Dietary Soy Protein and Estrogen Replacement Therapy Improve Cardiovascular Risk Factors and Decrease Aortic Cholesteryl Ester Content in Ovariectomized Cynomolgus Monkeys

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Estrogen replacement therapy (ERT) decreases the progression of coronary artery atherosclerosis in monkeys. Dietary soy protein also retards the progression of atherosclerosis relative to animal proteins such as casein. Soy protein contains weakly estrogenic compounds called isoflavones or phytoestrogens that may be responsible for the cardioprotective effects. This study was designed as a 2 × 2 factorial to determine the magnitude of soy protein's effects on cardiovascular risk factors relative to casein and lactalbumin, with or without estradiol treatment. Ovariectomized female monkeys were randomized to four treatment groups based on past dietary cholesterol consumption, their origin, and past reproductive history, and studied for 7 months. The animals were divided into (1) a group fed casein and lactalbumin as the protein source (n = 14), (2) a group fed casein and lactalbumin as the protein source plus 17β-estradiol (E2) (n = 13), (3) a group fed soybean protein isolate as the protein source (n = 11), and (4) a group fed soybean protein isolate as the protein source plus E2 (n = 10). Soy protein compared with casein consumption resulted in a significant improvement in plasma lipid and lipoprotein concentrations, a significant improvement in insulin sensitivity and glucose effectiveness as determined by minimal-model analyses, and a decrease in arterial lipid peroxidation. E2-treated monkeys had a significant reduction in fasting insulin levels and insulin to glucose ratios, total body weight, and amounts of abdominal fat, and had smaller low-density lipoprotein (LDL) particles. In addition, E2 treatment resulted in a significant reduction ($P = .001$) in aortic cholesteryl ester content. A similar trend ($P = .14$) was found for soy protein compared with casein. There also was a significant interaction ($P = .02$) with soy and E2, such that animals consuming soy protein + E2 had the least arterial cholesteryl ester content. These results suggest that both ERT and dietary soybean protein have beneficial effects on cardiovascular risk factors. Interestingly, the two treatments affected different risk factors and together resulted in the greatest reduction in arterial cholesterol content. Further studies are needed to determine the active component of the soy protein and to assess its long-term effects on the cardiovascular system and other organ systems (such as the bones and reproductive system).

ALTHOUGH CORONARY HEART DISEASE (CHD) is more common in men than in women, it remains the leading cause of death in women in western societies. The rate of CHD mortality is much lower among Asians (both men and women) compared with Westerners.[1] One major difference between the two populations is dietary: Asians obtain most of their protein from soybeans, whereas for Westerners meat products are the primary protein source. Although the antiatherogenic effects of dietary soy protein in comparison to casein have been known for many years,[2] the active component(s) responsible for these effects remain uncertain. Speculations have been made concerning changes in amino acid composition and/or phosphorylation, protein digestibility, saponins,[3,4] and isoflavones.[5]

Isoflavones are a group of compounds found in soybeans that have estrogenic activity, and thus have been termed phytoestrogens. The two primary estrogenic compounds in soybeans are genistein and daidzein (and their conjugates).[6] These compounds bind to the estrogen receptor with low affinity and, depending on the tissue, may exert either estrogenic or antiestrogenic effects.[7] In addition to exerting effects through the estrogen receptor, genistein also exerts biologic effects by inhibiting tyrosine kinase activity and subsequent signal transduction.[8,9]

In a recent study,[5] we compared plasma lipid and lipoprotein responses in monkeys fed soy protein either containing the phytoestrogens or with the phytoestrogens removed by alcohol extraction. The phytoestrogen-intact soy protein diet (1) reduced total and low-density lipoprotein (LDL) plus very–low-density lipoprotein cholesterol concentrations in both male and female monkeys, and (2) increased high-density lipoprotein (HDL) cholesterol (HDLC) concentrations in females. These results suggest that the components in the unextracted soy protein are active agents in improving plasma lipids.

The studies just described provide evidence that soy phytoestrogens may be cardioprotective. The purpose of the present study was to investigate further, using the same animal model, the effects of dietary soy protein on cardiovascular risk factors and to determine whether estrogen, with its well-known cardioprotective effects,[10,11] modified the effects of dietary soy protein with its phytoestrogens. We compared the effects of soy protein isolate versus casein and lactalbumin alone and in combination with 17β-estradiol (E2) on plasma lipids, carbohydrate metabolism, obesity, and arterial lipid peroxidation and cholesterol accumulation.

MATERIALS AND METHODS

Design

Fifty-six adult female cynomolgus monkeys (*Macaca fascicularis*) were initially assigned to this study. These monkeys had been part of a breeding colony supported by the National Heart, Lung, and Blood

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Submitted August 27, 1996; accepted December 16, 1996.

