Soy Protein Intake by Perimenopausal Women Does Not Affect Circulating Lipids and Lipoproteins or Coagulation and Fibrinolytic Factors¹,²

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ABSTRACT Soy protein favorably alters serum lipids and lipoproteins in hypercholesterolemic individuals, thereby reducing cardiovascular disease risk. The primary purpose was to determine the effect of soy protein (40 g/d) on circulating lipids and lipoproteins or coagulation and fibrinolytic factors in normocholesterolemic and mildly hypercholesterolemic perimenopausal women. We also determined the contribution of coagulation and fibrinolytic and other factors (e.g., body size and composition; serum estrogens, ferritin, iron; dietary intake) to lipid profiles. Subjects were randomly assigned to treatment: isoflavone-rich soy (n = 24), isoflavone-poor soy (n = 24), or whey control (n = 21). We measured circulating lipids and lipoproteins at baseline, wk 12 and wk 24, and coagulation/fibrinolytic factors at baseline and wk 24. Coagulation and fibrinolytic factors were not adversely affected by treatment. Treatment did not alter lipid profiles in mildly hypercholesterolemic (n = 30) or in all subjects combined. Time significantly (P < 0.001) affected serum total cholesterol, triacylglycerol, LDL cholesterol and HDL cholesterol concentrations. We could not attribute changes over time to various factors, but at baseline accounted for 57% of the variability in HDL cholesterol (P ≤ 0.0001) and for 50% in the total to HDL cholesterol ratio (P ≤ 0.0001). Dietary vitamin E and % energy from fat had positive effects, whereas plasma plasminogen activator inhibitor-1, fibrinogen, body weight and serum ferritin had negative effects on HDL and total to HDL cholesterol. Isoflavone-rich or isoflavone-poor soy protein had no effect on lipid profiles or coagulation and fibrinolytic factors, whereas the effect of time suggested that the hormonal milieu during the menopausal transition may have overridden any detectable treatment effect on lipids. The relationship between coagulation factors and serum lipids should be examined further as indices of cardiovascular disease risk in midlife women.


KEY WORDS: • fibrinogen • isoflavones • lipoprotein • plasminogen activator inhibitor-1

Perimenopause is considered a critical period in which preventive efforts should be initiated to reduce the menopause-associated increase in cardiovascular disease (CVD) risk (1). Soy protein with isoflavones (estrogen-like substances structurally similar to 17 β-estradiol) has been reported to favorably alter lipid profiles in perimenopausal (2) and post-menopausal (3,4) women. In contrast, studies with extracted isoflavones (5–8) have not shown a beneficial effect on circula-

1 Supported by Hatch Act and State of Iowa funds, a U. S. Department of Agriculture special grant, and an Iowa State University research grant. The soy protein isolates (Supro 675 HG and 675 IF) were donated by Protein Technologies International, a DuPont Business (St. Louis, MO); whey protein (ProMod) by Ross Laboratories (Columbus, OH); cranberries by Ocean Spray Cranberries, Inc. (Lakeville-Middleboro); and the flavorings and extracts by Tone Brothers, Inc. (Ankeny, IA).

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4 Abbreviations used: CVD, cardiovascular disease; PAI-1, plasminogen activator inhibitor-1; SPI–, isoflavone-poor soy protein isolate; SPI+, isoflavone-rich soy protein.
The primary aim of this study was to determine whether 24 wk of isoflavone-rich soy protein (SPI+) exerts an effect on lipid and lipoprotein concentrations or on coagulation and fibrinolytic factors in perimenopausal women. We hypothesized that SPI+ would have a modest beneficial effect on lipid profiles but would not exert a negative effect on coagulation or fibrinolytic factors, as is common with estrogen replacement therapy (12). The secondary aim was to determine the contribution of coagulation and fibrinolytic factors [plasma fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and factor VII antigen], along with other pertinent factors (such as body size and composition; physical activity; blood pressure; serum estrogens, ferritin and iron; dietary intake of fat, alcohol, fiber and antioxidants), to the change in lipid and lipoprotein concentrations and to their baseline values in these perimenopausal women.

