Soy Protein Reduces the Arterial Low-Density Lipoprotein (LDL) Concentration and Delivery of LDL Cholesterol to the Arteries of Diabetic and Nondiabetic Male Cynomolgus Monkeys

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We have previously shown that soy protein consumption improves lipoprotein concentrations and reduces the progression of atherosclerosis in cynomolgus monkeys. The mechanism for these beneficial effects is unclear. The purpose of this study was to determine potential mechanisms for the atheroprotective effects of soy and to determine if these effects extend to diabetic monkeys. We designed an experiment with a 2 × 2 factorial design in which adult male monkeys (N = 23) were fed an atherogenic diet with a protein source of either soy isolate or casein and lactalbumin, and the monkeys were either control or streptozotocin-induced diabetic. Diabetics had significantly increased fasting glucose and glycated hemoglobin (GHb) levels; this relationship was not affected by the type of dietary protein. Diabetics also had increased total (TC) and low-density lipoprotein cholesterol (LDLC) concentrations. However, soy consumption significantly reduced TC and LDLC concentrations in both control and diabetic monkeys. Plasma and arterial LDL metabolism was determined by injecting ^{125}I-LDL at 48 hours and ^{131}I-tyramine cellobiose LDL at 1 hour prior to necropsy. This allowed a determination of the arterial LDL concentration, permeability, and arterial LDL delivery. An increase in the whole-body plasma LDL fractional catabolic rate (FCR) was found with soy. Soy significantly reduced the arterial LDL concentration across all arterial sites by an average of 50%. Soy also significantly reduced the delivery of LDLC to all arterial sites by an average of 40%. While this was primarily due to the lower plasma LDLC concentration, LDL permeability in the carotid bifurcation and internal carotid arteries was also reduced. There was no additional effect of diabetes. These beneficial effects on plasma and arterial LDL metabolism would be expected to reduce atherosclerosis and were found in both control and diabetic monkeys.

CARDIOVASCULAR MORBIDITY and mortality are higher in men compared with women at all ages. These differences are thought to be mediated, in part, by differences in sex hormones.[1] Multiple lines of evidence implicate both endogenous and exogenous estrogens as antiatherogenic and protective against coronary heart disease (CHD).[2,3] There is extensive evidence that estrogen replacement therapy reduces the risk of CHD in postmenopausal women[4] and atherosclerosis in various animal models[2,3] by about 50%. Estrogen treatment of men also appears to be atheroprotective,[5-7] although this therapy has not been used for that purpose, primarily due to concerns regarding adverse side effects such as feminization and thrombosis.[8,9] It is possible that the recently described estrogens with tissue-selective agonistic or antagonistic properties, as well as soy phytoestrogens, may be cardioprotective in males without effects on the reproductive system.[10,11]

Epidemiologic and cross-cultural studies[12-14] and numerous animal studies[15-18] support the notion that soy consumption is cardioprotective. For example, Japanese men who consume relatively large amounts of soy have about one sixth the risk of CHD as US men.[19] In a recent meta-analysis, the effects of soy on plasma lipids and lipoproteins were reviewed.[13] Although the results were not dichotomized by gender, it is apparent that both men and women experience substantial decreases in plasma triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDLC) and modest to minimal increases in high-density lipoprotein cholesterol (HDLC). Furthermore, the effects of dietary soy on TC and LDLC were most pronounced among individuals with the highest baseline TC concentrations. Recent studies have suggested that many of the beneficial effects of soy may be mediated by the isoflavones or phytoestrogens present in the soy protein.[16,17] Depending on the tissue, the isoflavones may exert either estrogenic or antiestrogenic effects.[20] We have shown that soy protein, containing isoflavones, acts as an estrogen agonist with regard to plasma lipoprotein metabolism and effects on the cardiovascular system.[15,16] However, soy isoflavones appear to be estrogen antagonists for reproductive tissues in females[21] and males.[11]

We have reported that a soy protein diet compared with a casein protein diet with all other macronutrients equivalent results in lower TC and LDLC concentrations and, importantly, decreased atherosclerosis in both male and female monkeys.[11,15] In previous studies of mammalian estrogens in female monkeys, we have shown that one mechanism by which estrogens may be atheroprotective is by decreasing the degradation and accumulation of LDL by the artery wall.[22-24] Thus, we proposed that soy protein may also act at the level of the artery wall to reduce LDL accumulation in male monkeys. Furthermore, as diabetics are at increased risk of atherosclerosis,[25] we sought to determine if soy consumption would also improve plasma lipoproteins and decrease arterial LDL accumulation in diabetic monkeys.

MATERIALS AND METHODS

Study Population and Diet Composition

Twenty-six adult male cynomolgus monkeys (*Macaca fascicularis*) aged 12 to 24 years were studied for 14 weeks. All monkeys were originally imported from Institut Pertanian (Bogar, Indonesia) and were

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pair-housed at the Comparative Medicine Clinical Research Center at Wake Forest University School of Medicine. These monkeys were used previously in experimental studies involving dietary cholesterol. Therefore, a femoral artery biopsy was obtained to determine the preexisting arterial cholesterol content (described later) and baseline lipids were determined for randomization to 1 of 4 treatment groups.