Supported in part by Grant No. KO1RR00072 (J.D.W.) from the National Center for Research Resources, Grant No. PO1HL45666 from the National Heart, Lung, and Blood Institute, and Grants No. AG00578 (W.T.C.) and AG10816 (W.T.C. and J.D.W.) from the National Institute on Aging and the Office for Research on Women's Health, all from the National Institutes of Health, Bethesda, MD.

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0026-0495/97/4606-0017$03.00/0

Institute at the Bowman Gray School of Medicine. The monkeys were randomized into four treatment groups (n = 14 each) based on origin (colony-born *v* caught wild), lifetime dietary cholesterol consumption (cholesterol days = number of days eating dietary cholesterol × amount of dietary cholesterol in milligrams cholesterol per kilocalorie), and reproductive history (number of births and time since ovariectomy). The monkeys were 5 to 20 years of age. Eight monkeys had been ovariectomized 1 year previously, and the remainder were ovariectomized 3 weeks before the start of treatment. Six monkeys died of causes unrelated to experimental procedures early in the study, and two monkeys were found at necropsy to have some ovarian tissue and elevated estradiol levels. Although inclusion of these two animals in the data analysis did not result in a major change for any variable, data are reported for only 48 monkeys.

The treatment period was 7 months. All monkeys consumed a moderately atherogenic diet (0.2 mg cholesterol/kcal, 37% calories from fat) before the study and consumed diets containing the same amount of cholesterol and fat throughout the study. During the experimental period, the diets contained the following: (1) casein and lactalbumin as the protein source (casein group, n = 14), (2) casein and lactalbumin as the protein source and micronized E2 (Estrace; Mead Johnson, Princeton, NJ) added to the diet at the equivalent of 1 mg/d for a woman (per 1,800 kcal) (casein + E2 group, n = 13), (3) soybean protein isolate as the protein source with an isoflavone dose of 148 mg/d for a woman (per 1,800 kcal) (soy group, n = 11), and (4) soybean protein isolate as the protein source and E2 as just described (soy+E2 group, n = 10). Dietary compositions are depicted in Table 1.

Blood samples were collected for clinical measurements during the baseline period and at several time points during the treatment period. Before necropsy, animals were sedated with ketamine hydrochloride (15 mg/kg intramuscularly) and then anesthetized deeply with sodium pentobarbital (80 mg/kg body weight intravenously) before exsanguination. The cardiovascular system was flushed via the left ventricle with 1 L lactated Ringer's solution. A 3-cm section of the abdominal aorta (with adventitia removed) was excised and stored frozen at −70°C for analyses.

All procedures involving animals were conducted in compliance with state and federal laws, standards of the Department of Health and Human Services, and guidelines established by the Institutional Animal Care and Use Committee. Ovariectomies were performed while the animals were sedated with ketamine hydrochloride (15 mg/kg body weight intramuscularly) and butorphanol tartrate (0.05 mg/kg intramuscularly). Blood sampling and computed tomographic (CT) scans were performed while the animals were sedated with ketamine hydrochloride (15 mg/kg intramuscularly).

Clinical Measurements

Blood samples for determination of plasma lipids were collected into tubes containing EDTA (final concentration, 1 mg/mL) after the animals were fasted overnight. Total plasma cholesterol (TPC),[12] HDLC,[13] and triglycerides[14] were determined at baseline and after 2, 4, and 6 months of treatment. Analyses for TPC, HDLC, and triglycerides are in full standardization with the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program. Mean LDL particle size (molecular weight) was determined by column chromatography[15] after 6 months of treatment.

Blood samples for determination of glucose, insulin, and glycated plasma protein (fructosamine) were collected at baseline and after 4 and 7 months of treatment. As described previously,[16] glucose concentrations were determined by the glucose oxidase method on a Glucose Analyzer 2 (Beckman Instruments, Brea, CA), insulin concentrations were measured by radioimmunoassay (Incstar, Stillwater, MN), and fructosamine concentrations were measured by the second-generation fructosamine assay.

Insulin sensitivity and glucose effectiveness were determined after 7 months of treatment by the frequently sampled intravenous glucose tolerance test (minimal-model analysis) as previously described,[16] with the addition of a third-phase insulin infusion at 20 minutes.[17] In brief, after baseline sampling, glucose (0.5 g/kg) is injected intravenously followed by sampling every minute from 2 to 8 minutes and every 2 minutes from 8 to 20 minutes. Insulin (0.015 U/kg) is then injected intravenously followed by frequent sampling up to 240 minutes. Minimal-model analyses were made in a subset of animals. Because this analysis requires greater than 20 mL blood and other blood samples were being taken regularly, we decided to study only animals weighing more than 3 kg.