SUBJECTS AND METHODS

Research design and treatment. This 24-wk double-blind study compared the effects of SPI+ (Protein Technologies International, St. Louis, MO), isoflavone-poor soy protein isolate (SPI−; Protein Technologies International), and whey protein (control; Ross Laboratories, Columbus, OH) on circulating lipids and lipoproteins or coagulation and fibrinolytic factors in perimenopausal women. Each perimenopausal woman was randomly assigned to one of three treatments: SPI+ (80.4 mg/d aglycone components; n = 24), SPI− (4.4 mg/d aglycone components; n = 24) or control (n = 21). The whey protein served as a control for soy protein (SPI+ and SPI−), whereas SPI− served as a control for isoflavones (SPI+). Detailed written and verbal instruction on how to avoid other isoflavone-containing foods was provided for all subjects. The women (premenopausal) were asked to consume a total of 40 g protein/d provided in a jumbo muffin and as protein powder and to limit the remaining daily protein intake to one serving (i.e., 85 g of meat or meat substitute). The muffins were baked in the Human Metabolic Unit of the Center for Designing Foods to Improve Nutrition at Iowa State University. The subjects were instructed to consume the muffin and protein powder as a meal replacement and not as a supplement, because the treatment vehicle supplied ~2.89 MJ (500 kcal)/d. Because many of the women used supplements routinely, they were instructed to stop taking their own before the study began to obviate potential confounding due to ingestion of various supplement formulations. We then provided a daily over-the-counter vitamin and mineral supplement to ensure that all subjects were exposed to the same dose of supplemental vitamins and minerals. The women were informed of their rights as volunteers in this study, signed consent forms and were given a medical release form to be signed by their physicians before baseline testing. The study protocol and consent forms were approved by Iowa State University Human Subjects Review Committee (Institutional Review Board 01, Assurance ID M1361). Every 6 wk, the women were required to visit the Human Metabolic Unit for testing, replenishment of supplies, compliance checks and for submitting 24-h urine samples.

Subject screening, selection and characteristics. Subjects were recruited throughout the state of Iowa through newspaper and bulletin board advertisements, local television news stories and newspaper articles. Telephone interviews were conducted to screen potential perimenopausal women to ensure that they met our inclusion and exclusion criteria: experiencing ≥10 hot flushes and/or night sweats per wk, had irregular menses or cessation of menses for <1 y, had one or both ovaries remaining, had a body mass index (kg/m2) between 19 and 31, were willing to be randomly assigned to treatment and were able to participate for 24 wk. Women were excluded if they had a chronic disease (i.e., heart disease or osteoporosis), were taking medications chronically, had taken sex hormone therapy during the past 12 mo, had a history of an eating disorder or menstrual disorder and/or were excessive exercisers (>10.46 MJ (>2500 kcal) expenditure/wk). Once potential subjects qualified after the initial screening, blood was drawn to ensure that follicle-stimulating hormone concentrations were ≥30 IU/L (17); 22 were excluded on this basis. Women continuing participation due to inability to tolerate treatment (n = 6), medical conditions preventing continuance (n = 2), death (n = 1) or death in the family (n = 1); we excluded one woman due to noncompliance. Because of our strict inclusion and exclusion criteria, we recruited women in four waves or cohorts, with subjects randomly assigned to treatment within each cohort. The cohorts began in January 1997, May 1997, September 1997 and March 1998.

Data collection and measurement. Information on health and medical history and physical activity was obtained using interviewer-administered questionnaires. The health and medical history was used to rule out women with chronic or acute conditions or diseases and those who chronically used various drugs (nonprescription, prescription or illegal). The Paffenbarger Physical Activity Recall (18) was used to obtain information on weekly physical activity during their previous year. Each recreational activity was summed to provide an estimate of weekly energy expenditure. Dietary intake was assessed at baseline, wk 12 and wk 24 using 5-d food records. To assist subjects in quantifying portion sizes, two-dimensional food portion visual aids (Nutritional Consulting Enterprises, Morgan & Posner, 1981, Framingham, MA) were provided. Food records were analyzed by trained nutrition students using the Nutritionist IV computerized nutrient database program (Version 4.1, 1995; First DataBank, San Bruno, CA). The vitamin and mineral supplement provided to subjects was not included in these analyses.

Anthropometry data included measurement of height (using a stadiometer) and weight (using a balance beam scale; Health-o-meter, Bridgeville, IL). Dual-energy X-ray absorptionometry via QDR-2000+ (Hologic, Waltham, MA) was used to assess total body composition (lean and fat mass). Two researchers trained by Hologic performed all scans; one of these researchers analyzed all total body scans with software provided by the manufacturer (Version 710; 1992; Waltham, MA). Trained personnel used a random-digit sphygmomanometer (Marshall Electronics, Skokie, IL) to measure resting blood pressure.

Each subject collected a 24-h urine sample in polyethylene containers the day before each visit to the Human Metabolic Unit. After the first morning’s void, all urine was collected, including the next morning’s void. Total volume was measured and recorded for each morning’s void. Aliquots of urine were collected on a 5-mL basis for shipment to Fujicco Ltd. (Kobe, Japan), where urinary isoflavones were analyzed by reversed-phase HPLC to monitor compliance.

