

Soy Protein Intake, Cardiorenal Indices, and C-Reactive Protein in Type 2 Diabetes With Nephropathy

A longitudinal randomized clinical trial

LEILA AZADBAKHT, PHD^{1,2}
 SHAHNAZ ATABAK, MD³
 AHMAD ESMAILZADEH, PHD^{1,2}

OBJECTIVE — Several short-term trials on the effect of soy consumption on cardiovascular risks are available, but little evidence exists regarding the impact of long-term soy protein consumption among type 2 diabetic patients with nephropathy. To determine the effects of long-term soy consumption on cardiovascular risks, we measured C-reactive protein (CRP) and kidney function indexes among type 2 diabetic patients with nephropathy.

RESEARCH DESIGN AND METHODS — This longitudinal randomized clinical trial was conducted among 41 type 2 diabetic patients with nephropathy (18 men and 23 women). Twenty patients in the soy protein group consumed a diet containing 0.8 g protein/kg body weight (35% animal proteins, 35% textured soy protein, and 30% vegetable proteins) and 21 patients in the control group consumed a similar diet containing 70% animal proteins and 30% vegetable proteins for 4 years.

RESULTS — Soy protein consumption significantly affected cardiovascular risks such as fasting plasma glucose (mean change in the soy protein versus control groups: -18 ± 3 vs. 11 ± 2 mg/dl; $P = 0.03$), total cholesterol (-23 ± 5 vs. 10 ± 3 mg/dl; $P = 0.01$), LDL cholesterol (-20 ± 5 vs. 6 ± 2 mg/dl; $P = 0.01$), and serum triglyceride (-24 ± 6 vs. -5 ± 2 mg/dl; $P = 0.01$) concentrations. Serum CRP levels were significantly decreased by soy protein intake compared with those in the control group (1.31 ± 0.6 vs. 0.33 ± 0.1 mg/l; $P = 0.02$). Significant improvements were also seen in proteinuria (-0.15 ± 0.03 vs. 0.02 ± 0.01 g/day; $P = 0.001$) and urinary creatinine (-1.5 ± 0.9 vs. 0.6 ± 0.3 mg/dl, $P = 0.01$) by consumption of soy protein.

CONCLUSIONS — Longitudinal soy protein consumption significantly affected cardiovascular risk factors and kidney-related biomarkers among type 2 diabetic patients with nephropathy.

Diabetes Care 31:648–654, 2008

Dietary intervention has long been considered challenging in the treatment of renal disease. A typically prescribed diet for nephropathy contains 0.8 g protein/kg body weight with 70% of total dietary protein as animal protein (1,2). Such a diet might adversely affect

blood cholesterol and atherosclerosis (3). It seems that not only the quantity but also the type of protein have important implications in renal disease (4).

The precise mechanisms leading to the progression of renal injury are not yet completely understood. Activated immu-

nity and inflammation are relevant factors in the pathogenesis of diabetes and its microvascular complications, including nephropathy. In addition to inflammation, some investigators have suggested that dyslipidemia may be a factor having an influence on renal impairment in diabetic patients (5). This new pathogenic perspective leads to some therapeutic considerations that can be considered in clinical treatments for diabetic nephropathy (6). Several studies have shown that substitution of soy protein for animal protein might have beneficial effects on lipid profiles (4,7,8), circulating levels of inflammatory biomarkers (9,10), and renal function indexes (4,11). However, most data available in this regard come from short-term clinical trials, and no study has assessed the long-term consumption of soy protein as a stable component of the diet. Furthermore, how the body responds to soy consumption after a long period of time is not completely understood. Therefore, the current longitudinal clinical trial was performed to determine the long-term effects of soy consumption on lipid profiles, renal function indexes, and C-reactive protein (CRP) levels among type 2 diabetic patients with nephropathy.

RESEARCH DESIGN AND METHODS

A total of 50 diabetic patients with nephropathy (22 men and 28 women) was recruited for this study. Type 2 diabetes was defined as fasting plasma glucose ≥ 126 mg/dl (two times repeated) or taking oral glucose-lowering agents or insulin (12). All subjects had proteinuria with total urinary protein excretion between 300 and 1,000 mg/day, serum creatinine between 1 and 2.5 mg/dl, and blood urea nitrogen between 20 and 40 mg/dl. Their systolic and diastolic blood pressures were >140 and 90 mmHg, respectively. Nephropathy was established by the above-mentioned characteristics. Mean age of the patients at baseline ($n = 50$) was 62.1 ± 12.1 years. The diagnosis of retinopathy was based

From the ¹Department of Nutrition, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran; the ²Food Security and Nutrition Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; and ³Modarres Hospital, School of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Address correspondence and reprint requests to Leila Azadbakht, PhD, Department of Nutrition, School of Public Health, Isfahan University of Medical Sciences, Isfahan, P.O. Box 81745, Iran. E-mail: azadbakht@hlth.mui.ac.ir.

Received for publication 8 November 2007 and accepted in revised form 31 December 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 9 January 2008. DOI: 10.2337/dc07-2065. Clinical trial reg. no. NCT00555490, clinicaltrials.gov.