A 2 × 2 factorial design was used with the 4 treatment groups as follows: (1) nondiabetics fed casein-lactalbumin protein (CAS, n = 6), (2) nondiabetics fed soy protein (SOY, n = 6), (3) diabetics fed casein-lactalbumin protein (CAS + DM, n = 7), and (4) diabetics fed soy protein (SOY + DM, n = 7). Before induction of diabetes, baseline measurements were collected for plasma lipid and carbohydrate measures. All monkeys consumed the same baseline diet (for 8 months prior to study) that contained casein-lactalbumin as the protein source, 0.20 mg cholesterol/kcal, and 18%, 32%, and 50% of calories as protein, fat, and carbohydrate, respectively.

Treatment diets were designed to be identical in composition except for protein type (Table 1). One diet contained casein-lactalbumin as the protein source and the other diet contained soy as the protein source. Monkeys were fed 120 kcal/kg body weight per day. All diets included moderate amounts of cholesterol (0.21 mg/kcal) to generate TC concentrations of approximately 6.5 to 7.8 mmol/L and had a nutrient content (as percent of calories) of protein, fat, and carbohydrate of 21%, 39%, and 40%, respectively. The soy protein diet (Protein Technologies, St Louis, MO) contained 9.26 mg genistein and daidzein per 120 kcal (139 mg/1,800 kcal). The monkeys were fed treatment diets for 14 weeks.

Diabetes was induced in monkeys randomized to the diabetic groups with streptozotocin 30 mg/kg according to procedures previously reported.[26] One monkey from the CAS + DM group and 2 monkeys from the SOY + DM group died following treatment with streptozotocin, leaving 6 and 5 monkeys in each group, respectively. Exogenous insulin requirements were assessed based on glucose measurements (from tail blood sampling from nonsedated animals) using glucometer measurements 3 times per week. Nonfasting blood glucose levels were maintained at 100 to 300 mg/dL. Control animals were manipulated similarly and received similar injections (approximately 0.3 mL) of saline rather than insulin, to avoid differences in animal handling between groups. All procedures involving animals were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and guidelines established by the Institutional Animal Care and Use Committee. Blood samples were obtained while the monkeys were anesthetized with ketamine hydrochloride (10 to 15 mg/kg intramuscularly). Blood samples were collected after an 18-hour fast and before administration of the morning insulin dose (in diabetic animals).

Table 1. Diet Composition (g/kg diet)

Ingredient	CAS	SOY
Casein, USP	107.4	—
Lactalbumin	105.0	—
Soy protein isolate*	—	210.0
DL-Methionine	—	5.0
Dextrin	110.0	110.0
Sucrose	109.6	109.6
Wheat flour, self-rising	200.0	200.0
Applesauce, sweetened	45.0	45.0
Alphacel	50.0	51.7
Lard	76.0	76.0
Butter, lightly salted	68.0	70.0
Safflower oil (linoleic)	22.0	16.0
Dried egg yolk	20.0	20.4
Complete vitamin mix	25.0	25.0
Ausman-Hayes mineral mix	50.0	50.0
Calcium carbonate	4.9	3.9
Calcium phosphate	7.1	7.4
Composition		
Protein (% of energy)	21.4	21.4
Carbohydrate (% of energy)	39.9	39.9
Fat (% of energy)	38.7	38.7
Saturated (% of fat)	44.9	44.9
Monounsaturated (% of fat)	36.7	36.7
Polyunsaturated (% of fat)	18.4	18.4
Cholesterol (mg/kcal)	0.21	0.21
Isoflavones (mg/1,800 kcal)	—	138.9*

*Provided by SUPRO 670-HG (Protein Technologies, St Louis, MO).

Assessment of Carbohydrate Status

In addition to glucometer measures of blood glucose for diabetic management, experimental measures of carbohydrate and insulin metabolism (fructosamine, glycated hemoglobin [GHb], and fasting glucose and insulin) were obtained at baseline and 14 weeks. Glycated plasma protein (fructosamine) concentrations were measured by Nitro Blue colorimetric methodology (Roche Biomedical, Nutley, NJ), and GHb was analyzed with automated affinity high-performance liquid chromatography (Primus, Kansas City, MO) as previously reported in monkeys.[15,27] Glucose determinations were made using the glucose oxidase method, and insulin was determined from frozen plasma by radioimmunoassay (IncStar, Minneapolis, MN).[15]

Plasma Lipids and Lipoproteins

At baseline and 14 weeks, blood was collected into vacutainer tubes containing EDTA and immediately placed on ice, and the plasma was separated by low-speed centrifugation at 4°C. TC, HDLC, and triglyceride concentrations were determined as described previously.[24] After 12 weeks of treatment, lipoprotein(a) [Lp(a)] concentrations were measured using an enzyme-linked immunosorbent assay[22] and LDL molecular weight was determined by column chromatography.[28]

LDL Metabolism

LDL particles for use in labeling were isolated from pooled plasma obtained from 8 male monkeys consuming a similar casein-lactalbumin–based diet. Three blood pools of approximately 160 mL each were collected in tubes containing aprotinin and PPACK (D-phenylalanyl-L-prolylarginine chloromethyl-ketone) at a final concentration of 25 kallikrein inhibitory U/mL and 1 µmol/L, respectively, to limit the degradation of apolipoprotein B by proteolysis and 1 mg/mL Na_2EDTA to prevent oxidation.[22,24] The serine protease inhibitor phenyl methyl sulfonyl fluoride and the antioxidant butylated hydroxytoluene (BHT) were added to isolated plasma at a final concentration of 0.5 mmol/L and 20 µmol/L, respectively. LDL (1.020 to 1.063 g/mL) was isolated by differential ultracentrifugation followed by exhaustive dialysis against buffer (0.9% NaCl and 2 mmol/L EDTA, pH 7.4).[24] LDL protein was determined[29] using bovine serum albumin as a standard.

