Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women[1–3]

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ABSTRACT

Background: Soy consumption is known to reduce plasma total cholesterol and LDL cholesterol in hypercholesterolemic subjects, but the responsible soy components and the effects in normocholesterolemic subjects remain unclear.

Objective: The effects of soy isoflavone consumption on plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, apolipoprotein A-I, apolipoprotein B, and lipoprotein(a) concentrations and on LDL peak particle diameter were examined in normocholesterolemic, premenopausal women.

Design: Thirteen healthy, normocholesterolemic, free-living, premenopausal female volunteers took part in this randomized, crossover-controlled trial. Each subject acted as her own control. Three soy isoflavone intakes (control: 10.0 ± 1.1; low: 64.7 ± 9.4; and high: 128.7 ± 15.7 mg/d), provided as soy protein isolate, were consumed for 3 menstrual cycles each. Total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol were measured over the menstrual cycle. Apolipoprotein A-I, apolipoprotein B, lipoprotein(a), and LDL peak particle diameter were evaluated in the midluteal phase.

Results: Total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations changed significantly across menstrual cycle phases ($P < 0.005$). During specific phases of the cycle, the high-isoflavone diet lowered LDL cholesterol by 7.6–10.0% ($P < 0.05$), the ratio of total cholesterol to HDL cholesterol by 10.2% ($P < 0.005$), and the ratio of LDL to HDL cholesterol by 13.8% ($P < 0.002$).

Conclusions: Isoflavones significantly improved the lipid profile across the menstrual cycle in normocholesterolemic, premenopausal women. Although of small magnitude, these effects could contribute to a lower risk of developing coronary heart disease in healthy people who consume soy over many years. *Am J Clin Nutr* 2000;71:1462–9.

KEY WORDS Soy, isoflavone, phytoestrogen, lipids, cholesterol, LDL, HDL, triacylglycerol, apolipoprotein A-I, apolipoprotein B, lipoprotein(a), women, menstrual cycle

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States, accounting for >960 000 deaths in 1995 (1). Approximately 1 of every 5 people in the United States has some form of CVD, and ≈$274 billion was spent in direct and indirect costs of CVD in 1998 (1). Much research effort has therefore been directed toward developing new treatments and methods of prevention.

In the past 3 decades, numerous studies have shown that soy consumption improves plasma lipids in animals and humans. Addition of soy to the diet or substitution of soy protein for animal protein improves the lipid profile in a variety of animal species (2–4) as well as in men and women, particularly those with elevated total cholesterol concentrations (5–11). A few studies have shown minimal effects, particularly in normocholesterolemic populations (6, 11–13).

The components of soy responsible for the favorable effects on cholesterol concentrations and their mechanisms of action remain unknown (10). Of great interest have been the effects of phytoestrogenic isoflavones. Animal studies that specifically compared the effects of isoflavone-rich soy with isoflavone-free soy found decreased total cholesterol and LDL cholesterol (14, 15), increased (3, 14, 16) or unchanged HDL cholesterol (3, 15, 17), increased (14) or unchanged triacylglycerol (3, 14, 17), increased (14) or unchanged apolipoprotein (apo) A-I (14), unchanged apo B (14), decreased (14) or unchanged lipoprotein(a) [Lp(a)] (14, 17), and decreased (14) or unchanged (14, 17) LDL molecular weight. These data are consistent with those from a recent study that showed decreased total cholesterol and LDL-cholesterol, and unchanged HDL-cholesterol concentrations, triacylglycerol, and Lp(a) concentrations in hypercholesterolemic postmenopausal women and men consuming an isoflavone-rich soy protein compared with an isoflavone-free soy protein (18). At this time, there are no published reports of human studies comparing the effects of isoflavone-rich compared with isoflavone-free soy protein in normocholesterolemic subjects, although 2 human studies that

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compared the effects of purified isoflavones with a placebo in normocholesterolemic and mildly hypercholesterolemic subjects showed no effects of isoflavone supplementation on plasma lipid (19, 20) or Lp(a) (20) concentrations.