Abbreviations: ARB, angiotensin receptor blocker; CRP, C-reactive protein; GFR, glomerular filtration rate.

© 2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

on indirect ophthalmoscopic examination (after pupil dilatation with topical 0.5% tropicamide) and fundus photographs. In total, 60% of the patients ($n = 30$) had a family history of diabetes (having at least one first-degree relative with a diagnosis of diabetes at age ≥ 30 years). Mean durations of diabetes and hypertension among these patients were 10 ± 3 and 8 ± 2 years, respectively, and mean A1C level was $6.2 \pm 0.5\%$, showing good glycemic control. No other diabetes complications were reported by the patients. Eighty-one percent of them were using oral glucose-lowering agents, and 23% were using insulin. All were taking anti-hypertensive medications: 40% ($n = 20$) were using ACE inhibitors, 44% ($n = 22$) were using angiotensin receptor blockers (ARBs), and 76% ($n = 38$) were using diuretics; 20% ($n = 10$) were using both diuretics and ARBs, 18% ($n = 9$) were using both diuretics and ACE inhibitors, and 8% ($n = 4$) were using all three medications; and 12% ($n = 6$) were using both ARBs and ACE inhibitors. After matching for age, BMI, and diabetes duration, we randomly divided the patients into two groups: soy protein group ($n = 25$) and control group ($n = 25$). All patients provided written informed consent.

In this longitudinal randomized clinical trial study, the soy protein group followed a diet containing 0.8 g protein/kg body weight (35% animal proteins, 35% soy protein, and 30% vegetable proteins) and the control group consumed a similar diet with 0.8 g protein/kg body weight containing 70% animal and 30% vegetable proteins for 4 years. Both diets had 2,000 mg sodium and 2,000 mg potassium. Patients in the soy protein group used textured soy protein (Sobhan textured soy protein; Sobhan, Behshahr, Iran). They also received education regarding the preparation of their meals with soy protein. A dietitian explained that soy protein should be washed and soaked for 30 min and then cooked in boiling water with turmeric, lemon juice, and tomato paste for 10 min. Based on our analysis, the nutrient composition (per 30 g) of soy protein consumed by the study participants was as follows: protein (15 g), fat (0.3 g), fiber (10 g), total phytoestrogen (84 mg), sodium (9 mg), magnesium (91 mg), calcium (83 mg), copper (0.5 mg), potassium (1 mg), iron (2.3 mg), and phosphorus (223 mg). The soy protein we used in the current study was a commercially available product. The protein and fat contents of the soy were mea-

sured according to the methods of Kjeldahl and Soxhlet, respectively (13). Fiber content was determined gravimetrically as the difference in weights of a test sample before and after extraction in a solution. Calcium, magnesium, iron, copper, and phosphorus contents of soy protein were analyzed by atomic absorption based on a titrimetric method. Sodium and potassium contents of soy protein were measured by flame photometry and its isoflavones by high-performance liquid chromatography (13).

Calorie requirements of each participant were calculated individually on the basis of equations suggested by Institute of Medicine Food and Nutrition Board (14). Each patient was visited every 3 months for 45–60 min. Patients called the dietitian whenever they had questions regarding their diets. Dietitians called patients to remind them about soy intake and recording 3-day dietary records and to encourage them to follow their diets. Patients recorded their physical activities for 3 days every 3 months. They also recorded 3-day dietary records every 3 months. Diet compliance was assessed by dietary records and plasma phytoestrogen levels. Medications and their dosage were also reported for each patient during the study.

All measurements were done at baseline and every 6 months for up to 4 years. Body weight was measured while the subjects were minimally clothed without shoes using digital scales and recorded to the nearest 0.1 kg. Height was measured in a standing position, without shoes, using a tape meter while the shoulders were in a normal state. Blood pressure was measured twice after the participants sat for 15 min.

Twelve-hour fasting blood samples were collected into tubes containing 0.1% EDTA and were centrifuged at 4°C and 500g for 10 min to separate the plasma. Blood glucose was measured on the day of blood collection by an enzymatic colorimetric method using glucose oxidase. Renal function indexes including urinary and serum creatinine, blood and urinary urea nitrogen, and proteinuria were all measured. All subjects provided a 24-h urine sample at baseline and every 6 months. Each patient was taught the correct method for collecting a 24-h urine sample. Both urinary and serum concentrations of creatinine were determined by the Jaffe method (15), performed in a Hitachi 705 automatic analyzer (Boehringer

Mannheim, Mannheim, Germany) that was set to record the mean absorbance in the interval of 60–140 s after the start of the reaction. The absorbance was measured bichromatically with primary and secondary wavelengths (505/570 nm). Blood and urinary urea nitrogen levels were analyzed by enzymatic methods; the enzyme urease converts urea to ammonia and carbonic acid. Proteinuria was assessed by using trichloroacetic acid and sulfosalicylic acid (15). Glomerular filtration rate (GFR) was calculated on the basis of the following formula: $\text{GFR} = [140 - \text{age (years)}] \times [\text{weight (kilograms)}] / 72 \times (\text{serum creatinine})$ (16). Serum total cholesterol and triacylglycerol concentrations were measured by using commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran) adapted to a Selectra autoanalyzer. HDL cholesterol was measured after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. LDL cholesterol was calculated according to the Friedewald method (17). It was not calculated when the serum triacylglycerol concentration was >400 mg/dl. All samples were analyzed when internal quality control checks met the acceptable criteria. Plasma phytoestrogen levels were measured by high-performance liquid chromatography according to Franke et al. (18) to check soy consumption compliance. Inter- and intra-assay coefficients of variation were both $<5\%$ for all measurements.

