, saturated/monounsaturated/polyunsaturated fat as well as milligram cholesterol consumption.

Daily physical activities of the subjects were also noted on three consecutive days and Harris-Benedict formula (66.5+(13.75xbody mass)+(5.03xheight)-(6.75xage)) was used to calculate their basal metabolic rate. Before the soy diet was initiated, daily energy requirements of the subjects were calculated as the sum of the energy consumed on daily physical activities and the basal metabolic rate. 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 the soy protein constituted 30% of the total protein intake. The soy products were provided in the forms of soy flour, soybeans and soy bean sprouts.

Compliance with the diet was checked with daily phone calls and weekly visits by the dietitian of the study and the subjects were reminded to consume all foods in recommended amounts to minimize changes in body weight.

Blood samples and laboratory determinations

Blood samples were drawn after at least 12 hours of fasting from a large antecubital vein without interruption of venous flow before inclusion and at 6th weeks of 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 autoanalyser). Plasma levels of apolipoprotein A1, apolipoprotein B and lipoprotein (a) were measured with rate nephelometric method using Beckman Immage Immunochemistry systems (Beckman Coulter, Inc. Fullerton, CA, USA). Plasma TM levels were measured by two-site ELISA with two monoclonal antihuman TM antibodies (ELISA, Asserachrom Thrombomodulin, Diagnostica Stago, France). In all tests the laboratory technicians were blinded to the clinical status of the patient.

Brachial artery ultrasound 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. Right brachial artery was imaged at a location of 3-7cm 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 minutes. Blood flow was increased through the brachial artery after deflation of the cuff. Brachial artery diameter was measured after deflation in the first 15 seconds during reactive hyperemia. Endothelium dependent dilatation was calculated as the % increase in diameter at reactive hyperemia compared to baseline diameter.

Statistics

The Statistical Package for the Social Science (SPSS 9.1 version for Windows) was employed for statistical analysis. Student's paired t-test was used for within-groups comparisons of the parameters at baseline with that at 6 weeks. Values were expressed as mean ± SD or percentages as appropriate. Percentages of change (versus baseline) was calculated as [(Value at 6. week-Value at baseline)/Value at baseline] X 100%. Statistical significance was set at $p<0.05$.

RESULTS

Twenty hypercholesterolemic male subjects with a mean age of 50±12 (range 30-72) were included in the study. The daily energy consumption, calculated based on the daily requirements, was 1844kcal/day before the soy protein diet was instituted and 1845kcal/day during the soy diet ($p>0.05$). The details of the characteristics of food consumption before and during the soy protein diet are summarized in Table 1. Total protein, fat, carbohydrate and dietary fiber consumptions remained stable during the study diet, however, the protein from animal source was significantly lower ($p<0.001$). During the study diet mean soy protein intake was 19.9±2.2g/day and as a total plant source protein (including soy) was significantly higher during the soy protein diet ($p<0.001$). Saturated fat consumption was significantly lower and accordingly polyunsaturated fat consumption significantly higher during diet (both $p<0.001$). The cholesterol intake with the previous diet was below 200mg/day (on average 160mg/day) as suggested by

Table 1. 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**
Plant source protein (excluding soy)	36.4±8.9	32.2±10.7*
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**
Monounsaturated	21.3±5.9	21.7±5.5
Polyunsaturated	9.4±3.3	19.4±4.7**
Carbohydrates (g/day)	277.5±56.1	279.5±56.8
Cholesterol (mg/day)	160.0±48.5	94.6±24.4**
Dietary fiber (g/day)	5.6±0.9	5.8±0.9

**p<0.05, **p<0.001 (compared to pre-diet value)*

NCEP/AHA step 2 diet and was decreased to 94mg/day during soy consumption (p<0.001).

Physical characteristics and lipid parameters of the subjects at study entry and after 6 weeks of soy protein diet are summarized in Table 2. The weight, waist/hip ratio and skin fold thickness of the subjects did not change with soy protein diet, however there was a slight but significant decrease in the 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 also noted in T-C/HDL-C and apolipoprotein B levels (p<0.001, and p=0.039). High-density lipoprotein cholesterol, apolipoprotein A and lipoprotein (a) levels were not affected with soy protein diet (p>0.05). The mean changes in T-C was -15%, LDL-C was -20% and TG was -14%. When the baseline T-C levels were divided into quartiles (<237mg/dl, 237-254mg/dl, 254-271mg/dl and >271mg/dl) the most significant decrease was noted in patients with the highest baseline cholesterol levels (-15%, -12%, -14% and -21% respectively).

The results of brachial artery ultrasound studies before and after soy protein diet are shown in Table 3.

Table 2. The 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

BMI: Body mass index, T-C: Total cholesterol, HDL: High density lipoprotein cholesterol, LDL: Low density lipoprotein cholesterol

Table 3. Ultrasound examinations 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
EBD (%)	8.2±0.6	12.6±0.6	0.002

EDD: Endothelium dependent dilatation

There was 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). The EDD was also significantly improved with soy protein diet (p=0.002).

The mean plasma TM level, which was 49±22ng/dl before soy protein diet, significantly decreased to 44(17ng/dl after diet (p=0.004).

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 reporting insignificant effect in 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. In our study the diet consumed by the subjects throughout the study period was consistent with the recommendations of the NCEP/AHA Step 2 for lowering plasma cholesterol concentrations with the cholesterol intake limited to <200 mg/dl. The properties of the diet including total protein, fat, carbohydrate and energy were similar with or without the addition of soy. The fiber content of both diets was also similar (≅6g/day). The replacement of soy protein with animal protein in the diet resulted in significant decreases in the animal source protein and saturated fat consumption.

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). In the same meta-analysis HDL-C increased by 2.4% and TG decreased by 10.5%; however, none of these parameters were statistically significant. In subjects with moderate hypercholesterolemia (259-333mg/dl) the decrease in T-C was 7.4%, whereas subjects with severe hypercholesterolemia (>335mg/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. However, the soy protein intake in our study was associated with low cholesterol and saturated fat consumption. Furthermore, subjects included in our study were all hypercholesterolemic, a criteria increasing the hypocholesterolemic effect of soy diet. The hypocholesterolemic effect of soy protein has been shown to be minimal or negligible in normocholesterolemic subjects (25). Our results support the idea of the hypocholesterolemic effect of soy diet to be higher in the patients with higher baseline T-C levels.

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

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 in structure and function to estradiol. 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 non-reproductive 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 alcohol-extractable component of soy protein lowers plasma cholesterol other than the amino acid composition and contribute to its antiatherosclerotic effects (31).

The data related to the effects of soy protein on endothelial markers are limited. Genistein 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 co-cultured 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 was 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 non-lipid 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). Postmenopausal monkey on a phytoestrogen-rich diet for six months exhibited normal coronary artery vasodilation in response to locally administered acetylcholine, whereas a vasocontrictive response was seen in 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 brachial artery in response to hyperemia-induced vasodilation have previously been demonstrated to be a reliable method of non-invasive evaluation of endothelial function (4). Endothelial function assessed by this method has been shown to be impaired in several conditions including hypercholesterolemia, coronary artery disease, after high fat consumption and 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 soy protein diet on EDD of the brachial artery. Our data indicated that 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) itself.

In conclusion, it is possible to decrease the cholesterol content of the diet significantly while the amount of total protein remains the same using a soy diet. Soy protein diet significantly decreases plasma T-C, LDL-C, TG and apolipoprotein B levels. Furthermore, 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 of soy on the endothelium, but most probably a combination of both. However there are still many questions remaining to be answered related to the effects of soy on cardiovascular system.

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