The main purpose of this study was to examine the effects of consumption of 3 soy isoflavone intakes, given as soy protein isolates, on plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, apo A-I, apo B, and Lp(a) concentrations and on LDL peak particle diameter in healthy, normocholesterolemic, premenopausal women. A secondary purpose was to evaluate the effects of specific phases of the menstrual cycle on plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerol concentrations.

SUBJECTS AND METHODS

Study design

This study was part of a larger study investigating the effects of soy isoflavone consumption on reproductive hormones in premenopausal women (21). The protocol was approved by the University of Minnesota Institutional Review Board Human Subjects Committee.

The study used a randomized crossover design in which each woman acted as her own control. Every subject consumed 3 soy protein isolates each for 3 menstrual cycles plus 9 d, with a 2–3-wk washout between diet periods during which she resumed an ad libitum diet. The actual length of the entire study for each subject depended on her individual menstrual cycle lengths. The average length of study was 11.4 ± 1.9 mo, including the washout periods.

Subjects

Healthy premenopausal women between the ages of 18 and 35 y were recruited from the University of Minnesota campus and surrounding areas. The women were interviewed over the telephone and in person and completed detailed questionnaires and health histories. All subjects reported normal menstrual cycles, had not taken antibiotics or oral contraceptives for 6 mo before beginning the study, were not taking any medications regularly, were relatively sedentary, were 90–120% of ideal body weight, had a stable body weight over the previous year and a body mass index (BMI; in kg/m^2) between 18 and 25, were not vegetarian, consumed diets low in fiber and soy, had no history of food allergies, were nonsmokers, and were not pregnant or lactating. Routine blood and urine tests were done and physical exams were performed by a physician to confirm that the subjects had no cardiac, gynecologic, hepatic, renal, or endocrine disorders. After a 2-h orientation to the study, women who met the inclusion criteria and wanted to participate signed informed consent forms.

Soy isoflavone supplementation

The subjects lived at home and consumed their usual diets. They received detailed dietary instructions to avoid alcohol, vitamin and mineral supplements, foods high in fiber, and foods known to be rich in phytoestrogens (such as legumes, bean sprouts, and flaxseed). The women consumed each of 3 soy protein isolates as a beverage powder (control, low isoflavone, and high isoflavone) providing 0.15 ± 0.01, 1.01 ± 0.04, and 2.01 ± 0.03 mg isoflavones·kg body wt^{-1}·d^{-1}, respectively (10.0 ± 1.1, 64.7 ± 9.4, and 128.7 ± 15.7 mg isoflavones/d, respectively), expressed as unconjugated phytoestrogen units. Isoflavones were analyzed by HPLC, as described previously (22). The 3 soy protein isolates (Supro Brand Isolated Soy Protein; Protein Technologies International, St Louis) had similar macronutrient compositions; differing isoflavone contents were achieved by alcohol extraction of the protein isolate. Although the soy protein isolate used as the control was intended to be isoflavone free, it contained a very low concentration of isoflavones because of incomplete alcohol extraction. The nutrient and isoflavone compositions of the 3 powders were described previously (21). The amount of soy powder consumed per day was 75–100 g, depending on the subject's body weight, and provided an average of 1.21 MJ (290 kcal), 53 g protein, 15 g carbohydrate, 1.9 g fat, and no cholesterol. Participants and laboratory personnel were blinded to the specific soy protein isolates provided.

Plasma collection

Every other day during the third menstrual cycle of each diet period, a 10-h fasting venous blood sample was obtained. Blood was drawn into evacuated tubes containing EDTA at the same time each morning (±30 min) to minimize daily fluctuations in plasma lipid concentrations. Within 30 min of collection, samples were centrifuged at 3000 × *g* for 8–10 min at 4°C, plasma was removed, and sodium azide and aprotinin were added to concentrations of 1 g/L and 1 mg/L, respectively. All samples were immediately dispensed as aliquots, frozen, and stored at −70°C until analyzed.

Determination of ovulation

To determine the day of ovulation, subjects performed home urinary luteinizing hormone (LH) testing (OvuQUICK Self-Test; Quidel Corporation, San Diego) beginning on day 9 of each menstrual cycle until detection of the LH surge. Each subject also measured her daily basal body temperature during diet period 1 to validate the LH surge results. Subjects whose LH test results were ambiguous performed basal body temperature measurements in diet periods 2 and 3 as well. In most cases, the day of ovulation was assumed to be the day after the LH surge, as determined by a positive LH test result. If the LH test results were ambiguous, plasma hormones, urinary LH, and basal body temperature charts were used as adjuncts, allowing for precise identification of the day of ovulation by a reproductive endocrinologist (WRP).