Statistical methods

Statistical analysis was performed using SPSS for Windows (version 13.0; SPSS, Chicago IL) and SAS (version 8.2; SAS Institute, Cary, NC). The Kolmogorov-Smirnov test was used to assess whether the variables were normally distributed. A skewed variable (CRP) was treated as a log-transformed value in all analyses and reported as the geometric mean. To avoid long tables, we used the mean of two measurements in each year to obtain one value for each year. Two-way ANOVA was applied to assess the effect of time (baseline, first year, second year, third year, and fourth year), group, and the interaction term between time and group. For comparing mean changes of the variables in the soy protein and control groups, Student's *t* test was used. For executing this procedure, the difference between the fourth year and the first year was calculated as a new variable for each group and then the means of these two new variables were compared by using

Table 1—Nutrient intakes of the study participants

Nutrient intake	Soy protein group					Control group					P values		
	Baseline	1st year	2nd year	3rd year	4th year	Baseline	1st year	2nd year	3rd year	4th year	T	G	T · G
Energy (kcal/day)	2,408 ± 280	2,321 ± 236	2,409 ± 278	2,506 ± 295	2,390 ± 391	2,311 ± 205	2,364 ± 254	2,409 ± 284	2,519 ± 315	2,436 ± 319	0.13	0.13	0.11
Protein (g/day)	56 ± 25	55 ± 17	56 ± 19	54 ± 23	57 ± 26	58 ± 22	56 ± 17	57 ± 21	55 ± 16	58 ± 23	0.11	0.14	0.13
Fat (g/day)	58 ± 25	56 ± 17	55 ± 19	59 ± 23	54 ± 21	63 ± 17	62 ± 19	62 ± 22	64 ± 25	60 ± 16	0.03	0.01	0.19
Fiber (g/day)	15 ± 3	17 ± 4	16 ± 5	15 ± 5	18 ± 6	14 ± 2	23 ± 3	27 ± 4	26 ± 3	24 ± 5	0.01	0.02	0.01
SFA (g/day)	13 ± 2	12 ± 4	13 ± 8	14 ± 7	12 ± 6	14 ± 7	17 ± 8	16 ± 5	19 ± 6	17 ± 5	0.04	0.04	0.10
MUFA (g/day)	19 ± 5	18 ± 7	19 ± 6	20 ± 7	17 ± 4	21 ± 9	19 ± 8	18 ± 5	20 ± 6	19 ± 6	0.15	0.19	0.25
PUFA (g/day)	23 ± 7	24 ± 6	19 ± 5	23 ± 8	22 ± 6	26 ± 8	22 ± 7	24 ± 8	23 ± 9	24 ± 8	0.33	0.29	0.26
Soy protein (g/day)	0	16 ± 9	17 ± 11	15 ± 8	14 ± 8	0	0	0	0	0	0.01	0.01	0.01
Soy isoflavones (mg/day)	0	43 ± 15	48 ± 19	39 ± 17	36 ± 13	0	0	0	0	0	0.01	0.01	0.01

Data are means ± SD. G, group; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; T, time.

the Student's *t* test. To adjust for potential confounders, we used ANCOVA with changes in lipid profiles, plasma glucose, body weight, and phytoestrogen intake as covariates. Further adjustments were also made for changes in plasma phytoestrogen levels.

RESULTS— In total, 9 patients (of 50) were excluded from the study for different reasons: 4 subjects for not following the diet ($n = 3$ in the soy protein group vs. $n = 1$ in the control group) and 5 for starting dialysis ($n = 3$ in the soy protein group vs. $n = 2$ in the control group). Finally, 41 patients (20 in the soy protein group and 21 in the control group) with type 2 diabetes completed the entire 4-year trial. There were 18 men (43%) and 23 women (57%) in the study. The mean ± SD age of patients who completed the whole trial ($n = 41$) was 62.0 ± 12.0 years (soy protein group [$n = 20$] 61.9 ± 11.8 years and control group [$n = 21$] 62.1 ± 12.1 years), 67% of whom ($n = 27$ of 41) had a positive family history of diabetes (70% [$n = 14$] in the soy protein group vs. 61% [$n = 13$] in the control group). The proportions of patients using ACE inhibitors, ARBs, and diuretics in the soy protein group were 40% ($n = 8$), 50% ($n = 10$), and 100% ($n = 20$), respectively. These proportions in the control group were 57% ($n = 12$), 57% ($n = 12$), and 85% ($n = 18$), respectively. Of the patients, 73% had retinopathy (34% in the soy protein group and 39% in the control group). Nutrient intakes of participants based on the mean of the 3-day dietary records at baseline and during each year are shown in Table 1. Soy protein was well tolerated. Patients' activity levels remained unchanged across all study periods.