Plasma concentrations of estradiol were measured by radioimmunoassay using commercially available kits (Diagnostic Products, Los Angeles, CA) at the laboratory of Dr Mark Wilson (Yerkes Regional Primate Center, Atlanta, GA).[18] Samples were collected 4 hours after feeding (peak plasma concentrations of estradiol) after 5 months of treatment.

Body weight, body mass index, and subscapular and triceps skinfold thicknesses were determined at baseline and at 2 and 6 months as described previously.[16] Body mass index (kilograms per meter squared) was estimated as the ratio of body weight in kilograms to trunk length in meters squared. Subscapular to triceps ratios were calculated as an

Table 1. Diet Composition

Ingredient/ Composition	Casein	Casein + E2	Soy	Soy + E2
Ingredient (g/100 g)				
Casein	10.10	10.10	—	—
Lactalbumin	10.10	10.10	—	—
Soy protein isolate	—	—	20.00	20.00
Methionine	—	—	0.50	0.50
Dextrin	9.20	9.20	9.20	9.20
Sucrose	9.20	9.20	9.20	9.20
Wheat flour	25.80	25.80	26.00	26.00
Wheat bran	5.60	5.60	5.60	5.60
Applesauce	4.50	4.50	4.50	4.50
Lard	5.30	5.30	5.30	5.30
Beef tallow	4.00	4.00	4.30	4.30
Butter	3.50	3.50	3.50	3.50
Safflower oil	2.30	2.30	1.70	1.70
Olive oil	0.10	0.10	—	—
Dried egg yolk	2.20	2.20	2.20	2.20
Complete vitamin mix	2.50	2.50	2.50	2.50
Modified Ausman-Hayes mineral mix	5.00	5.00	5.00	5.00
Calcium carbonate	0.60	0.60	0.50	0.50
E2 (micronized)	—	0.00023	—	0.00023
Composition*				
Protein	21.2	21.2	21.2	21.2
Carbohydrate	42.2	42.2	42.2	42.2
Fat	36.6	36.6	36.6	36.6
Saturated	15.7	15.7	15.7	15.7
Monounsaturated	13.8	13.8	13.8	13.8
Polyunsaturated	7.1	7.1	7.0	7.0
Cholesterol (mg/kcal)	0.2	0.2	0.2	0.2
Phytoestrogens (mg/1,800 kcal)	—	—	148.4	148.4
Estradiol (mg/1,800 kcal)	—	1.01	—	1.01

*Expressed as % kcal unless otherwise noted.

indication of central fat distribution. CT scans were made at 6 months (General Electric Model CT9800, 120 kVp, 120 MAS, 2-second scan time; General Electric, Stamford, CT). A single 1-cm scan at the umbilicus was performed for each animal. The quantity of abdominal fat was determined using a General Electric CT software package and a density range of −140 to −40 Hounsfield units to identify fat.[19]

Arterial Assessments

Lipid extracts of samples of abdominal aorta were prepared using chloroform/methanol (2:1 vol/vol) by the method of Folch et al.[20] Total and free cholesterol concentrations were then determined enzymatically.[21] Cholesteryl ester content was determined as the difference between measured total and free cholesterol.

Lipid peroxidation was assessed in aortic samples by the thiobarbituric acid–reactive substances (TBARS) method as described previously.[22,23] Briefly, tissue homogenates (0.1 mL) were combined with 0.2 mL 8.1% sodium dodecyl sulfate, 1.5 mL 20% acetic acid (pH 3.5), and 1.5 mL 0.8% thiobarbituric acid, diluted to 4 mL with distilled water, and heated at 95°C for 1 hour. One milliliter of water and 5 mL *n*-butanol:pyridine (15:1 vol/vol) were added. Absorbance at 532 nm was measured in the *n*-butanol layer since malondialdehyde (MDA), a secondary product of lipid peroxidation, changes color in the presence of thiobarbituric acid. Values (TBARS) are reported as nanomoles of MDA equivalents per gram wet weight, as described previously.[23]

Statistical Analyses

Reported values are the mean ± SEM. All analyses were made using BMDP Statistical Software (Programs 2V and 7D, Version 7.0; BMDP, Los Angeles, CA). Logarithmic transformation of arterial cholesterol values and an inverse transformation of TBARS values (1/TBARS) were used to reduce skewness and equalize variances. Values for arterial cholesterol and TBARS are untransformed values in the original units derived from retransforming the means of the transformed data. Data were analyzed using two-way ANOVA or analysis of covariance to match the 2 × 2 factorial study design. Thus, *P* values in the tables are for the main effect of protein (casein *v* soy), the main effect of E2 treatment (no *v* yes), and the interaction of protein and E2 (P × E2). Pearson product-moment correlations were used to assess relationships among variables. Statistical significance was set at 95%.