Two different labeling protocols were used. Half of each LDL pool (approximately 40 mg) was labeled with ^{125}I using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce, Rockford, IL) and injected into animals 48 hours before necropsy to determine the arterial concentration of LDL.[23,24,30,31] The other half was coupled to ^{131}I-tyramine cellobiose (TC)-LDL[24] and injected 1 hour before necropsy to determine arterial LDL permeability.[30,31] The specific activities for ^{125}I-LDL and ^{131}I-TC-LDL were 333 ± 32 and 75 ± 50, respectively. Each labeled LDL preparation was filter-sterilized (0.45-µm Millipore filter, Bedford, MA) prior to injection to 7 or 8 monkeys within 1 week of labeling (all LDLs were dialyzed and stored in the dark at 4°C under nitrogen to avoid oxidative damage while in the laboratory).

Before injection of labeled LDL, indwelling catheters were inserted into the right femoral vein. Animals were then fitted with a nylon mesh

jacket and attached to a flexible metal tether (Alice King Chatham Medical Arts, Los Angeles, CA) to facilitate collection of multiple blood samples.[22] ^{125}I-LDL ($2.4 \pm 0.14 \times 10^8$ cpm/kg) was injected 48 hours prior to necropsy. Subsequent blood samples were collected from the catheter into tubes containing EDTA (0.1% final concentration) at 4, 10, 15, 20, 30, 40, and 60 minutes and 2, 4, 7, 20, 26, and 48 hours after injection to determine the plasma LDL decay. The plasma fractional catabolic rate (FCR) of LDL was calculated from exponents and coefficients determined by the biexponential equation fitted to data for the decline of protein-bound radioactivity in the plasma.[22] ^{131}I-TC-LDL ($5.7 \pm 1.1 \times 10^7$) was injected 1 hour prior to necropsy. Blood samples were collected 4, 10, 15, 20, 30, 40, 50, and 60 minutes after injection to determine the area under the curve (AUC) for plasma decay of ^{131}I-TC-LDL.[30,31]

After collection of the final blood sample, the animals were deeply anesthetized with sodium pentobarbital (80 mg/kg body weight intravenously). The cardiovascular system was flushed via the left ventricle with 1 L lactated Ringer solution containing 2.7 mmol/L EDTA and 50 μmol/L BHT to prevent oxidative damage. The following arteries were removed: thoracic and abdominal aorta, common iliac, femoral, carotid, internal carotid, and left anterior descending coronary arteries, and carotid bifurcations. The arteries were then placed in half-strength Karnovsky solution for 24 hours to provide adequate fixation for radiolabeled LDL.[22,23] Fixation in half-strength Karnovsky solution removes TCA-soluble ^{125}I from arterial samples.[32] Thus, most of the ^{125}I measured in the artery after 48 hours represents primarily undegraded LDL.

The arterial concentration of undegraded LDL (micrograms of LDLC per gram) was calculated as the ratio of ^{131}I radioactivity in the tissue (cpm per gram) versus plasma (cpm per milliliter) at the time of necropsy multiplied by the plasma LDLC concentration of the individual animals.[23] LDL permeability (microliters per gram per hour) was determined by dividing the tissue radioactivity (cpm per gram) by the plasma AUC (cpm per hour per milliliter) as described previously.[30,31] While some of the ^{131}I-TC-LDL that was injected 1 hour prior to necropsy may have been metabolized, TC is a residualized label and metabolites of ^{131}I-TC would be trapped intracellularly.[30,31] Radioactivity levels in all samples were corrected for overlap of the energy spectra of the 2 isotopes, for background radioactivity, and for isotopic decay. Samples were counted for approximately 60 minutes, for a 2σ counting error of less than 1.0% for ^{125}I and less than 3.0% for ^{131}I. Background was counted until a minimum of 10,000 counts accumulated, resulting in a 2σ counting error of less than 2%.

Arterial Cholesterol and Isoprostane Determinations

Arterial cholesterol content was determined in the right femoral artery biopsy obtained at baseline and in sections of the left femoral and carotid artery, carotid bifurcation, and abdominal aorta obtained at necropsy. Lipid extracts of arterial tissue were prepared using the method of Folch et al.[33] TC and free cholesterol concentrations were determined enzymatically as described previously.[34] Esterified cholesterol was calculated as the difference between measured TC and free cholesterol.