Means for cardiovascular risk factors are provided separately by groups in Table 2. Fasting plasma glucose (values for the fourth year in the soy protein versus control groups: 121 ± 42 vs. 147 ± 57 mg/dl; $P_{\text{time}} = 0.03$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.02$), total cholesterol (201 ± 35 vs. 218 ± 38 mg/dl; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.01$), LDL cholesterol (128 ± 14 vs. 158 ± 31 mg/dl; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.09$), and serum triglyceride (224 ± 43 vs. 232 ± 49 mg/dl; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.14$) levels declined significantly in the soy protein group compared with those in the control group. CRP, a marker of inflammation, also de-

creased significantly by soy protein consumption (2.4 ± 0.1 vs. 3.9 ± 0.2 mg/l; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.01$). Proteinuria (513 ± 39 vs. 725 ± 81 mg/day; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.1$), urinary urea nitrogen (12.8 ± 3.6 vs. 19.5 ± 5.6 mg/dl; $P_{\text{time}} = 0.04$, $P_{\text{group}} = 0.02$, and $P_{\text{time} \cdot \text{group}} = 0.08$), and urinary creatinine (3.1 ± 0.3 vs. 5.2 ± 0.4 mg/dl; $P_{\text{time}} = 0.01$, $P_{\text{group}} = 0.01$, and $P_{\text{time} \cdot \text{group}} = 0.01$) were improved significantly in the soy protein group.

Mean changes in cardiorenal risk factors and CRP are indicated in Fig. 1. Soy protein consumption significantly affected fasting plasma glucose (mean change in the soy protein versus control groups: -18 ± 3 vs. 11 ± 2 mg/dl; $P = 0.03$), total cholesterol (-23 ± 5 vs. 10 ± 3 mg/dl; $P = 0.01$), LDL cholesterol (-20 ± 5 vs. 6 ± 2 mg/dl; $P = 0.01$), and serum triglyceride (-24 ± 6 vs. -5 ± 2 mg/dl; $P = 0.01$) concentrations. CRP decreased significantly by soy protein intake (1.31 ± 0.6 vs. 0.33 ± 0.1 mg/l; $P = 0.02$). Changes in proteinuria (-0.15 ± 0.03 vs. 0.02 ± 0.01 g/day; $P = 0.001$) and urinary creatinine were also significant (-1.5 ± 0.9 vs. 0.6 ± 0.3 mg/dl) in comparing the soy protein and control groups. The significant findings of proteinuria disappeared after controlling for changes in blood lipid profiles; however, adjusting for other covariates such as changes in plasma glucose, phytoestrogen intake, and body weight had minimal effects on this finding. Significant changes in urinary creatinine and urinary urea nitrogen were not affected by controlling for changes in covariates. Further adjustments for changes in plasma phytoestrogen levels made soy impacts on fasting plasma glucose marginally significant ($P = 0.06$), with other dependent variables minimally affected.

CONCLUSIONS— The results of the present study, performed among a group of type 2 diabetic patients with nephropathy, showed that 4-year soy substitution in the diet resulted in significantly lower levels of fasting blood glucose, total cholesterol, LDL cholesterol, and triglycerides. The circulating level of CRP, the most popular inflammatory marker, decreased after 4-year soy consumption. In addition to cardiovascular risks, renal indexes also improved significantly.

Although several short-term studies regarding the effect of soy on kidney-related biomarkers and cardiovascular

risk factors are available (4,7,8), to our knowledge this is the first study in which such effects have been assessed for a long period of time (4 years). Thus, the results would be useful in interpreting the stability of soy effects. As shown in the current study, the beneficial effects of soy consumption on cardiovascular risks and kidney-related biomarkers were stable with long-term soy consumption. As diabetic nephropathy is a progressive disease, we expected that the conditions of these patients would have gotten worse after 4 years, but because of medical and dietary control, their conditions improved in some respects.

It seems that soy protein consumption might have a favorable effect on diabetic nephropathy through its impact on serum lipid levels (4,19). The association between hypercholesterolemia and diabetic nephropathy was demonstrated earlier (20). The results of the present study are also in line with those of previous studies in indicating that the effects of soy protein consumption on proteinuria could be mediated through its impact on lipid profiles. Plasma glucose levels have also been reported to modulate kidney function (21). Our results do not support this result because soy consumption affected proteinuria independently of changes in plasma glucose. The interaction between prevailing blood glucose levels and protein intake in the regulation of the renal response to a protein load has not been identified exactly (22).

The beneficial effects of soy protein on cardiovascular risks might be mediated through its impact on abdominal fat. Sites et al. (23) suggested that soy protein-containing isoflavones may prevent the accumulation of fat in the abdominal depot. Their study showed a significant effect of soy intake on total and subcutaneous abdominal fat. Even after adjusting for total body fat, they found a strong trend toward an effect of soy to prevent visceral fat accumulation. This finding could have important implications in the prevention of insulin resistance and cardiovascular disease.