RESULTS

The group means for age, past dietary cholesterol consumption as assessed by cholesterol days, and estradiol concentration are shown in Table 2. Although there were no significant differences among treatment groups with respect to age and cholesterol days ($P > .34$), the number of cholesterol days was considerably higher in the soy+E2 group. As expected, estradiol concentrations were significantly higher in the casein+E2 and soy+E2 groups compared with those not receiving E2 treatment ($P < .001$). However, there was also an effect of protein, such that animals consuming soy had lower estradiol concentrations.

Group means for plasma lipid and lipoprotein concentrations in the baseline and treatment period are shown in Table 3. TPC concentrations were significantly lower in monkeys consuming the soy protein diet. E2 treatment was associated with significantly higher triglyceride concentrations, as well as smaller LDL particles. HDLC concentrations and the TPC:HDLC ratio were affected by both protein source and E2. The soy diet was associated with significantly higher HDLC concentrations, whereas E2 treatment was associated with lower HDLC concentrations. There was also a significant interaction between protein source and E2 for HDLC concentrations, such that HDLC concentrations were higher in monkeys consuming soy with no E2 treatment.

Assessments of carbohydrate metabolism are shown in Table 4. Monkeys treated with E2 had significantly lower fasting insulin concentrations and insulin to glucose ratios. There was no effect of E2 or protein source on fasting glucose or fructosamine levels. In the subset of animals studied using minimal-model analysis, insulin sensitivity was 50% to 90% higher in animals consuming the soy diet compared with the casein groups (Fig 1). In addition, glucose effectiveness was significantly improved in animals fed the soy diet, especially the soy+E2 group, where there was a significant interactive effect ($P = .04$).

The effects of treatment on body weight and body fat measurements are shown in Table 5. Body weight, body mass index, and the subscapular to triceps ratio were all significantly lower in monkeys treated with E2. In addition, body fat (total abdominal fat and subcutaneous and intraabdominal fat) as assessed by CT scans was significantly lower with E2 treatment. Although not statistically significant, there was a similar trend toward lower values for the soy protein group compared with the casein groups.

Measures of adiposity were highly correlated with each other for all monkeys. For example, intraabdominal fat was significantly associated with both total abdominal and subcutaneous fat ($r = .99$ and $r = .94$, respectively, $P < .001$), body weight ($r = .92$, $P < .001$), and skinfold ratio ($r = .51$, $P < .001$). Measures of adiposity were also related to fasting insulin concentrations, with the strongest association with intraabdominal fat ($r = .77$, $P < .001$). Insulin sensitivity determined by the minimal model was negatively associated with adiposity; however, this relationship was strongest in animals treated with E2 ($r = -.35$ for all animals, $P < .05$; $r = -.73$ and $r = -.63$ for casein+E2 and soy+E2 groups; and $r = -.19$ and $r = -.46$ for casein and soy groups).

Arterial assessments are depicted in Table 6 and Fig 2. Arterial cholesterol measurements are shown as unadjusted means and as means adjusted for cholesterol days and the baseline TPC:HDLC ratio. Cholesterol days and the TPC:HDLC ratio were used as covariates for arterial cholesterol,

Table 2. Age, Lifetime Dietary Cholesterol History, and E2 Concentrations

Parameter	Casein (n = 14)	Casein + E2 (n = 13)	Soy (n = 11)	Soy + E2 (n = 10)	*P* (protein/E2/P × E2)
Age (yr)	11.3 ± 1.2	10.6 ± 1.2	12.4 ± 1.6	10.9 ± 1.3	.61/.43/.78
Cholesterol days*	584 ± 145	607 ± 151	568 ± 151	856 ± 203	.47/.34/.42
E2 (pmol/L)	28 ± 16	885 ± 51	2 ± 2	697 ± 92	.03/<.001/.10

NOTE. Results are the mean ± SEM.

*Number of days eating dietary cholesterol × amount of dietary cholesterol.

Table 3. Effects of Treatment on Plasma Lipid and Lipoprotein Measurements

Parameter	Casein (n = 14)	Casein + E2 (n = 13)	Soy (n = 11)	Soy + E2 (n = 10)	P (protein/E2/P × E2)
TPC (mmol/L)					
Baseline*	6.00 ± 0.65	6.70 ± 0.70	6.26 ± 0.65	6.28 ± 0.80	NS
Treatment†	6.83 ± 0.39	7.63 ± 0.41	4.73 ± 0.44	5.35 ± 0.47	<.001/.11/.73
HDLC (mmol/L)					
Baseline*	1.86 ± 0.13	1.78 ± 0.18	1.84 ± 0.13	1.71 ± 0.23	NS
Treatment†	1.73 ± 0.10	1.03 ± 0.10	1.84 ± 0.10	1.73 ± 0.13	<.001/<.001/.02
Triglyceride (mmol/L)					
Baseline*	0.49 ± 0.03	0.43 ± 0.02	0.49 ± 0.10	0.38 ± 0.03	NS
Treatment†	0.43 ± 0.05	0.69 ± 0.05	0.55 ± 0.06	0.65 ± 0.06	.45/<.001/.19
LDL molecular weight (g/μmol)					
Treatment*	2.86 ± 0.13	2.36 ± 0.18	2.67 ± 0.13	2.43 ± 0.12	.70/.02/.39