Arterial isoprostane levels were measured as an index of oxidant stress using negative ion selective ion monitoring gas chromatography–mass spectroscopy as reported previously.[35,36] Briefly, femoral artery sections from the biopsy and at necropsy were extracted using the method of Folch et al,[33] including 0.1 mmol/L DTPA, 80 μmol/L BHT, and 2 mmol/L triphenylphosphine in the extraction to prevent further oxidation. F2-isoprostane levels were measured from the organic layer using a Ribermag R10-10C quadrupole mass analyzer interfaced to a Hewlett Packard model 5890 Series II gas chromatograph (Palo Alto, CA) as described previously.[35]

Data Analysis

Data are presented as the mean ± SEM. Statistical analyses were performed using BMDP Statistical Software (Version 7.0; BMDP, Los Angeles, CA). A 2-way ANOVA was used to detect differences among treatment groups to match the 2×2 factorial study design. *P* values in the Tables are for the main effect of protein (CAS *v* SOY), the main effect of induced diabetes (no *v* yes), and the interaction of protein and diabetes (Prot × DM). If baseline measures were available, analyses were made with baseline covariates, and adjusted means are shown along with *P* values for covariate analyses. Logarithmic transformations were performed if variances among groups were unequal, and *P* values from these analyses are reported. For arterial LDL metabolism studies, each arterial site was analyzed separately first. As no interactive effect of protein or DM was found, analyses were performed to determine the overall effect of protein. Repeated-measures ANOVA was used to determine the effects of protein across all arterial sites. Statistical significance was set at a *P* level of .05 or less.

RESULTS

There were no differences in age or pretreatment measures of carbohydrate or lipid metabolism (Tables 2 and 3). As expected, the induced diabetics were hyperglycemic, with significantly increased fasting glucose and measures of antecedent glycemic control (eg, fructosamine and GHb) compared with nondiabetic monkeys (all $P < .05$; Table 2). There were no effects of protein on the measures of glycemic control. Additionally, there were no significant treatment effects for fasting plasma insulin or body weight.

Changes in plasma lipid and lipoprotein measures are shown in Table 3. Diabetic animals had significantly higher TC and LDLC ($P = .02$ and $P = .03$, respectively) and tended to have higher Lp(a) and triglyceride (both $P = .08$) compared with nondiabetics. However, soy consumption resulted in a significant reduction in TC, LDLC, and the TC:HDLC ratio and a significant increase in HDLC in both diabetic and nondiabetic animals (all $P \leq .01$; Table 3). The type of dietary protein also influenced the plasma LDL FCR. Monkeys consuming soy protein had a significantly increased plasma LDL FCR compared with monkeys consuming casein protein, regardless of their diabetic status. The plasma LDL FCR correlated negatively with plasma LDL concentrations in all animals ($r = -.52$, $P < .02$).

Arterial cholesterol determinations are shown in Table 4. There was no significant difference in the baseline (biopsy) femoral artery cholesterol content among the groups. While the short treatment period (14 weeks) resulted in a significant increase in femoral artery cholesterol content ($P < .05$), there was no treatment effect. Due to the short period of study, chosen to allow evaluations of arterial LDL metabolism, there were no significant treatment effects in the arterial cholesterol content for any artery. However, there was a tendency ($P = .08$) for reduced cholesterol content in the abdominal aorta with soy and a tendency for an interaction effect ($P = .09$) between soy and diabetes. Similar findings were found for free and esterified cholesterol (data not shown).

Data for the arterial LDLC concentration (Table 5) and permeability (Table 6) are presented for each arterial site. Soy consumption resulted in a significant reduction in the arterial LDLC concentration (which represents primarily undegraded LDL) in the internal carotid artery, carotid bifurcation, and

Table 2. Effect of Soy and Diabetes on Carbohydrate Measures and Body Weight (mean ± SEM)

Parameter	SOY (n = 6)	SOY + DM (n = 5)	CAS (n = 6)	CAS + DM (n = 6)	*P* (Prot/DM/ Prot × DM)
Age (yr)	18 ± 1	19 ± 1	16 ± 1	17 ± 1	NS
Glucose (mg/dL)					
Baseline	44 ± 1	51 ± 2	46 ± 2	44 ± 3	.38/.33/.08
14 weeks	56 ± 3	175 ± 56	56 ± 2	173 ± 27	.97/.0005/.98
Insulin (μU/mL)					
Baseline	25 ± 4	52 ± 16	29 ± 5	26 ± 4	.19*/.13*/.08*
14 weeks	28 ± 8	41 ± 14	35 ± 7	34 ± 7	NS
Fructosamine (μU/mL)					
Baseline	158 ± 9	151 ± 7	155 ± 5	159 ± 2	NS
14 weeks	188 ± 8	263 ± 31	172 ± 7	278 ± 34	.98/.001*/.51
GHb (%)					
Baseline	5.4 ± 0.2	5.4 ± 0.4	5.5 ± 0.3	5.1 ± 0.3	NS
14 weeks	5.6 ± 0.3	7.2 ± 0.8	5.4 ± 0.2	7.0 ± 1.0	.74/.02*/.98
Body weight (kg)					
Baseline	5.0 ± 0.1	5.2 ± 0.2	5.3 ± 0.3	4.9 ± 0.2	NS
14 weeks	5.4 ± 0.1	5.1 ± 0.2	5.4 ± 0.2	5.2 ± 0.2	NS

*Treatment measures and *P* values adjusted for baseline measures.

abdominal aorta, with similar trends in all other arterial sites. Soy consumption resulted in a similar reduction in the arterial LDLC concentration in both diabetics and nondiabetics, with no significant interactive effect. Thus, data for diabetics and nondiabetics were combined and are shown in Fig 1A. There was a significant effect of protein across all sites for the arterial LDLC concentration ($P = .025$), with an average reduction of 50%. There were also regional differences (effect of site, $P < .0001$), with higher LDLC concentrations in the coronary artery and carotid bifurcation and lower concentrations in the aorta and iliac arteries.