Fasted blood was drawn in the early morning from each subject at baseline, wk 12 and wk 24 and serum (1000 × g for 15 min at 4°C) or plasma (1200 × g for 10 min at 4°C) was separated for measurement of blood analytes. Serum was frozen and stored for less than 2 wk at −20°C for analysis by Quest Diagnostics (St. Louis, MO), a certified clinical laboratory. Quest performed the serum iron, 17β-estradiol, estrone, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride measurements at each time point. Using the Friedewald equation, LDL cholesterol concentrations were calculated: (total cholesterol) − ([HDL cholesterol] + [triglyceride]/5). To convert the cholesterol (total, LDL-C and HDL-C) values to mmol/L, we multiplied by 0.02586; to convert the triglyceride values to mmol/L, we multiplied by 0.01129. Serum ferritin was determined at Iowa State using an enzyme-linked immunosassay kit obtained from RAMCO Labs (Houston, TX); intra-assay variability was 9.8%. Plasma was frozen and stored at −80°C for Lp(a), factor VII antigen, factor VII coagulant activity, PAI-1 and fibrinogen analyses, conducted under the supervision of Dr. Larry Brace in the Hematology and Coagulation Laboratory at the University of Illinois at Chicago. Plasma Lp(a) [TintElize Lp(a)] concentrations were measured at baseline, wk 12 and wk 24, using an immunoenzymetric method with affinity-purified polyclonal antibodies against Lp(a), according to the manufacturer’s guidelines (Biopool International, Ventura, CA). Lp(a) concentrations were read using an automated microparticle reader (EL311x; Bio-Tek Instruments, Winooksi, VT); intra-assay variability was 8.7%. The remaining coagulation and fibrinolytic factors were measured from baseline and wk 24 plasma.
samples. To measure factor VII antigen (Asserachrom VII:Ag) concentrations, we used an enzyme immunoassay procedure for the quantitative determination of factor VII by the sandwich technique according to guidelines from Diagnostica Stago (Asnieres-Sur-Seine, France). Factor VII antigen concentrations were read with the automated plate reader; intra-assay variability was 5.4%. Plasma factor VII coagulant activity (Staclot VIIa-rTF) was quantitatively determined by measuring the clotting time of plasma after exposure to recombinant soluble tissue factor according to the manufacturer’s guidelines (Diagnostica Stago, Asnieres-Sur-Seine, France). Because factor VII coagulant activity is cold temperature-sensitive, plasma samples were not placed on ice but were centrifuged and kept at room temperature until frozen at −80°C. We did not measure factor VII coagulant activity in samples from the last cohort due to technical difficulties. PAI-1 (Stachrom PAI) was quantitatively determined by the synthetic chromogenic substrate method according to guidelines from Diagnostica Stago. Factor VII coagulant activity, PAI-1 and fibrinogen were analyzed in the Hematology and Coagulation Laboratory using the STA-R-automated coagulation analyzer (Diagnostica Stago) by a certified Diagnostica Stago technician. Because this system is automated and was newly installed, intra-assay variability was not available and we could not obtain these values from Diagnostica Stago.

**Statistical analyses.** Statistical analyses were performed with PC SAS (Version 8, 1999; Cary, NC); results were considered statistically significant at \( P < 0.05 \). Descriptive statistics included means for normally distributed data (total cholesterol, LDL cholesterol, and total to HDL cholesterol; age; body size and composition; and blood pressure) and medians for data that were not normally distributed (triaclyglycerol, HDL cholesterol, and Lp(a); physical activity; dietary nutrient intake; and coagulation and fibrinolytic factors). Pearson correlation analysis was performed to examine the simple relationship between lipid and lipoprotein concentrations and various lipid-related factors at baseline. In determining a treatment effect, cohort was included in all analyses as an obligatory variable to account for the necessary random assignment of subjects to treatment within each cohort (loss of three degrees of freedom). Repeated measures ANOVA was used to determine the effect of treatment on total cholesterol, triacylglycerol, LDL cholesterol, HDL cholesterol, the ratio of total to HDL cholesterol and Lp(a), with their respective baseline values included in each analysis to account for individual differences at baseline. Because one outlier was found in the control group for Lp(a), we removed her data from the repeated measures analysis for determining a treatment effect on Lp(a). Residual analysis indicated nonconstancy of error variance for the triacylglycerol, HDL cholesterol and Lp(a) regression models. Thus, these values were log-transformed for the repeated measures ANOVA, Pearson correlation analysis and regression analyses, markedly improving the residual plots for the latter. In an analogous manner, we also determined in separate ANOVA whether treatment had an effect in mildly hypercholesterolemic [total cholesterol \( \geq 5.69 \) mmol/L (\( \geq 220 \) mg/dL)] subjects (n = 30). ANOVA was used to determine the effect of treatment on fibrinogen, factor VII antigen, factor VII coagulant activity and PAI-1. Because fibrinogen and PAI-1 were not normally distributed, these values were log-transformed for the ANOVA.