We used naturally occurring isoflavones in the form of isolated soy protein in our intervention trial instead of purified isoflavones. The isoflavones and fiber content of the soy protein and probably its inositol-derived substances such as lipintol and pinitol might have beneficial effects on the blood lipid level and glycemic control (24,25). The improvement in

Table 2—Cardiovascular risk factors, CRP, and markers of renal function among type 2 diabetic patients with nephropathy

P values	T	G	T	Control group				Soy protein group				Measurements	
				4th year	3rd year	2nd year	1st year	Baseline	4th year	3rd year	2nd year		1st year
0.19	0.14	0.11	73 ± 10	69 ± 9	73 ± 10	71 ± 9	72 ± 8	71 ± 10	73 ± 10	72 ± 9	70 ± 10	71 ± 9 ¹	Weight (kg)
0.15	0.10	0.14	148 ± 67	147 ± 58	150 ± 49	155 ± 64	153 ± 71	147 ± 49	149 ± 52	153 ± 68	148 ± 55	150 ± 64	SBP (mmHg)
0.28	0.31	0.25	93 ± 43	94 ± 39	96 ± 42	95 ± 36	91 ± 41	93 ± 29	94 ± 33	90 ± 26	92 ± 32	96 ± 23	DBP (mmHg)
0.02	0.01	0.03	147 ± 57	146 ± 61	145 ± 51	142 ± 49	137 ± 54	121 ± 42	129 ± 36	132 ± 43	130 ± 32	141 ± 55	FBG (mg/dl)
0.01	0.01	0.01	228 ± 48	227 ± 56	225 ± 53	221 ± 45	218 ± 38	201 ± 35	207 ± 38	209 ± 35	216 ± 39	225 ± 48	TC (mg/dl)
0.14	0.01	0.01	232 ± 49	228 ± 42	239 ± 36	235 ± 45	238 ± 39	224 ± 43	231 ± 37	236 ± 40	239 ± 42	249 ± 51	TG (mg/dl)
0.09	0.01	0.01	158 ± 31	156 ± 29	148 ± 11	153 ± 20	151 ± 15	128 ± 14	132 ± 26	138 ± 19	141 ± 21	149 ± 16	LDL-C (mg/dl)
0.19	0.28	0.38	45 ± 19	43 ± 15	40 ± 22	46 ± 17	43 ± 11	53 ± 31	50 ± 20	52 ± 25	47 ± 19	49 ± 14	HDL-C (mg/dl)
0.08	0.02	0.04	19.5 ± 5.6	18.9 ± 5.2	18.5 ± 3.7	17.8 ± 4.8	18.1 ± 4.5	12.8 ± 3.6	14.3 ± 4.5	15.7 ± 3.4	16.0 ± 2.8	17.2 ± 3.4	UUN (mg/dl)
0.10	0.01	0.01	725 ± 81	719 ± 73	717 ± 65	684 ± 59	691 ± 71	513 ± 39	543 ± 45	603 ± 67	621 ± 63	667 ± 58	Proteinuria (mg/day)
0.21	0.19	0.09	1.61 ± 0.7	1.54 ± 0.6	1.51 ± 0.6	1.45 ± 0.5	1.49 ± 0.5	1.41 ± 0.2	1.49 ± 0.3	1.44 ± 0.3	1.53 ± 0.4	1.56 ± 0.5	Serum creatinine (mg/dl)
0.01	0.01	0.01	5.2 ± 0.4	5.4 ± 0.4	4.8 ± 0.4	4.6 ± 0.2	4.5 ± 0.3	3.1 ± 0.3	3.5 ± 0.4	3.8 ± 0.3	4.0 ± 0.4	4.8 ± 0.3	Urine creatinine (mg/dl)
0.29	0.32	0.26	22 ± 5	21 ± 4	20 ± 3	20 ± 4	19 ± 3	18 ± 3	18 ± 4	19 ± 4	19 ± 3	20 ± 2	BUN (mg/dl)
0.39	0.41	0.23	81 ± 35	75 ± 29	86 ± 36	80 ± 30	78 ± 23	88 ± 33	82 ± 29	79 ± 16	89 ± 23	84 ± 19	GFR (ml/min)
0.01	0.01	0.01	3.9 ± 0.2	3.8 ± 0.1	3.6 ± 0.3	3.7 ± 0.1	3.5 ± 0.2	2.4 ± 0.1	2.5 ± 0.08	3.1 ± 0.1	3.2 ± 0.1	3.8 ± 0.1	CRP (mg/l)
0.17	0.01	0.01	1.08 ± 0.03	1.11 ± 0.04	1.06 ± 0.03	1.10 ± 0.03	1.07 ± 0.03	1.29 ± 0.03	1.27 ± 0.04	1.19 ± 0.03	1.33 ± 0.03	1.02 ± 0.02	Plasma phytoestrogens (µmol/l)

Data are means ± SD. BUN, blood urea nitrogen; DBP, diastolic blood pressure; FBG, fasting plasma glucose; G, group; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; SBP, systolic blood pressure; T, time; TC, total cholesterol; TG, serum triglyceride; UUN, urinary urea nitrogen.