Soy also reduced arterial LDL permeability, but only in the carotid bifurcation and internal carotid artery (Table 6). As with the arterial LDLC concentration, there was no effect of diabetes and no interactive effect between protein and soy. However, there was a reduction in the carotid bifurcation (28%) and internal carotid artery (33%) with soy.

To determine the amount of LDLC delivered to arterial sites, LDL permeability was multiplied by the plasma LDLC concentration (Fig 1B). Across all arterial sites, soy reduced the delivery of LDL cholesterol by 38% ($P = .05$), with significant reductions in the carotid bifurcation (50%) and internal carotid (56%) and coronary (49%) arteries. There was also a significant effect of arterial site, again greatest in the coronary artery and

Table 3. Effect of Soy and Diabetes on Plasma Lipid and Lipoprotein Measures (mean ± SEM)

Parameter	SOY (n = 6)	SOY + DM (n = 5)	CAS (n = 6)	CAS + DM (n = 6)	*P* (Prot/DM/ P × DM)
TC (mg/dL)*					
Baseline	405 ± 54	338 ± 53	340 ± 54	375 ± 75	NS
14 weeks	211 ± 30	288 ± 33	304 ± 30	384 ± 30	.007/.02/.95
HDLC (mg/dL)*					
Baseline	39 ± 6	35 ± 9	42 ± 7	37 ± 8	NS
14 weeks	71 ± 6	66 ± 6	45 ± 6	57 ± 6	.01/.55/.15
LDLC (mg/dL)*					
Baseline	367 ± 58	303 ± 59	298 ± 58	338 ± 77	NS
14 weeks	139 ± 33	223 ± 36	259 ± 33	326 ± 32	.004/.03/.81
TC:HDLC*					
Baseline	12.5 ± 3.0	12.3 ± 3.1	9.9 ± 2.4	12.8 ± 3.6	NS
14 weeks	3.5 ± 1.3	4.6 ± 1.4	7.5 ± 1.3	9.2 ± 1.3	.005/.31/.85
Triglyceride (mg/dL)*					
Baseline	25 ± 2	18 ± 2	27 ± 7	28 ± 7	NS
14 weeks	27 ± 18	84 ± 20	21 ± 18	34 ± 18	.15/.08/.26
LDL molecular weight (μg/μmol)*					
Baseline	4.0 ± 0.43	3.6 ± 0.14	3.7 ± 0.26	3.6 ± 0.16	NS
12 weeks	3.9 ± 0.18	3.9 ± 0.20	3.6 ± 0.18	3.5 ± 0.18	NS
Lp(a)					
12 weeks	19 ± 1	27 ± 3	23 ± 3	35 ± 9	.29/.08/.68
Plasma LDL FCR (pools/h)					
14 weeks	.035 ± .002	.030 ± .003	.027 ± .003	.025 ± .003	.04/.2/.46

*Treatment measures and *P* values adjusted for baseline measures.

Table 4. Effect of Treatment on Arterial Cholesterol Content (mg/g, mean ± SEM)

Site	SOY (n = 6)	SOY + DM (n = 5)	CAS (n = 6)	CAS + DM (n = 6)	*P* (Prot/DM/ Prot × DM)
Baseline femoral	0.97 ± 0.18	1.06 ± 0.53	0.75 ± 0.10	1.22 ± 0.28	NS
Treatment femoral*	2.35 ± 0.95	3.65 ± 2.37	2.25 ± 0.75	2.10 ± 0.50	NS
Carotid bifurcation	7.55 ± 2.43	6.39 ± 1.42	8.64 ± 1.83	8.82 ± 3.50	NS
Carotid	5.86 ± 1.89	3.27 ± 1.33	6.73 ± 2.47	5.92 ± 1.67	NS
Abdominal aorta	4.81 ± 1.15	2.89 ± 0.24	4.87 ± 0.90	6.53 ± 1.20	.08/.90/.09

*Treatment measures and *P* values adjusted for baseline measures.

least in the internal carotid artery. There was a significant group × site interaction, with little effect at the carotid (4%), whereas all other sites showed a range between 30% and 56%.

No significant effects were found for femoral artery isoprostane concentrations at baseline (1.92 ± 0.31, 2.04 ± 0.33, 2.22 ± 0.36, and 2.80 ± 0.57 for SOY, SOY + DM, CAS, and CAS + DM, respectively). After 14 weeks of treatment, these levels were significantly increased ($P = .02$) and there was a tendency ($P = .10$) for reduced oxidative stress with soy consumption (2.36 ± 0.43, 3.00 ± 0.47, 3.51 ± 0.42, and 3.40 ± 0.44 for SOY, SOY + DM, CAS, and CAS + DM, respectively).