Stepwise multiple regression was used to determine the effect of contributors to each lipid and lipoprotein outcome at baseline and to their change from baseline to wk 24: concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol, Lp(a) and the total to HDL cholesterol ratio. Classes of variables in modeling the lipid and lipoprotein outcomes included values for age, body size and composition (weight, lean mass, fat mass or percentage body fat), resting blood pressure (diastolic, systolic), dietary factors [fat (total g and as % of total energy), polyunsaturated fat, fiber, alcohol, vitamin C, vitamin E and iron], physical activity and circulating analytes (serum ferritin, iron, 17 β-estradiol, estrone; plasma PAI-1, fibrinogen, factor VII antigen). Factor VII coagulant activity was not included in the regression models due to the reduced sample size. Residual analyses indicated that the model assumptions of independence of residuals, normality of error terms and homogeneity of residual variance were satisfied for these regression models. No notable multicollinearities emerged among the independent variables, as indicated by the low variance inflation factors in the regression analyses.

**RESULTS**

**Compliance to dietary treatment.** Self-reported consumption of muffins (87% of subjects consumed 100%) and powder (84% consumed 100%) and urinary excretion of isoflavones (genistein and daidzein) in each group reflected excellent adherence to the dietary treatment. These self-reports were corroborated by urinary isoflavone excretion, with significant (\( P \leq 0.0001 \)) differences among groups at wk 12 and wk 24 but not at baseline (\( P = 0.62 \)). Median (min–max) urinary isoflavone concentrations (mg/L) at baseline, wk 12 and wk 24, respectively, for each of the three treatment groups were: S+I = 0.04 (0–0.9), 0.99 (0.09–6.0), 2.10 (0.4–16.9); S+I = 0.06 (0–0.8), 0.14 (0–1.7), 0.27 (0.06–2.5); and control = 0.06 (0–0.2), 0.05 (0–0.2), 0.08 (0–0.4). We removed one control subject’s data from all subsequent analyses due to her urinary excretion of isoflavones during treatment being similar to women in the S+I group. Counts of vitamin and mineral supplements indicated that the women in each treatment group were 95% compliant. Additional information on compliance is described in more detail elsewhere (20).

**Lipid and lipoprotein and coagulation and fibrinolytic factor concentrations: descriptive data and ANOVA.** Baseline characteristics of these perimenopausal women are presented in Table 1. The expected potential contributors to lipid and lipoprotein concentrations included age, body size and composition, physical activity, resting blood pressure, dietary intake of selected nutrients, plasma coagulation and fibrinolytic factors, and serum estrogens, ferritin and iron. None of these characteristics at baseline were significantly different among the treatment groups, and, therefore, results from the three groups are reported as one. During the course of treatment, we documented an increase in dietary intake of energy [0.472 MJ (114 kcal); \( P = 0.014 \)] and of protein (27 g; \( P = 0.014 \)), whereas the increase in carbohydrate (3 g; \( P = 0.68 \)) and the decrease in fat (2 g; \( P = 0.42 \)) were not significant. There were no differences among the three treatment groups with respect to change in dietary intake. Body weight increased by 1.5% to 2.5% in each of the three groups during the course of treatment as noted in footnote 2 in Table 1, but there were no differences in weight gain among the groups. In addition, weight gain was not affected by treatment (\( P = 0.69 \)) and weight gain or changes in dietary intake did not remain in any of the lipid and lipoprotein models when we examined their change from baseline to wk 24.

Total and LDL cholesterol concentrations and the ratio of total to HDL cholesterol are presented as means ± SEM as they were normally distributed. Triacylglycerol, HDL cholesterol and Lp(a) were not normally distributed and, thus, median values are presented, with the 25th and 75th percentile bars indicating variability (Fig. 1). Treatment had no significant effect on circulating total cholesterol (\( P = 0.96 \)), triacylglycerol (\( P = 0.90 \)), LDL-C (\( P = 0.76 \)), HDL-C (\( P = 0.99 \)), Lp(a) (\( P = 0.052 \)) concentrations, or on the ratio of total to HDL cholesterol (\( P = 0.90 \)) as determined by repeated measures ANOVA. However, treatment had a significant effect on total cholesterol (\( P = 0.0002 \)), triacylglycerol (\( P \leq 0.0001 \)), LDL cholesterol (\( P = 0.0007 \)), HDL cholesterol (\( P \leq 0.0001 \)), and the ratio of total to HDL cholesterol (\( P \leq 0.0001 \)). By wk 24,
HDL cholesterol decreased and total to HDL cholesterol increased in all groups, triacylglycerol increased and total cholesterol decreased in the control group, and LDL-C increased particularly in the SPI+ group. Although Lp(a) concentrations increased from wk 12 to wk 24 in the control group, this was not significant because of the large variability in these values, particularly for this group. The only outcome in which cohort had a significant (P = 0.0055) effect was on LDL-C, with a significant cohort × time interaction (P = 0.0001). In examining only the hypercholesterolemic [total cholesterol ≥ 5.69 mmol/L (≥220 mg/dL)] subjects (n = 30), we found no effect of treatment on circulating total cholesterol (P = 0.73), triacylglycerol (P = 0.33), LDL cholesterol (P = 0.76), HDL cholesterol (P = 0.79), the ratio of total to HDL cholesterol (P = 0.77), or Lp(a) (P = 0.51) concentrations, whereas time significantly affected total cholesterol (P = 0.0022), triacylglycerol (P = 0.0001), LDL cholesterol (P = 0.038), HDL cholesterol (P = 0.0012) and the ratio of total to HDL cholesterol (P ≤ 0.0001). Treatment had no effect on coagulation or fibrinolytic factors (Table 2), but factor VII coagulant activity increased significantly (P ≤ 0.0001) from baseline to wk 24; cohort (P = 0.042) had a significant effect on factor VII coagulant activity.