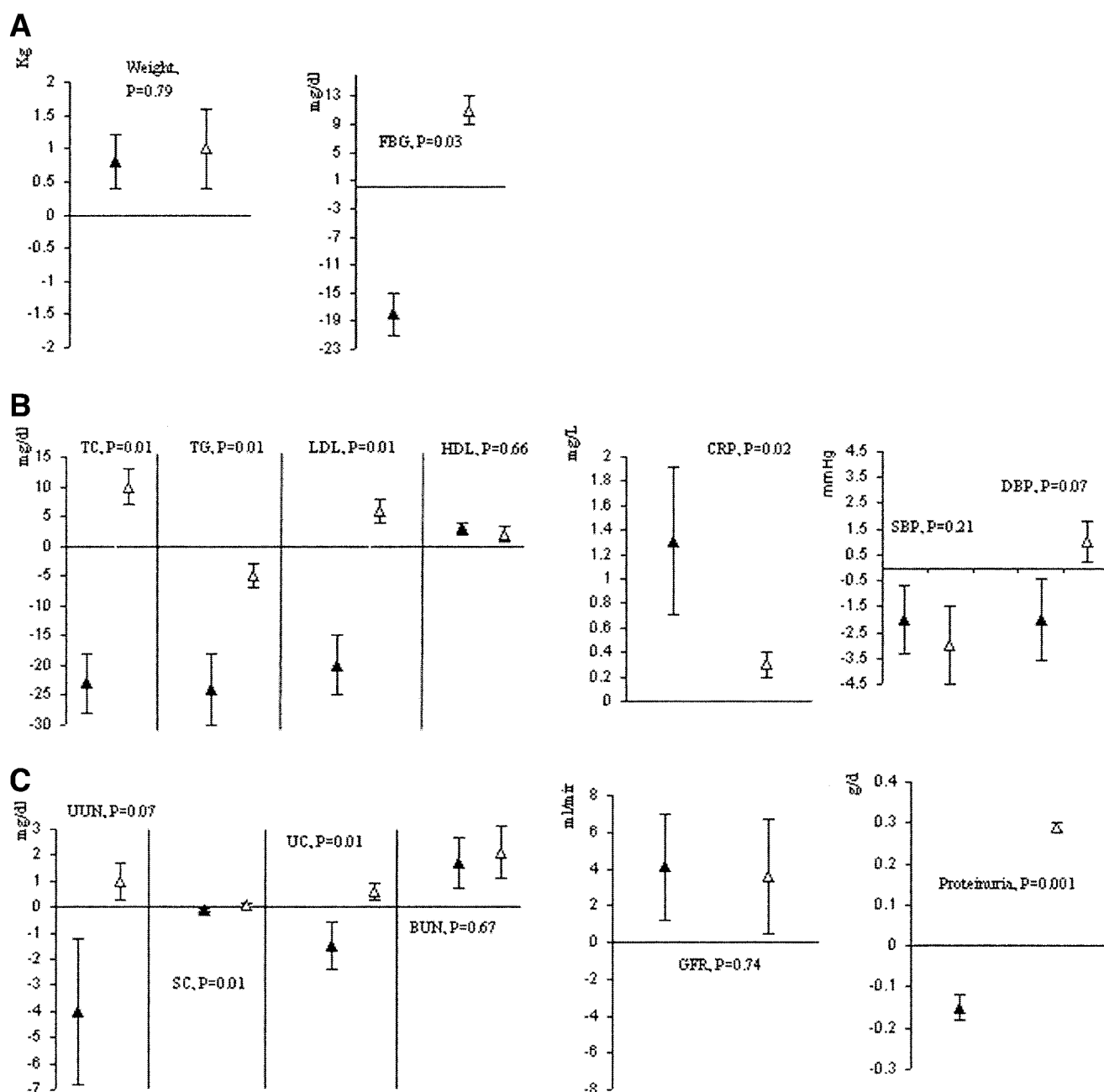


Figure 1— Mean \pm SD changes from baseline in cardiorenal indexes and CRP levels. A: Mean changes in weight and FPG. B: Mean changes in cardiovascular risks (lipid profiles, blood pressure, and CRP). C: Mean changes in renal function indexes. A statistically significant difference was found comparing the soy protein and control groups with regard to changes in fasting plasma glucose, total cholesterol, triglyceride, LDL, and CRP levels and also proteinuria and urinary creatinine ($P < 0.05$ for all). BUN: blood urea nitrogen; DBP, diastolic blood pressure; FPG, fasting plasma glucose; SBP, systolic blood pressure; SC, serum creatinine; TC, total cholesterol; TG, serum triglyceride; UC, urine creatinine; UUN, urinary urea nitrogen.

blood lipid and glucose levels might explain, to some extent, improvements we observed in kidney function.

Diabetic patients have been reported to have high circulating levels of inflammatory markers (1); however, few studies have assessed the effect of soy consumption on inflammatory biomarkers, particularly among diabetic patients. Our findings suggest that 4-year soy substitu-

tion in the diet decreased CRP significantly in the soy protein group compared with the control group. The decline in cardiovascular risks and also renal failure in the soy protein group might be explained by improvements in inflammatory status. We have studied only CRP from among a large number of inflammatory markers because of cost limitations. For accurate judgment, assessing the im-

pact of soy consumption on other inflammatory markers seems warranted.