DISCUSSION

We have previously shown that consumption of soy protein compared with casein-lactalbumin improves plasma lipoprotein concentrations and coronary artery atherosclerosis in juvenile male monkeys.[16] In this study, we extended those findings to include adult male monkeys. In addition to significant improvements in plasma lipoprotein concentrations, we found that soy protein compared with casein reduces the progression of atherosclerosis by decreasing LDL delivery to the arteries of both control and diabetic male monkeys. For most arterial sites, this reduced LDL delivery could be accounted for by the lower concentration of LDLC in plasma. However, for 2 arterial sites, reduced arterial permeability contributed to the reduced LDL delivery. These changes in arterial LDL metabolism would be expected to result in a decreased progression of atherosclerosis.

Soy consumption significantly reduced TC, LDLC, and the TC:HDLC ratio and increased HDLC concentrations as compared with casein. Soy also increased the whole-body plasma FCR (Table 3). This is consistent with previous reports in which both an increase in LDL receptor mRNA was found in mononuclear cells[37] and an increase in LDL receptor activity[38,39] was found with soy consumption. There is evidence that components removed by alcohol extraction may mediate LDL receptor regulation. In a study by Kirk et al,[40] C57BL/6 mice that were fed alcohol-extracted soy protein were found to have higher LDLC and atherosclerosis compared with C57BL/6 mice fed intact soy protein, while no difference was found in LDLC or atherosclerosis between either diet in LDL receptor–deficient mice, suggesting the involvement of the LDL receptor in the regulation of LDLC by soy protein consumption. In a study in postmenopausal women, Baum et al[37] found increases in LDL receptor mRNA in mononuclear cells with increasing amounts of isoflavones (in intact soy protein), suggesting a dose-response effect of isoflavones.

We have previously found that mammalian estrogens also increase the LDL FCR,[24,41] which was associated with an increased uptake of LDL particles by the liver. With mammalian estrogens, despite the increased uptake of LDL particles by the liver, hepatic cholesterol concentrations are reduced and biliary cholesterol production is increased.[42] Our preliminary data (Wagner et al, unpublished, October 1999) suggest that similar effects on hepatic cholesterol metabolism occur with soy as with estrogens, resulting in one mechanism for decreasing plasma cholesterol concentrations. In addition, we have found that soy reduces dietary cholesterol absorption,[43] resulting in a second mechanism for the decrease in lipids.

To determine changes in arterial metabolism that might contribute to a reduced progression of atherosclerosis rather than changes that result from differences in atherosclerosis,[44] we used a short (14-week) period of treatment. As in our earlier studies with mammalian estrogens,[2,22] this short study period resulted in minor treatment effects in atherosclerosis as assessed by arterial cholesterol (Table 4). Also, as these animals were used in previous studies of dietary cholesterol, we obtained a baseline femoral artery sample and baseline lipoprotein measures to ensure that there were no differences in pretreatment variables. As expected, there was an increase in cholesterol content with time in the femoral artery, but no treatment effect. Thus, results for the studies of arterial lipoprotein metabolism

Table 5. Effect of Treatment on Arterial LDL Concentration (μg LDLC/g, mean ± SEM)

Site	SOY (n = 6)	SOY + DM (n = 5)	CAS (n = 6)	CAS + DM (n = 6)	*P* (Prot/DM/ Prot × DM)
Coronary artery	353 ± 131	305 ± 102	595 ± 195	615 ± 179	.10/.93/.84
Carotid	96 ± 38	93 ± 40	185 ± 60	180 ± 63	.11/.94/.98
Carotid bifurcation	82 ± 26	69 ± 18	215 ± 48	230 ± 81	.01/.98/.80
Internal carotid	33 ± 8	28 ± 5	76 ± 18	86 ± 26	.009/.90/.67
Thoracic aorta	128 ± 53	71 ± 21	164 ± 38	227 ± 71	.08/.96/.26
Abdominal aorta	97 ± 23	67 ± 14	141 ± 34	182 ± 49	.03/.87/.31
Iliac	100 ± 45	58 ± 21	139 ± 46	157 ± 41	.11/.78/.48
Femoral	138 ± 34	131 ± 47	154 ± 36	269 ± 70	.14/.29/.23

Table 6. Effect of Treatment on Arterial LDL Permeability (μL/g · h, mean ± SEM)

Site	SOY (n = 6)	SOY + DM (n = 5)	CAS (n = 6)	CAS + DM (n = 6)	*P* (Prot/DM/ Prot × DM)
Coronary artery	0.711 ± 0.211	0.425 ± 0.037	0.691 ± 0.069	0.630 ± 0.096	NS
Carotid	0.241 ± 0.033	0.602 ± 0.356	0.307 ± 0.015	0.256 ± 0.016	NS
Carotid bifurcation	0.218 ± 0.024	0.243 ± 0.024	0.362 ± 0.027	0.287 ± 0.023	.001/.32/.06
Internal carotid	0.143 ± 0.033	0.144 ± 0.024	0.225 ± 0.020	0.201 ± 0.021	.01/.65/.62
Thoracic aorta	0.234 ± 0.040	0.218 ± 0.021	0.280 ± 0.018	0.242 ± 0.020	NS
Abdominal aorta	0.261 ± 0.011	0.256 ± 0.037	0.298 ± 0.030	0.274 ± 0.035	NS
Iliac	0.431 ± 0.202	0.184 ± 0.028	0.262 ± 0.042	0.276 ± 0.058	NS
Femoral	0.560 ± 0.177	0.263 ± 0.072	0.361 ± 0.075	0.338 ± 0.032	NS

can be compared among treatments without any confounding by differences in the extent of atherosclerosis.