*Mean ± SEM, unadjusted.
†Mean ± SEM, treatment values adjusted for baseline values.

since they are known to influence atherosclerosis progression. In previous studies from our group, these two variables account for approximately 55% of the variability in atherosclerosis extent (M.S. Anthony and T.B. Clarkson, unpublished data, April 1993). Although there was no effect of treatment on total or free cholesterol content, cholesteryl ester content was significantly lower in E2-treated groups, with a similar trend for soy protein compared with casein. In addition, there was a significant interaction between E2 and soy, with the lowest cholesteryl ester content in the soy+E2 group (Fig 2).

Arterial lipid peroxidation levels (TBARS) were also significantly lower in monkeys consuming soy (Table 6). As with cholesteryl ester content, there was a similar trend for an interaction between E2 and soy, with the lowest TBARS values in the soy+E2-treated monkeys. To determine if TBARS levels were dependent on the amount of cholesteryl ester present in the artery, TBARS data were covaried by the cholesteryl ester values. The same treatment differences were found ($P = .05$).

DISCUSSION

The major findings of this study include improvements in a number of CHD risk factors with either soy protein consumption or E2 treatment, including (1) improved plasma lipid and lipoprotein concentrations with consumption of soy protein compared with casein; (2) improved carbohydrate metabolism with E2 treatment and soy protein consumption; (3) lower body weight and abdominal fat content with E2 treatment, with a similar trend for soy protein; and (4) lower arterial lipid peroxidation (TBARS) with soy protein.

In addition to improvement in various CHD risk factors with both E2 and soy protein, there was a significant reduction ($P = .001$) in aortic cholesteryl ester content (Fig 2) with E2 treatment, with a similar trend for soy protein compared with casein ($P = .14$). There was also a significant interaction ($P = .02$) with soy and E2, such that the soy+E2 group had the lowest arterial cholesteryl ester content.

In previous studies of estrogen replacement therapy (ERT) in monkeys, we found a 50% to 70% reduction in the progression of coronary artery atherosclerosis, as assessed by the amount of intimal area, without significant improvement in plasma lipid and lipoprotein concentrations.[11,24] These findings are consistent with studies in women in which ERT is also associated with a 50% reduction in CHD risk, yet only 25% of this effect was due to beneficial changes in plasma lipid concentrations.[10] Thus, the lack of a beneficial effect of E2 on TPC and HDLC concentrations in the current study (Table 3) is consistent with past studies. However, as reported previously in monkeys[23,25] and women,[26,27] ERT is associated with an increase in plasma triglyceride concentrations and a reduction in LDL size.

In contrast to the effects of E2 on plasma lipids, soy protein

Table 4. Effects of Treatment on Carbohydrate and Insulin Measurements

Parameter	Casein (n = 14)	Casein + E2 (n = 13)	Soy (n = 11)	Soy + E2 (n = 10)	P (protein/E2/P × E2)
Glucose (mmol/L)					
Baseline*	3.11 ± 0.11	3.39 ± 0.33	3.33 ± 0.22	3.61 ± 0.33	NS
Treatment†	4.22 ± 0.50	4.16 ± 0.50	4.66 ± 0.56	4.72 ± 0.61	.22/.97/.89
Insulin (pmol/L)					
Baseline*	287 ± 50	287 ± 50	280 ± 43	258 ± 50	NS
Treatment†	208 ± 29	165 ± 29	230 ± 36	143 ± 36	.92/.005/.38
Insulin to glucose ratio					
Baseline*	88 ± 14	83 ± 9	87 ± 13	69 ± 9	NS
Treatment†	54 ± 6	40 ± 6	50 ± 8	31 ± 9	.24/.005/.60
Fructosamine (μmol/L)					
Baseline*	185 ± 7	181 ± 6	203 ± 4	184 ± 8	NS
Treatment†	158 ± 8	154 ± 8	146 ± 9	166 ± 10	.99/.26/.06

*Mean ± SEM, unadjusted.
†Mean ± SEM, treatment values adjusted for baseline values.