The amount of soy protein intake in the present study was ~ 16 g/day. Higher amounts might have produced better results for cardiovascular risks and kidney-related indexes. However, large doses may be associated with some other health risks (26). In some situations, excessive soy protein intake could do more harm

than good; existing evidence suggests that genistein can stimulate estrogen receptor-positive breast cancers to grow (26).

We recommended textured soy protein in the current study. Previous studies have shown that other soy products such as soy nut might have more beneficial effects on cardiovascular risks (7), inflammatory markers (8), and oxidative stress (27) than textured soy protein. Further studies are required to assess the effects of soy nut among type 2 diabetic patients with nephropathy.

We did not evaluate the effects of soy protein according to estrogen receptor genotype in our participants; in some studies responses to isoflavone consumption have varied according to the estrogen receptor genotype (28). Also, further studies might be warranted to assess the effect of soy consumption on different complications of diabetic patients with nephropathy while taking "equol producer" or "equol nonproducer" status into account (28).

Several points need to be clarified in the current study. Poor adherence to a specified diet has been reported in the management of type 2 diabetes (29). In the current study, the adherence to both diets was good on the basis of the results of 3-day dietary records (12 records every year) and plasma phytoestrogen levels. Adherence to the diets was supported by the close relationship between the dietitian and patients via monthly phone calls encouraging them to follow their diets. Patients were also receiving recommendations to adhere to their diets from their physician every 3 months during the study.

Patients' mean A1C was 6.2%, suggesting good glycemic control. This result could be attributed to the oral glucose-lowering agents and insulin injections they were receiving. Because of the good glycemic control in these patients, one might assume that diabetes was not established. However, the patients were typical of type 2 diabetic patients (mean duration of diabetes was 10 years), and normal values of A1C might have resulted just from medication use or insulin injections. As we know, even a few days of normal plasma glucose within a month would result in near-normal A1C values. The population studied is not grossly different from patients usually studied in Europe or North America. Also, the method of measurement of A1C did not differ significantly from the usual techniques. However, the accuracy of measurements might

be different. A1C levels reported from Iranian studies are in the range of 5–9%, and most are for diabetic subjects not using medications or insulin. Finally, the finding of mean A1C of 6.2% might warrant further investigations.

Hypertension was present in all patients (mean duration of hypertension was 8 years). Even the diets we prescribed did not affect hypertension substantially. One possible explanation for persistent hypertension among these patients is involvement of injuries to vessels in its etiology. As mentioned above, most of these diabetic patients had retinopathy; a consequence of diabetes resulting from deterioration of vessels (30). Another point that must be taken into account is that the response of blood pressure to hypertensive medications usually takes more time, particularly when several factors are involved in its etiology (30). Mean values of serum creatinine in our study might raise the question of whether some patients had hypertensive nephropathy instead of diabetic nephropathy. It should be kept in mind that the creatinine clearance is more important than serum creatinine for interpretation of the situation in these patients. It is hard to differentiate whether their nephropathy was the result of hypertension or diabetes. What is clear is that all subjects had diabetes first and, after awhile, they developed nephropathy (they had been seeing their physician [S.A.] for a long time). All patients had stage 1 or 2 nephropathy. Therefore, progression to the point of needing dialysis takes time. Both groups had minimal progression (compared with what is expected) in nephropathy during the 4 years, which could be attributed to use of medications and the stage of nephropathy. None of the patients were in the third stage of nephropathy. The control group received all medications and treatments that the case group was receiving except for the soy protein.

One of the limitations of the current study is that urinary urea nitrogen and urinary creatinine were measured as concentrations rather than as 24-h excretions. Concentrations of these variables are less relevant to kidney function than 24-h excretion.

In summary, longitudinal soy protein consumption has beneficial effects on cardiovascular risk factors, CRP, and kidney-related indexes among type 2 diabetic patients with nephropathy.

References

1. Joana LS, Karr CS, Hutchins AM, Lampe JW: Influence of soybean processing, habitual diet, and soy dose on urinary isoflavonoid excretion. *Am J Clin Nutr* 68 (Suppl.):1492s–1495s, 1998
2. Anderson JW, Blake JE, Turner J, Smith BM: Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. *Am J Clin Nutr* 68:1347S–1353S, 1998
3. Anderson JW, Johnstone BM, Cook-Newell ME: Meta-analysis of effects of soy protein intake on serum lipids in humans. *N Engl J Med* 333:276–282, 1995
4. Azadbakht L, Shakerhosseini R, Atabak S, Jamshidian M, Mehrabi Y, Esmail-Zadeh A: Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *Eur J Clin Nutr* 57: 1292–1294, 2003
5. Anderson JW: Nutritional management of diabetes mellitus. In *Modern Nutrition in Health and Disease*. 10th ed. Shils ME, Young VA, Eds. Philadelphia, Lippincott Williams & Wilkins, 2004, p. 1052
6. Henry RR: Protein content of the diabetic diet. *Diabetes Care* 17:1502–1513, 1994
7. Azadbakht L, Kimiagar M, Mehrabi Y, Esmailzadeh A, Hu FB, Willett WC: Soy inclusion in the diet improves features of the metabolic syndrome: a randomized cross-over study in postmenopausal women. *Am J Clin Nutr* 85:735–741, 2007
8. Azadbakht L, Kimiagar M, Mehrabi Y, Esmailzadeh A, Hu FB, Willett WC: Soy consumption, markers of inflammation and endothelial function: a cross-over study in postmenopausal women with metabolic syndrome. *Diabetes Care* 30: 967–973, 2007
9. Huang Y, Cao S, Nagamani M, Anderson KE, Grady JJ, Lu LJ: Decreased circulating levels of tumor necrosis factor- α in postmenopausal women during consumption of soy-containing isoflavones. *J Clin Endocrinol Metab* 90:3956–3962, 2005
10. Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D, Vidgen E: Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. *Metabolism* 51:919–924, 2002
11. Jibani MM, Bloodworth LL, Foden E, Griffiths KD, Galpin OP: Predominantly vegetarian diet in patients with incipient and early clinical diabetic nephropathy: effects on albumin excretion rate and nutritional status. *Diabet Med* 8:949–953, 1991
12. Harris TJ, Cook DG, Wicks PD, Cappuccio FP: Impact of the new American Diabetes Association and World Health Organization diagnostic criteria for diabetes on subjects from three ethnic groups