The majority of cholesterol accumulating in arteries is derived from plasma lipoproteins.[45,46] LDL entering the arteries may be retained in the extracellular space, degraded by cells in the artery, or efflux from the artery[30,32,44,47]; these removal processes together with the rate of LDL entry determine arterial concentrations of LDL. In this study, we used 2 different LDL labeling procedures to determine total arterial concentrations of LDLC (^{125}I-LDL injected 48 hours prior to necropsy, which represents primarily undegraded LDL) and LDL permeability (^{131}I-TC-LDL injected 1 hour prior to necropsy). The ^{131}I measured in the arteries (microliters per gram per hour), represents arterial permeability to LDL and is related to changes in endothelial function/permeability. If this parameter is multiplied by the plasma LDLC concentration, then an estimate of total LDLC delivery (micrograms of LDLC per gram per hour) to the artery wall is obtained.[30,31]

Soy consumption resulted in significant reductions in the arterial LDLC concentration in a number of arterial sites (Table 5), with an overall effect for all arterial sites when analyzed by repeated-measures ANOVA (Fig 1A). This reduction in the

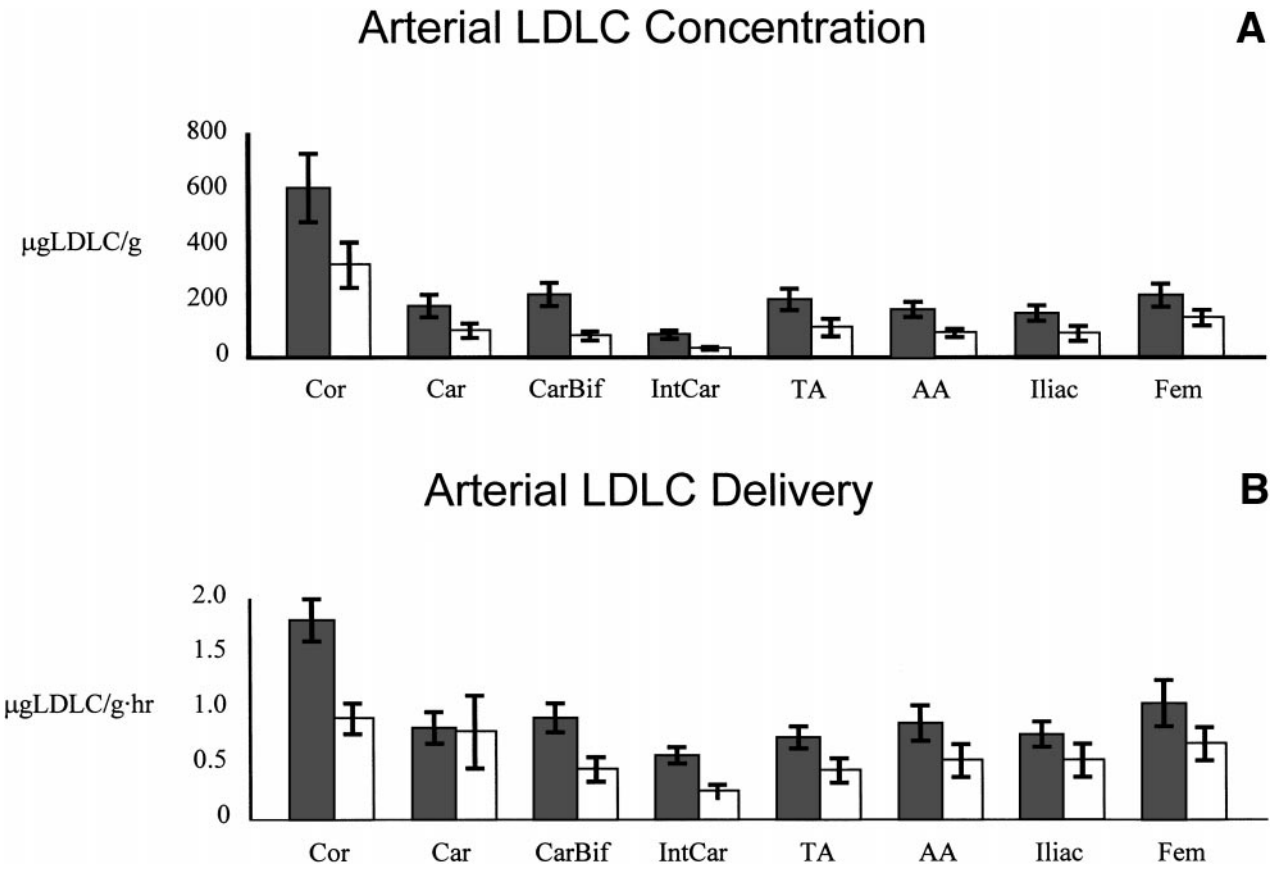

Fig 1. Effect of casein and soy protein consumption on arterial LDLC concentration (A) and delivery (B) for samples from the coronary (Cor), carotid (Car), carotid bifurcation (CarBif), internal carotid (IntCar), thoracic aorta (TA), abdominal aorta (AA), iliac, and femoral (Fem) arteries for monkeys consuming casein (■) and soy (□). Arterial LDLC concentration: protein × tissue site ANOVA: effect of protein, *P* = .025; effect of site, *P* < .0001; effect of protein × site interaction, *P* = .09. Arterial LDL delivery: protein × tissue site ANOVA: effect of protein, *P* = .05; effect of site, *P* < .0001; effect of protein × site interaction, *P* = .03.