Other procedures

To assess changes in dietary intake, 3-d food records were collected twice during each menstrual cycle (follicular phase: menstrual cycle days 7–9; luteal phase: days 7–9 after the LH surge). Food records were analyzed by using NUTRITIONIST IV for WINDOWS, version 4.0 (1995 First Databank, The Hearst Corporation, San Bruno, CA).

To assess changes in body weight and BMI, fasting body weight of subjects in hospital gowns was measured every 7–10 d. Changes in body composition were assessed by skinfold-thickness measurements made with a skinfold caliper (Cambridge Scientific Instruments, Ltd, Cambridge, MD) at the triceps, biceps, suprailiac, and subscapular sites on the subject's nondominant side at the beginning of the study and at the end of each diet period. To minimize measurement error, the same registered dietitian performed all skinfold-thickness measurements for a given subject. Body density was calculated from the sum of the 4 skinfold thicknesses and percentage body fat was determined by using a predictive equation (23).

Analytic methods

Plasma concentrations of total cholesterol and triacylglycerol were determined by using an enzymatic colorimetric method with commercially available enzyme reagents (Boehringer-Mannheim Cholesterol/HP; Boehringer Mannheim, Indianapolis). The method was previously adapted for microtiter plates (24). HDL cholesterol was isolated by selective precipitation of apo B–containing lipoproteins with phosphotungstic acid (6.1 mmol/L; Fisher Scientific, Pittsburgh) and magnesium chloride (20 mmol/L; Fisher Scientific), and analyzed for cholesterol content by using the plasma total cholesterol enzymatic assay. LDL-cholesterol concentration was calculated by using the Friedewald equation: LDL cholesterol = total cholesterol − [(HDL cholesterol) − (triacylglycerol/5)] (25). All samples from each subject were run in duplicate on the same day with 2 plasma pool controls and a standard curve on each plate. All plates for each assay were allowed to incubate for 60 min at room temperature before being read at 490 nm by an automated microplate reader. Intraassay variability was 0.7%, 0.8%, and 1.4% for total cholesterol, HDL cholesterol, and triacylglycerol, respectively, and interassay variability was 1.7%, 5.5%, and 4.9% for total cholesterol, HDL cholesterol, and triacylglycerol, respectively.

Plasma concentrations of apo A-I and apo B were determined by using an immunoturbidimetric method (26, 27) with commercially available reagents (Auto Apo AI and Auto Apo B; Bacton Assay Systems, Inc, San Marcos, CA). Lp(a) concentrations were determined via an enzyme-linked immunosorbent assay (28) that uses a polyclonal capture antibody immunospecific to apo(a) and a peroxidase-conjugated polyclonal detection antibody with recognition of the entire Lp(a) molecule (Donner Laboratory, Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA). LDL peak particle diameter was evaluated in whole plasma by using a nondenaturing-polyacrylamide-gradient-gel electrophoresis method described previously by Krauss and Burke (29). Intraassay variability was 0.8%, 0.7%, and 5.3%, and interassay variability was 3.9%, 5.3%, and 8.1% for apo A-I, apo B, and Lp(a) concentrations, respectively. Interassay variability for LDL peak particle diameter was 1.1%.

Data analysis

Before data analysis, each menstrual cycle was divided into 4 phases based on plasma hormone concentrations: early follicular (EF: cycle days 2 and 4), midfollicular (MF: cycle days 7 and 9), periovulatory (PO: ovulation −3, ovulation −1, and ovulation +1); and midluteal (ML: ovulation +5, ovulation +7, and ovulation +9). If blood was not drawn on the days corresponding to these phase definitions, interpolated values, based on values obtained on the surrounding days, were analyzed.