- living in the UK. *Nutr Metab Cardiovasc Dis* 10:305–309, 2000
13. Horwitz W: *Official Methods of Analysis of AOAC International*, Vol. II, 17th ed. Gaithersburg, MD, AOAC International, 2000
 14. Institute of Medicine, Food and Nutrition Board: *Dietary Reference Intake for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC, National Academies Press, 2002
 15. Burtis CA, Ashwood ER: *Tietz Fundamentals of Clinical Chemistry*. 4th ed. Philadelphia, WB Saunders, 1989, pp 53–79
 16. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
 17. Friedewld WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 8:499–502, 1972
 18. Franke AA, Custer LJ, Tanaka Y: Isoflavones in human breast milk and other biological fluids. *Am J Clin Nutr* 8: 1466S–1473S, 1998
 19. Dubois D, Chanson P, Timsit J, Chauveau D, Nochy D, Guillausseau PJ, Lubetzki J: Remission of proteinuria following correction of hyperlipidemia in NIDDM patients with non-diabetic glomerulopathy. *Diabetes Care* 17:906–908, 1994
 20. Mulec H, Johnson S, Bjorck S: Relationship between serum cholesterol and diabetic nephropathy. *Lancet* 335:1537–1538, 1990
 21. Wiseman MJ, Mangili R, Alberetto M, Keen H, Viberti GC: Glomerular response mechanisms to glycemic changes in insulin dependent diabetics. *Kidney Int* 31:1012–1018, 1987
 22. Jones SL, Kontessis P, Wiseman M, Dodds R, Bognetti E, Pinto J, Viberti G: Protein intake and blood glucose as modulators of GFR in hyperfiltering diabetic patients. *Kidney Int* 41:1620–1628, 1992
 23. Sites CK, Cooper BC, Toth MJ, Gastaldelli A, Arabshahi A, Barnes S: Effect of a daily supplement of soy protein on body composition and insulin secretion in postmenopausal women. *Fertil Steril* 88: 1609–1617, 2007
 24. Merz-Demlow BE, Duncan AM, Wangen KE, Xu X, Carr TP, Phipps WR, Kurzer MS: Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am J Clin Nutr* 1:1462–1469, 2000
 25. Kim JI, Kim JC, Kang MJ, Lee MS, Kim JJ, Cha JI: Effects of pinitol isolated from soybeans on glycemic control and cardiovascular risk factors in Korean patients with type II diabetes mellitus: a randomized controlled study. *Eur J Clin Nutr* 59:456–458, 2005
 26. Willett WC, Skerrett PK: *Eat, Drink and Be Healthy. The Harvard Medical School Guide to Healthy Eating*. New York, Simon and Schuster, 2005, p. 130–131, 2005
 27. Azadbakht L, Kimiagar M, Mehrabi Y, Esmaillzadeh A, Hu FB, Willett WC: Dietary soya intake alters plasma antioxidant status and lipid peroxidation in postmenopausal women with the metabolic syndrome. *Br J Nutr* 17:1–7, 2007
 28. Hall WL, Vafeiadou K, Hallund J, Bugel S, Reimann M, Koebnick C, Zunft H-JF, Ferrari M, Branca F, Dadd T, Talbot D, Powell J, Minihane A-M, Cassidy A, Nilsson M, Dahlman-Wright K, Gustafsson J-A, Williams, CM: Soy-isoflavone-enriched foods and markers of lipid and glucose metabolism in postmenopausal women: interactions with genotype and equol production. *Am J Clin Nutr* 83:592–600, 2006
 29. The Diabetes and Nutrition Study Group of the Spanish Diabetes Association (GSEDNu): Diabetes Nutrition and Complications Trial: adherence to the ADA nutritional recommendations, targets of metabolic control, and onset of diabetes complications: a 7-year, prospective, population-based, observational multicenter study. *J Diabetes Complications* 20: 361–366, 2006
 30. Ibrahim HN, Nath KA, Hostetter TH. *Handbook of Nutrition and the Kidney*. 3rd ed. Philadelphia, Lippincott-Raven, 1999, p. 167–199