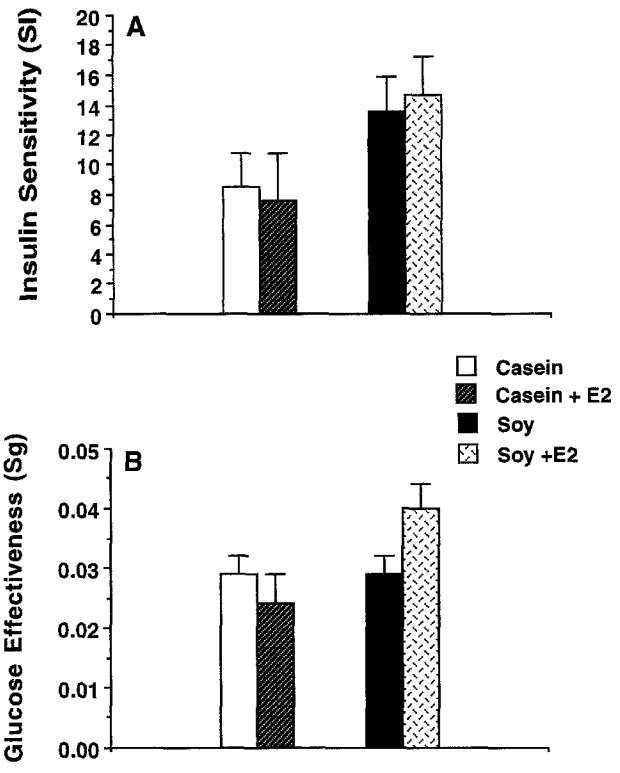

Fig 1. Minimal-model analyses providing measures of (A) insulin sensitivity (10^{-4} $min^{-1} \cdot \mu U^{-1} \cdot$ mL) and (B) glucose effectiveness (min^{-1}) for subsets of monkeys in the following groups: casein (n = 10), casein+E2 (n = 5), soy (n = 9), and soy+E2 (n = 8). Values are the mean ± SEM adjusted for baseline insulin to glucose ratios. Statistical analyses for insulin sensitivity: effect of protein, $P = .03$; effect of estradiol, $P = .95$; and protein × estradiol interaction, $P = .69$. Statistical analyses for glucose effectiveness: effect of protein, $P = .05$; effect of estradiol, $P = .40$; and protein × estradiol interaction, $P = .04$.

compared with casein/lactalbumin resulted in significantly lower TPC and higher HDLC concentrations. There was no effect of soy protein on triglyceride concentrations or LDL size. The lower TPC concentrations associated with dietary soy may be associated with an increase in LDL receptor activity, as shown previously in both rat liver[28] and human mononuclear cells.[29]

In addition to lipids and lipoproteins, insulin resistance, carbohydrate intolerance, and central obesity are also risk factors for CHD that may be affected by either ERT, protein source, or both. However, due in part to the various methods of assessing insulin resistance and the different types, doses, and routes of administration of estrogens and progestins used in ERT, previous reports of the effects of ERT on parameters assessing carbohydrate metabolism have yielded conflicting results. Some studies of ERT alone have reported lower fasting glucose and insulin concentrations[30-32] or improvement in insulin sensitivity,[33-35] whereas others have not.[16,36,37] However, a few large epidemiologic studies, including the Atherosclerosis Risk in Communities Study,[30] the Rancho Bernardo Study,[31] and the Postmenopausal Estrogen/Progestin Interventions Trial,[32] have reported small but significant improvements in glucose and insulin concentrations in subjects taking primarily conjugated equine estrogens with or without a progestin.

In the present study, neither E2 nor soy affected fasting glucose concentrations or fructosamine (which reflects antecedent glycemic control) (Table 5). However, consistent with the epidemiologic studies just described, both fasting insulin and the insulin to glucose ratio were reduced with E2, suggesting increased insulin sensitivity. Whereas there was no effect of soy versus casein protein on fasting glucose and insulin concentrations, insulin sensitivity and glucose effectiveness were increased with soy protein but not with E2 (Fig 1).

The improved insulin to glucose ratio seen with E2 and the lack of effect in the minimal-model studies is confusing. However, this may be due to the subset of animals chosen for minimal-model studies. Because of the amount of blood