Statistical analyses were performed by using SAS version 6.12 (30). The effect of diet on anthropometric and nutrient intake data were evaluated by using repeated-measures analysis of variance (ANOVA), controlling for subject and diet. Because of nonsignificant phase-by-diet interactions, the effect of menstrual cycle phase on plasma lipid concentrations was evaluated on all 3 diets combined, controlling for subject, diet, and menstrual cycle phase. The effect of diet on plasma lipid concentrations was evaluated within each menstrual cycle phase separately while controlling for subject and diet because of unequal variance between menstrual cycle phases. In addition, the effect of diet on plasma concentrations of apo A-I, apo B, and Lp(a) and on LDL peak particle diameter was evaluated in ML phase samples while controlling for subject and diet. After ANOVA, the Tukey procedure was performed on all endpoints to adjust the significance level for multiple comparisons. Results are expressed as means ± SDs or ± SEs. In the event of missing data, the least-squares mean is presented to account for the imbalance. *P* values < 0.05 were considered statistically significant.

TABLE 1
Prestudy subject characteristics[1]

Age (y)	26.3 ± 4.8
Body weight (kg)	64.0 ± 7.2
BMI (kg/m^2)	22.8 ± 1.8
Percentage body fat (%)[2]	28.8 ± 5.0
Menstrual cycle length (d)[3]	30.1 ± 2.7
Plasma lipids (mmol/L)	
Total cholesterol	3.87 ± 0.67 (149.5 ± 25.9)[4]
HDL cholesterol	1.20 ± 0.28 (46.2 ± 10.9)
LDL cholesterol	2.32 ± 0.56 (89.7 ± 21.6)
Triacylglycerol	0.77 ± 0.41 (67.9 ± 35.9)
Total cholesterol:HDL cholesterol	3.4 ± 0.9
LDL cholesterol:HDL cholesterol	2.1 ± 0.8

[1] $\bar{x}$ ± SD; n = 13.
[2] Determined from skinfold-thickness measurements.
[3] Determined from the average length of the 6 menstrual cycles immediately before the start of the study.
[4] Values in mg/dL in parentheses.

RESULTS

Of the 20 women who began the study, 6 left the study because of difficulties complying with required procedures. Therefore, 14 subjects completed the entire study. One of the 14 subjects was hypercholesterolemic (total cholesterol = 6.29 mmol/L, or 243 mg/dL) and was therefore excluded a priori from the lipid data analyses. The characteristics of the 13 remaining subjects are summarized in **Table 1**.

After reviewing reproductive hormone data, it was determined that one subject did not ovulate during the third menstrual cycle of both her low- and high-isoflavone diet periods. Therefore, the data from the PO and ML phases during these 2 anovulatory cycles were not included in the data analyses.

Because there was no effect of diet order, the results are presented by diet. The average number of days the subjects consumed the soy powders before lipid concentrations were measured was 60.2 ± 12.0, 58.0 ± 7.2, and 60.1 ± 8.7 d (mean ± SD) for the control, low-, and high-isoflavone diets, respectively.

Anthropometric and dietary data

There were no significant effects of diet on body weight, BMI, or percentage body fat. There were also no significant effects of diet on daily energy, macronutrient, or dietary fiber intake (**Table 2**). However, subjects consumed significantly more protein ($P < 0.001$) and significantly less dietary fiber ($P < 0.001$) during the study compared with prestudy diet records.

Effects of the menstrual cycle on plasma lipid concentrations

As shown in **Table 3**, there were significant effects of menstrual cycle phase on total cholesterol ($P < 0.001$), HDL cholesterol ($P < 0.005$), LDL cholesterol ($P < 0.0005$), ratio of total cholesterol to HDL cholesterol ($P < 0.001$), and ratio of LDL to HDL cholesterol ($P < 0.001$). In particular, HDL cholesterol

TABLE 2
Daily energy, macronutrient, and fiber intakes[1]