**Lipid and lipoprotein concentrations: regression analyses.** Because we did not demonstrate a treatment effect, we first explored what additional factors might be related to the documented change (due to time) in circulating lipid and lipoprotein concentrations. We also performed additional analyses to examine the influence of purported CVD-related factors on circulating lipid and lipoprotein concentrations at baseline, because few data are available for perimenopausal women. Coagulation factors were included as contributors to lipid and lipoprotein models due to their established relationship in acute cardiovascular syndromes (13). The models for change in lipid and lipoprotein concentrations are not presented in detail, because these overall models were not significant. For instance, only 3% of the change in total cholesterol (F = 0.48, P = 0.75) could be accounted for by cohort (P = 0.55), treatment (SPI+ vs. SPI−, P = 0.37; SPI+ vs. control, P2 = 0.77) and weight gain (P = 0.36). Likewise, <9% of the change in HDL cholesterol (F = 1.44, P = 0.23) was accounted for by cohort (P = 0.049), treatment (SPI+ vs. SPI−, P = 0.59; SPI+ vs. control, P = 0.38) and weight gain (P = 0.47). The remaining regression models for change in LDL cholesterol, triacylglycerol or Lp(a) did not provide any additional information. To determine the relationship between various factors and these lipid and lipoprotein outcomes at baseline, we have presented the two best (highest R2 and F values) models—HDL cholesterol and the total to HDL cholesterol ratio (Table 3). Also, because biological variability (21) of total and HDL cholesterol is less (6–7%) than that of LDL cholesterol (9%), triacylglycerol (28%) or Lp(a) (1–45%), we are only reporting the cholesterol-based models. After variable elimination was completed, almost 57% of the variability in HDL cholesterol (P ≤ 0.0001) was accounted for by dietary vitamin E, plasma PAI-1, dietary fat (% of total energy), body weight and serum ferritin. Almost 50% of the variability in serum total to HDL cholesterol (P ≤ 0.0001) was accounted for by plasma PAI-1, vitamin E and plasma fibrinogen.

**Lipid and lipoprotein concentrations: correlation analysis at baseline.** In addition to being related to HDL cholesterol and to the total to HDL cholesterol ratio in the regression models, correlation analysis indicated that plasma PAI-1 was positively related (r = 0.50, P ≤ 0.0001) to triacylglycerol. Likewise, in addition to being related to the total to HDL cholesterol ratio in regression analysis, fibrinogen was related to each lipid outcome (i.e., LDL cholesterol: r = 0.32, P = 0.0098; Lp(a): r = 0.34, P = 0.0065) except triacylglycerol. As well as being related to HDL in the regression model, dietary fat (% of total energy) was positively related to total cholesterol (r = 0.26, P = 0.032) and negatively related to Lp(a) (r = −0.24, P = 0.047). Vitamin E intake was negatively related to triacylglycerol (r = −0.31, P = 0.011), as well as being related to HDL cholesterol and the total to HDL cholesterol ratio using regression. Although total body weight was negatively related to HDL cholesterol using regression, it was likely the fat mass (r = −0.24, P = 0.03) component exerting the effect, because lean mass was not related to HDL cholesterol (r = −0.15, P = 0.21) using correlation analysis. However, lean mass was inversely related to both total cholesterol (r = −0.36, P = 0.0021) and LDL cholesterol (r = −0.30, P = 0.011), whereas body weight was not related to lipids except to HDL cholesterol.