Table 5. Effects of Treatment on Body Weight and Body Fat Measurements

Parameter	Casein (n = 14)	Casein + E2 (n = 13)	Soy (n = 11)	Soy + E2 (n = 10)	P (protein/E2/P × E2)
Body weight (kg)					
*Baseline**	3.84 ± 0.38	4.03 ± 0.42	3.96 ± 0.29	3.77 ± 0.28	NS
Treatment†	3.95 ± 0.14	3.59 ± 0.15	3.90 ± 0.15	3.27 ± 0.17	.11/.0001/.24
Body mass index (kg/m^2)					
*Baseline**	58.6 ± 5.0	58.5 ± 5.4	57.1 ± 2.6	58.4 ± 4.7	NS
Treatment†	49.1 ± 2.8	48.2 ± 2.8	51.4 ± 3.0	43.2 ± 3.2	.60/.048/.12
Subscapular to triceps skinfold ratio					
*Baseline**	2.5 ± 0.4	2.9 ± 0.1	2.4 ± 0.2	2.5 ± 0.3	NS
Treatment†	2.6 ± 0.2	2.2 ± 0.3	2.6 ± 0.3	2.2 ± 0.3	.93/.04/.97
Total abdominal fat (cm)					
Treatment‡	4,617 ± 568	3,619 ± 589	4,621 ± 672	1,825 ± 672	.16/.004/.16
Intraabdominal fat (cm)					
Treatment‡	2,516 ± 330	1,914 ± 342	2,448 ± 390	1,082 ± 390	.22/.01/.30
Subcutaneous abdominal fat (cm)					
Treatment‡	2,100 ± 266	1,706 ± 276	2,173 ± 314	744 ± 315	.14/.003/.09

*Mean ± SEM, unadjusted.

†Mean ± SEM, treatment values adjusted for baseline values.

‡Mean ± SEM, treatment values adjusted for baseline body weight.

Table 6. Effects of Treatment on Arterial Measurements

Parameter	Casein (n = 13)	Casein + E2 (n = 13)	Soy (n = 11)	Soy + E2 (n = 9)	P (protein/E2/P × E2)
Aortic lipid content (mg/g)*					
Total cholesterol					
Unadjusted	3.0 ± 1.0	2.2 ± 0.4	2.7 ± 0.5	2.6 ± 0.9	
Adjusted	2.06	1.90	2.60	1.60	.86/.13/.26
Free cholesterol					
Unadjusted	1.4 ± 0.3	1.3 ± 0.2	1.6 ± 0.3	1.8 ± 0.5	
Adjusted	1.15	1.18	1.45	1.31	.26/.81/.67
Cholesteryl ester					
Unadjusted	1.7 ± 0.8	0.9 ± 0.2	1.2 ± 0.2	0.7 ± 0.4	
Adjusted	0.84	0.63	1.11	0.20	.14/.001/.02
Lipid peroxidation (nmol/g)†	52.6	58.8	50.0	41.7	.04/.58/.16

*Means adjusted for baseline TPC:HDLC ratio and cholesterol days are in original units derived by retransforming the means of the transformed data.

†Means are in original units derived by retransforming the means of the transformed data.

samples needed, we studied only animals weighing more than 3 kg. As will be discussed, E2 treatment resulted in animals with lower body weights. Thus, we studied fewer animals in the E2 groups and removed those that were leaner and more likely most insulin-sensitive. Despite the sampling artifact in E2-treated animals, the increased insulin sensitivity with soy compared with casein was pronounced.

In vitro studies of genistein and daidzein (isoflavones found in soy protein) in insulin secretion[38,39] and intermediary carbohydrate metabolism have been reported.[40] As previous studies have shown with estradiol,[41] genistein[38,39] and, to a lesser extent, daidzein[39] increased insulin secretion from islet preparations and also increased glucose sensitivity.[38] Interestingly, Jonas et al[39] reported little effect of genistein on basal insulin release, but an augmented response was found in the presence of glucose or other nutrients. This may explain the lack of effect of the soy protein in our study under fasting conditions versus the improved sensitivity in response to a glucose challenge in the minimal-model studies.

Whether the effect of the soy protein on insulin sensitivity in this study was due to genistein is unknown. It is also unknown if the effects of genistein are due to its estrogenic activity or other effects on intracellular processes regulating carbohydrate metabolism. Genistein has been shown to inhibit tyrosine kinase activity for both platelet-derived growth factor and epidermal growth factor.[8,9] Insulin activity also is affected by tyrosine kinase activity. Activation of insulin receptor kinase plays an essential role for many, if not all, of the biological effects of insulin.[42,43] Further, insulin receptor tyrosine kinase plays a major role in signal transduction distal to the receptor.[42,44,45] Whether the effects on insulin sensitivity seen in the soy-treated groups were due to genistein-induced alterations in carbohydrate metabolism via inhibition of tyrosine kinase activity is unknown. However, this activity would seem to be inhibitory, and others have shown postreceptor effects of genistein independent of tyrosine kinase inhibition.[39,40]

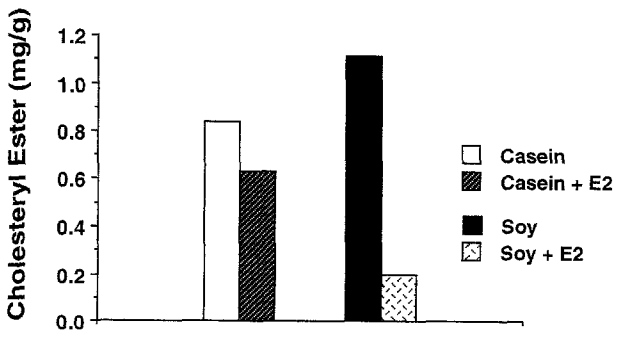

Fig 2. Effect of treatment on aortic cholesteryl ester content (mg/g). Values are the mean adjusted for baseline TPC:HDLC ratios and cholesterol days. Effect of protein, $P = .14$; effect of estradiol, $P = .001$; and protein × estradiol interaction, $P = .02$.