	Prestudy	Control diet	Low-isoflavone diet	High-isoflavone diet
Energy				
(MJ)	8.46 ± 0.51	9.53 ± 0.31	9.52 ± 0.31	9.27 ± 0.30
(kcal)	2021 ± 122	2277 ± 75	2275 ± 74	2216 ± 72
Protein (g)	69.5 ± 5.9[2]	115.0 ± 4.0	114.0 ± 4.0	117.7 ± 3.8
Carbohydrate (g)	278.8 ± 16.4	294.2 ± 9.1	296.9 ± 8.9	291.3 ± 8.7
Total fat (g)	73.8 ± 5.6	75.8 ± 3.1	73.7 ± 3.0	67.4 ± 2.9
SFA (g)	26.0 ± 2.3	23.7 ± 1.2	23.7 ± 1.2	21.2 ± 1.1
PUFA (g)	12.0 ± 1.5	12.4 ± 0.9	13.0 ± 0.9	11.0 ± 0.9
MUFA (g)	21.2 ± 2.2	20.0 ± 1.3	17.4 ± 1.3	15.8 ± 1.2
Cholesterol (mg)	200.2 ± 31.5	194.3 ± 19.7	238.5 ± 19.4	181.8 ± 18.9
Dietary fiber (g)	11.6 ± 0.9[2]	8.8 ± 0.4	8.9 ± 0.4	8.1 ± 0.4

[1] Least-squares mean ± SE. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.
[2] Significantly different from diet periods, $P < 0.001$.

peaked during the PO phase whereas total cholesterol and LDL cholesterol declined during the PO and ML phases. Accordingly, more favorable ratios of total to HDL cholesterol and LDL to HDL cholesterol were present during the PO and ML phases. No significant changes were noted for triacylglycerol.

Effects of soy isoflavone consumption on plasma lipid concentrations

As a result of significant phase effects, the effects of soy isoflavones on lipid concentrations were analyzed within menstrual cycle phase (**Table 4**). When compared with the control diet, the high-isoflavone diet lowered LDL cholesterol significantly by 7.6% and 10.0% in the MF and PO phases, respectively ($P < 0.02$). There were trends toward decreased LDL cholesterol with increased isoflavone consumption during the other phases. Although isoflavone consumption did not significantly affect total cholesterol or HDL cholesterol, there were trends toward decreasing total cholesterol with increased isoflavone consumption in all 4 phases and trends toward increasing HDL cholesterol with increased isoflavone consumption in the MF and PO phases. Isoflavone consumption did not significantly affect triacylglycerol concentrations.

When compared with the control diet, the high-isoflavone diet lowered the ratio of total cholesterol to HDL cholesterol by 10.2% ($P < 0.002$) and the ratio of LDL cholesterol to HDL cholesterol by 13.8% ($P < 0.002$) in the PO phase. There were also trends toward lower ratios in the EF, MF, and ML phases. Isoflavone consumption did not significantly affect concentrations of apo A-I, apo B, or Lp(a) or affect LDL peak particle diameter, all evaluated in ML-phase samples only (**Table 5**).

DISCUSSION

The purpose of this study was to investigate the effects of soy isoflavones on plasma lipid concentrations in premenopausal women. This study differed from previous studies in several important ways. Our subjects were healthy, free-living, premenopausal women with normal total cholesterol concentrations. Their diets were supplemented with 3 doses of isoflavones that were provided relative to individual body weights, and the isolated soy protein powders did not completely substitute for the protein in their habitual diets. The subjects consumed the diets for 2 menstrual cycles before any lipid measurements were taken. Blood samples were collected every other day for an entire menstrual cycle, menstrual cycle phases were carefully defined after evaluation of reproductive hormone concentrations, and anovulatory cycles were excluded.

As expected, lipid concentrations changed across the menstrual cycle. Numerous prior investigations assessing such cyclical changes yielded conflicting results, in part because of different study designs and cycle phase definitions (31–37). Nonetheless, our findings of increased total cholesterol and LDL

TABLE 3
Effects of the menstrual cycle on plasma lipid concentrations[1]