arterial LDLC concentration could be accounted for by the reduced concentration of LDLC in plasma, as the arterial concentration of LDL expressed as a percentage of the plasma level did not differ between groups (data not shown). There was no significant overall effect of dietary protein on arterial permeability to LDL (Table 6). However, when LDL delivery to the artery was determined (Fig 1B), there was an overall effect of soy across all arterial sites. This reduced delivery of LDLC across arterial sites with soy treatment was due to reduced plasma LDLC concentrations. Thus, the reduced concentration of LDL in plasma was translated into beneficial effects at the level of the artery and would be expected to reduce atherosclerosis.

For 2 individual arterial sites, the internal carotid artery and carotid bifurcation, arterial permeability to LDL was significantly reduced (Table 6), suggesting an effect of soy at the endothelial cell level, independent of plasma LDLC. It is interesting that the arterial sites associated with stroke (those found in the head and neck) seemed to have the greatest protection with soy. Although previous studies have found the permeability to LDL to differ among arterial regions, this heterogeneity only partially correlated with regional variation in the susceptibility to atherosclerosis.[30,31,44] Further studies will be needed to determine if soy consumption may also be protective against atherothrombotic stroke.

Another mechanism for decreased vascular disease with soy may be its antioxidant properties. Some studies have reported antioxidant properties of isoflavones or soy,[48-51] whereas others have not.[52,53] We reported previously that soy, compared with casein, reduces arterial lipid peroxidation in female monkeys.[15] F2-isoprostanes are now recognized as a specific and sensitive in vivo measure of oxidative stress[36,54] and have been shown to be increased with atherosclerosis[55] and diabetes.[56] Although not significant, there was a trend ($P = .10$) for reduced isoprostanes with soy. A recent study[53] reported no effect of isoflavone administration on urinary isoprostane levels. However, as discussed later, the addition of isoflavones does not appear to have the same cardiovascular benefits as consumption of intact soy protein.

Diabetes is a major risk factor for atherosclerosis, increasing the risk of cardiovascular disease about 2- to 4-fold.[25] Some of this increased risk is due to the adverse effects of diabetes on plasma lipids and lipoproteins.[57] As with human diabetics, monkeys with experimentally induced diabetes were also dyslipidemic, with significantly increased TC and LDLC and a tendency ($P = .08$) for increased triglyceride and Lp(a) (Table 3). As in the control monkeys, soy consumption also resulted in improved plasma lipoprotein concentrations in diabetic monkeys. These beneficial effects on plasma lipoproteins resulted in a reduced arterial LDL concentration in both diabetics and nondiabetics. Although differences among groups were not significant, in general, LDL concentrations across all arterial sites tended to be the greatest for the CAS + DM group and the least for the SOY + DM group, explaining the lack of overall effect of diabetes (Table 5). Similarly, the abdominal aortic cholesterol content tended to be greatest in the CAS + DM group and least in the SOY + DM group, with a tendency for a protein × diabetes effect ($P = .09$).

It is possible that with a longer treatment period, the arterial effects of diabetes would be more apparent. For example, we used the streptozotocin-induced diabetic monkey model previously and found that after 6 months of hyperglycemia, atherosclerosis was increased compared with control monkeys.[26] In that study, all monkeys consumed a casein-based diet very similar to the casein diet in this study. In the previous study, the increased atherosclerosis was also associated with an increased accumulation of radiolabeled TC-LDL, which was greatest and significant only in the femoral artery.[26] Interestingly, the greatest increase in arterial LDL (75%) in this study was in the femoral artery of animals in the CAS group. The increased LDL concentration in the femoral artery may reflect an increase in peripheral vascular disease noted in diabetics.[25]

In this study, we have found that soy protein as compared with casein is as atheroprotective in adult male monkeys as it is in female monkeys.[15] This may explain the decreased cardiovascular disease in Asian men compared with men eating a Western diet.[19] While we found a decrease in arterial LDLC concentrations across all arterial sites, the greatest decrease with soy was in the carotid bifurcation and internal carotid artery, suggesting that soy consumption will also decrease stroke due to atherothrombotic disease. Further, the cardiovascular benefits of soy were also present in diabetic monkeys.

While the goal of this study was not to determine which component of soy is responsible for the cardiovascular protection, we have shown that the removal of isoflavones by alcohol extraction decreases the benefit of soy protein.[16] However, the addition of isoflavones to a casein-based diet resulted in no improvement in plasma lipoproteins[58] and no improvement in arterial lipoprotein metabolism.[59] Thus, there appears to be some requirement for both the soy protein and the isoflavones. Studies are ongoing to determine the active component of soy.

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