Insulin concentrations and sensitivity are also known to be affected by body composition.[46] In support of this, we found significant associations among measures of adiposity and both fasting insulin concentrations and insulin sensitivity. In this study, E2 treatment resulted in animals that weighed less and had lower body mass indices and decreased anthropometric measures (Table 5). In addition, total and intraabdominal and subcutaneous fat depots were significantly reduced with E2 treatment. Lower body weight and/or fat has been shown previously in monkeys,[18] rats,[47] and women[30,32,48] treated with ERT.

The effects of the soy protein were not as robust as the effects of E2 on body weight and measures of adiposity ($P > .05$). However, animals in the soy+E2 group consistently weighed less and had the least amount of central fat. In addition, animals in the soy+E2 group also had the lowest fasting insulin concentrations and greatest insulin sensitivity. The changes in body weight seen in the current study are similar to those found by Arjmandi et al,[49] who compared E2 and soy protein isolate in ovariectomized rats. Both estrogen and soy prevented the ovariectomy-induced body weight gain in these rats, with the greatest effect due to E2. In addition, the abdominal fat mass was significantly smaller in soy-treated rats. We do not know whether the changes in body weight and body fat were due to changes in food consumption, activity levels, or some combination of the two. However, previous studies in rats[47] suggest that sex hormones can affect both food consumption and activity levels.

From the present studies, it is unclear if the improvements in insulin sensitivity are due to changes in abdominal fat, a direct action of E2 and soy on insulin-sensitive tissues, or a combination of the two. Since the animals in the soy-only group also had greater insulin sensitivity compared with those in the casein+E2 group yet had more abdominal fat, soy protein may affect carbohydrate metabolism through mechanisms other than by simply reducing fat content. Postulated mechanisms other than the previously reported effects on tyrosine kinase[38,39] include changes in insulin receptor number, affinity, and intracellular phosphorylation and alterations in the glucose transport apparatus.

Both estrogens[50,51] and isoflavones[52,53] in general, as well as genistein itself,[54] have antioxidant activity. Consistent with these previous results, in the present study, animals consuming soy had significantly lower amounts of aortic TBARS than those consuming casein (Table 6). Although there was not a significant effect of E2, monkeys in the soy+E2 group had the least amount of lipid peroxidation.

Since a reduction in the risk factors for CHD should result in an improvement in cardiovascular disease, it is not surprising that monkeys in the soy+E2 group had the lowest aortic cholesteryl ester content (Fig 2). Both soy and E2 reduced a number of CHD risk factors, and the combination resulted in a significant interactive effect with regard to aortic cholesteryl ester content ($P = .02$). It is important that these animals had preexisting atherosclerosis before the study began, and that the soy+E2 group had the greatest pretreatment dietary cholesterol exposure. Despite this, the soy+E2 group had the least amount of arterial cholesteryl ester, and after adjusting for pretreatment variables, this difference was highly significant. With more advanced lesions, there is an increase in extracellular lipid, particularly free cholesterol, which is more slowly mobilized during atherosclerosis regression[55] and thus may be less likely to be reduced with short-term treatment (over only 7 months). However, the cholesteryl ester content is more quickly mobilized and thus more responsive to interventions. This may explain the lack of treatment effect on free cholesterol and the marginal effect on total cholesterol content for the soy+E2 group.

In conclusion, this study suggests that both estradiol and dietary soy have beneficial effects on a number of CHD risk factors. Whereas soy protein was associated with improvements in lipid concentrations, insulin sensitivity, and lipid peroxidation, estradiol treatment was associated with improvements in fasting insulin concentration, LDL size, and central adiposity. However, the combination of the two treatments resulted in the greatest reduction in arterial cholesteryl ester content. This study was not designed to determine whether the beneficial effects of the soy diet were due to the phytoestrogen content or other components of the soybean protein. In addition, if genistein is the active component, further studies are needed to determine if the effects are due to its interaction with the estrogen receptor, tyrosine kinase inhibition, or other mechanisms.

ACKNOWLEDGMENT

The authors thank Vickie Hardy, Deborah Silverstein, Joel Collins, and Roberto Gonzalez for technical help, Karen Potvin Klein for editorial assistance, and Protein Technologies International (St Louis, MO) for providing the soy protein.

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