	Early follicular phase	Midfollicular phase	Periovulatory phase	Midluteal phase
Total cholesterol (mmol/L)	3.72 ± 0.03[2,a]	3.83 ± 0.03[b]	3.71 ± 0.03[a]	3.64 ± 0.03[a]
	(143.8 ± 1.1)	(147.9 ± 1.1)	(143.6 ± 1.2)	(140.9 ± 1.2)
HDL cholesterol (mmol/L)	1.17 ± 0.01[a]	1.21 ± 0.01[a,b]	1.24 ± 0.01[b]	1.18 ± 0.01[a]
	(45.2 ± 0.5)	(46.6 ± 0.5)	(47.8 ± 0.5)	(45.8 ± 0.5)
LDL cholesterol (mmol/L)	2.26 ± 0.03[a,b]	2.30 ± 0.03[a]	2.18 ± 0.03[b,c]	2.15 ± 0.03[c]
	(87.2 ± 1.0)	(88.9 ± 1.0)	(84.4 ± 1.0)	(83.2 ± 1.0)
Triacylglycerol (mmol/L)	0.64 ± 0.02	0.70 ± 0.02	0.64 ± 0.2	0.67 ± 0.02
	(56.9 ± 1.6)	(61.9 ± 1.6)	(56.3 ± 1.6)	(59.6 ± 1.6)
Total cholesterol:HDL cholesterol	3.25 ± 0.03[a]	3.25 ± 0.03[a]	3.08 ± 0.03[b]	3.17 ± 0.03[a,b]
LDL cholesterol:HDL cholesterol	1.99 ± 0.03[a]	1.98 ± 0.03[a]	1.83 ± 0.03[b]	1.89 ± 0.03[a,b]

[1] Least-squares mean ± SE; values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Values in mg/dL in parentheses.

TABLE 4
Effects of soy isoflavone consumption on plasma lipid concentrations[1]

	Control diet	Low-isoflavone diet	High-isoflavone diet
Total cholesterol (mmol/L)			
Early follicular phase	3.76 ± 0.05 (145.1 ± 3.0)[2]	3.75 ± 0.08 (144.3 ± 2.9)	3.64 ± 0.08 (140.6 ± 2.9)
Midfollicular phase	3.91 ± 0.06 (151.0 ± 2.2)	3.84 ± 0.06 (148.2 ± 2.2)	3.74 ± 0.06 (144.5 ± 2.2)
Periovulatory phase	3.85 ± 0.07 (148.7 ± 2.7)	3.79 ± 0.08 (146.3 ± 2.9)	3.60 ± 0.08 (139.1 ± 2.9)
Midluteal phase	3.76 ± 0.05 (145.1 ± 2.0)	3.63 ± 0.06 (140.2 ± 2.2)	3.63 ± 0.06 (140.3 ± 2.2)
HDL cholesterol (mmol/L)			
Early follicular phase	1.16 ± 0.04 (44.8 ± 1.6)	1.18 ± 0.04 (45.5 ±1.5)	1.16 ± 0.04 (44.9 ± 1.5)
Midfollicular phase	1.19 ± 0.03 (46.1 ± 1.3)	1.21 ± 0.03 (46.6 ± 1.3)	1.23 ± 0.03 (47.3 ± 1.3)
Periovulatory phase	1.23 ± 0.03 (47.3 ± 1.0)	1.26 ± 0.03 (48.6 ± 1.1)	1.26 ± 0.03 (48.8 ± 1.1)
Midluteal phase	1.20 ± 0.03 (46.4 ± 1.0)	1.19 ± 0.03 (45.9 ± 1.1)	1.20 ± 0.03 (46.3 ± 1.1)
LDL cholesterol (mmol/L)			
Early follicular phase	2.31 ± 0.06 (89.2 ± 2.4)	2.26 ± 0.06 (87.4 ± 2.2)	2.18 ± 0.06 (84.0 ± 2.2)
Midfollicular phase	2.38 ± 0.04 (91.7 ± 1.5)[a]	2.34 ± 0.04 (90.2 ± 1.5)[a]	2.20 ± 0.04 (84.9 ± 1.5)[b]
Periovulatory phase	2.30 ± 0.05 (88.7 ± 1.9)[a]	2.25 ± 0.05 (86.8 ± 2.0)[a]	2.07 ± 0.05 (79.8 ± 2.0)[b]
Midluteal phase	2.26 ± 0.04 (87.3 ± 1.7)	2.13 ± 0.05 (82.1 ± 1.8)	2.12 ± 0.05 (81.8 ± 1.8)
Triacylglycerol (mmol/L)			
Early follicular phase	0.63 ± 0.04 (55.7 ± 3.9)	0.64 ± 0.04 (56.9 ± 3.7)	0.66 ± 0.04 (58.1 ± 3.7)
Midfollicular phase	0.75 ± 0.04 (66.4 ± 3.3)	0.65 ± 0.04 (57.6 ± 3.3)	0.70 ± 0.04 (61.9 ± 3.3)
Periovulatory phase	0.71 ± 0.04 (62.9 ± 3.5)	0.61 ± 0.04 (54.3 ± 3.8)	0.59 ± 0.04 (52.4 ± 3.8)
Midluteal phase	0.65 ± 0.03 (57.1 ± 2.8)	0.69 ± 0.04 (61.2 ± 3.1)	0.69 ± 0.04 (60.9 ± 3.1)
Total cholesterol:HDL cholesterol			
Early follicular phase	3.27 ± 0.08	3.26 ± 0.08	3.23 ± 0.08
Midfollicular phase	3.36 ± 0.07	3.25 ± 0.07	3.15 ± 0.07
Periovulatory phase	3.24 ± 0.05[a]	3.07 ± 0.06[a,b]	2.91 ± 0.06[b]
Midluteal phase	3.24 ± 0.07	3.14 ± 0.08	3.11 ± 0.08
LDL cholesterol:HDL cholesterol			
Early follicular phase	2.01 ± 0.08	2.00 ± 0.07	1.96 ± 0.07
Midfollicular phase	2.05 ± 0.06	2.00 ± 0.06	1.87 ± 0.06
Periovulatory phase	1.96 ± 0.04[a]	1.84 ± 0.05[a,b]	1.69 ± 0.05[b]
Midluteal phase	1.98 ± 0.06	1.85 ± 0.07	1.83 ± 0.07