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**TABLE 1**

**Baseline characteristics in 69 perimenopausal women1,2**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.2 ± 3.6 (41.9–61.6)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>164.8 ± 5.0 (151.6–177.5)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.3 ± 9.0 (48.5–98.1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1 ± 3.2 (17.4–33.7)</td>
</tr>
<tr>
<td>Total body lean mass, kg</td>
<td>39.8 ± 6.8 (31.4–46.6)</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>37.2 ± 6.3 (18.4–59.1)</td>
</tr>
<tr>
<td>Energy expenditure, MJ/Jwk</td>
<td>4.33 (0.17–17.14)</td>
</tr>
<tr>
<td>Resting systolic blood pressure, mmHg</td>
<td>119 ± 11 (100–144)</td>
</tr>
<tr>
<td>Resting diastolic blood pressure, mmHg</td>
<td>71 ± 10 (54–98)</td>
</tr>
</tbody>
</table>

**Measure variable**

<table>
<thead>
<tr>
<th>Value</th>
<th>Median (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ</td>
<td>7.46 (4.07–10.64)</td>
</tr>
<tr>
<td>Fat, g</td>
<td>59.4 (26.8–99.8)</td>
</tr>
<tr>
<td>% of total energy</td>
<td>30.2 (14.8–45.8)</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>18.7 (7.6–48.4)</td>
</tr>
<tr>
<td>Polysaturated fat, g</td>
<td>10.5 (2.8–20.6)</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>18.7 (4.1–51.2)</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>116.8 (27.8–586.2)</td>
</tr>
<tr>
<td>Folate, µg</td>
<td>10.7 (1.5–29.7)</td>
</tr>
<tr>
<td>Alcohol intake, g/5d</td>
<td>246.7 (74–725)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD (min–max).
2 These characteristics at baseline were not significantly different among the three treatment groups.
3 Gain in body weight was 1.5 kg (+2.2%) in isoflavone-rich, 1.0 kg (+1.5%) in isoflavone-poor, and 1.7 kg (+2.5%) in control. Treatment had no effect (P = 0.69) on weight gain, which was not different among the groups.
4 Body composition was assessed by dual-energy X-ray absorptiometry.
5 Median value is reported for energy expenditure, which is a reflection of weekly recreational activity as assessed by the Paffenbarger physical activity recall questionnaire; likewise, median values are reported for serum analytes and dietary intake, since most were not normally distributed.
6 Dietary nutrient intake was assessed using a 5-d food diary collected prior to baseline testing.
7 Alcohol intake is based on 33 women who reported consuming alcohol during the 5-d recording of the diary.
DISCUSSION

Dietary intake of soy protein for 24 wk did not improve circulating lipid and lipoprotein concentrations in these 69 perimenopausal women or in subjects (n = 30) who were mildly hypercholesterolemic. In addition, SPI1 did not adversely affect coagulation and fibrinolytic factors, in contrast to the unfavorable effects reported with estrogen therapy (12). The lipid results are similar to previous studies that used extracted soy isoflavones rather than soy protein (5,6,8). However, our results do not agree with those studies that used isoflavone-containing soy protein (2–4,10). The differences may be due to other studies being of shorter duration (2,4,10), having a smaller sample size (2,4), using a crossover design (2,4), including clearly postmenopausal women (3,4) and having subjects at baseline with higher total cholesterol (3), higher LDL cholesterol (4,10) or lower HDL cholesterol and

FIGURE 1  Concentrations (mean ± SEM) of serum total cholesterol, LDL cholesterol and the ratio of total cholesterol to HDL cholesterol presented from baseline to wk 24 in each of three treatment groups of perimenopausal women: SPI+ (n = 24), SPI− (n = 24) and whey (control; n = 21) protein. Concentrations of serum triacylglycerol, serum HDL cholesterol and plasma Lp(a) from baseline to wk 24 in each of three treatment groups are presented as median values (error bars indicate 25th and 75th percentiles) because they are not normally distributed. Lipid and lipoprotein values at baseline were not significantly different among treatment groups. Repeated measures ANOVA indicated that treatment had no significant effect on circulating total cholesterol (P = 0.96), triacylglycerol (P = 0.90), LDL-C (P = 0.76), HDL-C (P = 0.99), Lp(a) (P = 0.052) concentrations on the ratio of total to HDL cholesterol (P = 0.90), whereas time significantly affected serum total cholesterol (P = 0.0002), triacylglycerol (P < 0.0001), LDL cholesterol (P = 0.0007), HDL cholesterol (P = 0.0001) and the ratio of total to HDL cholesterol (P = 0.0001); cohort had a significant (P = 0.0005) effect on LDL-C.

TABLE 2

Coagulation and fibrinolytic factor concentrations at baseline and wk 24 in perimenopausal women