[1] Least-squares mean ± SE; values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Values in mg/dL in parentheses.

cholesterol before ovulation, increased HDL cholesterol at ovulation, and more favorable ratios of total to HDL cholesterol and LDL cholesterol to HDL cholesterol during the PO and ML phases are consistent with those of most studies involving precise identification of the day of ovulation (34–37). It is likely that the menstrual cycle changes noted are directly related to fluctuations in sex steroid concentrations. Sex steroids can exert a direct influence on lipid metabolism through many mechanisms, including effects on hepatic lipase activity and apoprotein gene expression (38), as well as cyclic alterations in biliary cholesterol excretion (39), plasma volume (40), and cholesterol requirements for steroid (especially progesterone) synthesis (41). Regardless of the cause, these results illustrate the importance of controlling for menstrual cycle phase in studies attempting to detect small changes (<5%) in lipid concentrations in premenopausal women.

This is the first study to address the effects of isoflavone consumption on plasma lipids during carefully defined phases of the menstrual cycle. Our study showed that consumption of a soy protein isolate containing 129 mg isoflavones/d significantly lowered LDL cholesterol by 8–10% during the MF and PO phases. Trends could be seen toward the lowering of total cholesterol in all 4 phases and LDL cholesterol in the EF and ML phases. Changes in the ratios of total to HDL cholesterol and LDL cholesterol to HDL cholesterol paralleled the changes observed in total cholesterol and LDL cholesterol. Isoflavone consumption tended to lower the ratios in all phases, with statistical significance being reached in the PO phase. Although statistical significance was reached only with the high dose of 129 mg isoflavones/d, a clear dose response was seen for most endpoints, with the value for the 65 mg/d dose being between the values for the control and high dose. HDL cholesterol showed nonsignificant trends toward higher concentrations.

These data are consistent with those of Anthony et al (3, 14, 16) and Honore et al (17), who reported lower total cholesterol and LDL cholesterol and higher HDL cholesterol in nonhuman primates fed soy protein isolate containing isoflavones compared with soy protein isolate devoid of isoflavones. Our data are also consistent with those from a recent study showing lower total cholesterol and LDL cholesterol in men and women consuming soy protein isolate containing isoflavones compared with soy protein isolate devoid of isoflavones (18), although subjects were hypercholesterolemic, menstrual cycle was not controlled for, and soy effects were not seen when data from premenopausal women were analyzed separately. Studies in men and postmenopausal women given isoflavone tablets have shown no significant effects on lipids (19, 20).

Note that although there were no significant differences in nutrient intake among the 3 diet periods, there were nonsignificant differences in fat and cholesterol intakes. The Keys equation (42) predicts that these differences would account for ≈20% of the effect of isoflavone consumption on total cholesterol concentrations. Consider, however, that the Keys equation

TABLE 5
Effects of soy isoflavone consumption on plasma LDL peak particle size, lipoprotein(a), apolipoprotein A-I, and apolipoprotein B concentrations[1]

	Control diet	Low-isoflavone diet	High-isoflavone diet
LDL peak particle size (nm)	27.48 ± 0.07	27.71 ± 0.07	27.51 ± 0.07
Lipoprotein(a) (μmol/L)	0.52 ± 0.02	0.54 ± 0.02	0.52 ± 0.02
	(14.64 ± 0.58)[2]	(15.16 ± 0.63)	(14.59 ± 0.63)
Apolipoprotein A-I (g/L)	1.02 ± 0.02	1.02 ± 0.02	1.02 ± 0.02
Apolipoprotein B (g/L)	0.63 ± 0.01	0.63 ± 0.01	0.62 ± 0.01

[1] Least-squares mean ± SE.
[2] Values in mg/dL in parentheses.

was calculated from data collected in men and may not always reflect changes observed in women (42, 43), particularly across menstrual cycle phases.

In addition to favorable plasma lipids, increased concentrations of apo A-I, decreased concentrations of apo B and Lp(a), and increased LDL particle diameter have also been associated with decreased coronary artery disease risk (44–46). Our observation of no significant effects of isoflavones on these endpoints is consistent with other studies (17, 20, 47), although one study of female monkeys consuming very high doses of isoflavones showed increased apo A-I concentrations, decreased apo B and Lp(a) concentrations, and decreased LDL molecular weight (14).

There are several issues related to study design that may explain the differences between our results and those published previously. First, our study used a crossover design in which each subject acted as her own control. Some studies that showed no significant changes in lipid concentrations (20, 48) did not use such a design. The high intersubject variability in isoflavone metabolism (48–50) and lipid responses to dietary changes (51, 52) may have masked a true effect of diet in those studies.

Second, the isoflavone form and dose may influence the results. The subjects consumed the isoflavones as a constituent of a soy protein isolate, and it is possible that the alcohol extraction of the protein isolate may have removed other components, in addition to the isoflavones, that influence lipid metabolism (2). Although, the isoflavone doses used in this study were chosen to fall within the range of intakes reported previously in a typical Asian diet (53), recent data suggest the typical isoflavone intake to be far lower than reported previously, likely between 20 and 50 mg isoflavones/d (54). Therefore, our low dose was at the upper end of current estimates of typical Asian intake and our high isoflavone dose was significantly higher.

Third, the background diet is a critical factor. The studies in monkeys (14, 16, 17) that showed significant changes in lipids used mildly or moderately atherogenic diets. In our study, the subjects consumed ≈30% of energy from fat and <240 mg cholesterol/d, which approximate the recommendations by the National Cholesterol Education Program (55).

Fourth, each diet period in our study was >3 mo in length, whereas the other studies conducted in humans were from 1 to 2.5 mo in length. Although many lipid studies are 3–4 wk in length, some effects on lipid metabolism may occur after longer periods of exposure.

Fifth, we carefully controlled for menstrual cycle phase by determining ovulation and defining the 4 phases of the menstrual cycle on an individual basis by using reproductive hormone concentrations. Last, our subjects were normocholesterolemic, whereas most studies showing lipid-lowering effects of soy were performed in hypercholesterolemic subjects (6, 8). It is interesting that we found small yet statistically significant changes in a group with total cholesterol concentrations of 3.87 ± 0.67 mmol/L (150 ± 26 mg/dL).

This study is the first to show that soy isoflavones lower plasma LDL-cholesterol concentrations in normocholesterolemic, premenopausal women. Despite the high amount of isoflavones consumed, the changes in lipid concentrations were small and it is uncertain whether such small changes would provide physiologic benefits to healthy young women with normal cholesterol concentrations. It is possible, however, that over a lifetime, the small effects observed in our study, in combination with nonlipid effects (3, 19, 56), could slow the development of atherosclerosis and subsequent risk of CVD even in normocholesterolemic women.

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