<table>
<thead>
<tr>
<th>Measure1</th>
<th>Isoflavone-rich soy protein (SPI+)</th>
<th>Isoflavone-poor soy protein (SPI−)</th>
<th>Whey protein (control)</th>
<th>P values (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Wk 24</td>
<td>Baseline Wk 24</td>
<td>Baseline Wk 24</td>
<td>Treatment Cohort</td>
</tr>
<tr>
<td>Fibrinogen,2 g/L</td>
<td>3.3 (2.5–4.6)</td>
<td>3.5 (2.6–4.4)</td>
<td>3.2 (1.4–4.5)</td>
<td>3.2 (2.7–4.2)</td>
</tr>
<tr>
<td>Factor VII antigen,2 %</td>
<td>79.4 ± 12.1</td>
<td>76.8 ± 12.3</td>
<td>74.1 ± 12.0</td>
<td>75.0 ± 15.8</td>
</tr>
<tr>
<td>Factor VII coagulant activity,3 %</td>
<td>46.4 ± 32.5</td>
<td>76.7 ± 33.4</td>
<td>42.4 ± 30.6</td>
<td>73.4 ± 39.6</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1,4 µkat/L</td>
<td>283.4 (66.7–550)</td>
<td>283.4 (66.7–1,234)</td>
<td>333.4 (150–734)</td>
<td>300.1 (183–700)</td>
</tr>
</tbody>
</table>

1 Means ± SD are reported for normally distributed data (factor VII antigen and factor VII coagulant activity); medians (min–max) are reported for data (fibrinogen and plasminogen activator inhibitor-1) that are not normally distributed; these latter variables were log-transformed for ANOVA.
2 For fibrinogen and factor VII antigen, n = 20 in SPI+, n = 23 in SPI−, and n = 21 in control; n = 64.
3 Factor VII coagulant activity: n = 16 in SPI+, n = 15 in SPI−, n = 15 in control; n = 46; factor VII coagulant activity increased (P < 0.0001) from baseline to wk 24.
4 Plasminogen activator inhibitor-1: n = 21 in SPI+, n = 23 in SPI−, n = 21 in control; n = 65.
higher triacylglycerol (2–4) concentrations than our participants. Among those published to date, the study by Washburn et al. (2) is the most comparable, yet their perimenopausal women had more variable baseline total cholesterol values (mmol/L) than ours (mean ± SD = 5.38 ± 1.05 vs. 5.49 ± 0.85), perhaps partially explaining the disparate results. A myriad of differences in design make direct comparisons among studies difficult. Nonetheless, a meta-analysis of 38 trials in humans (9) indicated a decline in total (9%) and LDL (13%) cholesterol, and in triacylglycerol (10%), with an average soy protein intake of 47 g/d. This analysis reported that initial serum cholesterol concentrations determined the extent of response, with mildly hypercholesterolemic (5.2–6.6 mmol/L) exhibiting nonsignificant reductions. Hence, our lack of response is not entirely surprising because less than one half of these women were mildly hypercholesterolemic at baseline.

Significant changes in total cholesterol, triacylglycerol, LDL cholesterol, HDL cholesterol and the total to HDL cholesterol ratio during the 24 wk were due to a time effect (Fig. 1). The changes in lipid profiles could not be attributed to weight gain and treatment did not affect weight gain. However, time also affected (∆ weight gain and treatment did not affect weight gain. How-1). The changes in lipid profiles could not be attributed to LDL cholesterol, HDL cholesterol and the total to HDL cholesterol ratio. Overall model \(R^2 = 56.5\%\) (Adj \(R^2 = 52.9\%\)); \(F (5, 59) = 15.35\) (\(P < 0.0001\)).

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Parameter estimate</th>
<th>Percentage variance</th>
<th>(P) value</th>
<th>Variance inflation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol4</td>
<td>Overall model (R^2 = 56.5%) (Adj (R^2 = 52.9%)); (F (5, 59) = 15.35) ((P &lt; 0.0001))</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intercept</td>
<td>0.4779</td>
<td>0.0207</td>
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<tr>
<td>Dietary vitamin E</td>
<td>0.0142</td>
<td>13.62</td>
<td>0.0001</td>
<td>1.10</td>
</tr>
<tr>
<td>Plasma plasminogen</td>
<td>−0.0005</td>
<td>7.77</td>
<td>0.0019</td>
<td>1.17</td>
</tr>
<tr>
<td>activator inhibitor-1</td>
<td>0.0108</td>
<td>7.01</td>
<td>0.0031</td>
<td>1.05</td>
</tr>
<tr>
<td>Dietary fat (% of total energy)</td>
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<td>3.76</td>
<td>0.028</td>
<td>1.11</td>
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<tr>
<td>Body weight</td>
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<td>3.50</td>
<td>0.033</td>
<td>1.04</td>
</tr>
<tr>
<td>Serum ferritin</td>
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<td>16.56</td>
<td>0.0001</td>
<td>1.09</td>
</tr>
<tr>
<td>Total Cholesterol/HDL-C</td>
<td>Overall model (R^2 = 49.9%) (Adj (R^2 = 47.4%)); (F (3, 60) = 19.92) ((P &lt; 0.0001))</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intercept</td>
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<td>0.023</td>
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<tr>
<td>Plasma plasminogen</td>
<td>0.0028</td>
<td>16.56</td>
<td>0.0001</td>
<td>1.09</td>
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<tr>
<td>activator inhibitor-1</td>
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<td>10.72</td>
<td>0.0007</td>
<td>1.06</td>
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<tr>
<td>Dietary vitamin E</td>
<td>0.6112</td>
<td>10.61</td>
<td>0.0007</td>
<td>1.04</td>
</tr>
</tbody>
</table>

1 Squared semipartial type II correlation coefficient; accounts for shared variance among variables.
2 Variables left in models are significant at \(P < 0.10\).
3 Measures inflation in the variances of parameter estimates due to multicollinearities among regressors.
4 Because HDL-C values were not normally distributed, they were log-transformed for this analysis.

TABLE 3

Regression analyses: contributors to HDL cholesterol and the ratio of total to HDL cholesterol at baseline in perimenopausal women

Because lipid and lipoprotein concentrations exhibit biological variability (21), it would have been desirable to have drawn multiple blood samples, but this was not possible. Yet, we have no reason to believe that any given group exhibited more variability than another, because subjects were randomly assigned to treatment. Thus, the lack of treatment effect should not be questioned based on day-to-day variability. However, this study is unique in that we identified coagulation and fibrinolytic factors that contributed to the lipid profiles at baseline in these midlife women.

The following few paragraphs refer to relationships from the regression or Pearson correlation analyses at baseline among circulating lipids and lipoproteins and various factors for all subjects combined. Our finding of a positive relationship between plasma fibrinogen and LDL cholesterol has been reported in population (27,28) and cross-sectional (29,30) studies. These population studies, like our study, also have shown a positive association between fibrinogen and Lp(a) concentrations, signifying the involvement of thrombosis in atherogenesis. Fibrin, the end-product of fibrinogen conversion, provides an absorptive surface area within plaque for Lp(a) and LDL (31), suggesting the role of Lp(a) and fibrinogen in atherogenesis. Researchers (29) have also reported a negative correlation between fibrinogen and HDL cholesterol, supporting our finding of a positive relationship between fibrinogen and the total to HDL cholesterol ratio. Our findings that plasma PAI-1 was positively related to triacylglycerol and negatively related to HDL cholesterol are similar to that of the European Concerted Action on Thrombosis Angina Pectoris study (32). The direct relationship that we found between PAI-1 and triacylglycerol may be explained by the stimulatory effect of triacylglycerol-rich lipoproteins on PAI-1 release from hepatic cells (33). The HDL-associated hydrolase, paraoxo- nase (34), may inhibit lipid peroxidation of LDL particles; thus, HDL is thought to exhibit antioxidant properties. It is through this mechanism that HDL may attenuate LDL cholesterol-induced changes in generating fibrinolytic regulators,
such as PAI-1, from vascular endothelial cells (35). The negative association between serum ferritin and HDL cholesterol has been previously reported (15), although the role of iron stores in CVD remains controversial (36). The association of lower HDL cholesterol with higher iron stores (as reflected by serum ferritin) might indicate greater lipid peroxidation and, hence, greater removal of oxidized HDL particles via scavenger receptors (37). Although the iron bound to ferritin does not dissociate readily, perhaps it is a source of iron for redox reactions (38).

Our study confirms the idea that dietary factors are related to serum lipoproteins and lipids. High fat intakes, depending upon the specific fatty acid profile, may increase cholesterol (39), particularly LDL and HDL (40). At baseline, we found that as dietary fat (as % of total energy) increased, total and HDL cholesterol increased. The effect of dietary fat intake on Lp(a) is less well established, but we found that fat intake was negatively related to Lp(a), similar to the DELTA study (40), which showed an inverse association with saturated fat intake. The (n-3) fatty acids, rather than total fat as in our study, have been shown to reduce Lp(a) concentrations (41). It is likely that as total fat increases, (n-3) fatty acids also increase, but our nutrient analysis program did not provide these estimates. Estrogen therapy has been reported to decrease Lp(a) (42), but we found no association between either SPI+ or serum estrogens and Lp(a) concentrations. In our study, vitamin E was directly related to the total to HDL cholesterol ratio and triacylglycerol, but indirectly to HDL cholesterol, perhaps signifying the role of vitamin E in quenching lipid peroxidation. Yet, α-tocopherol has been shown to exhibit anti- or pro-oxidant activity for serum lipids, depending upon the reactivity of the oxidant and degree of radical influx (43). Vitamin E supplements have been shown to reduce intracellular lipid peroxide, leading to greater hepatic uptake of triacylglycerol-rich lipoproteins (44). The Nurses Health Study (45) results suggest that 2 y of vitamin E supplementation (> 100 IU/d) was associated with a 41% decline in coronary heart disease risk in women (n = 87,245) 34–59 y of age. We found that body weight contributed negatively to HDL cholesterol, as previously reported (46–48), likely attributable to the fat component. Most studies have reported a positive relationship between total cholesterol and fat mass (49), but our study indicated a negative relationship between lean mass and total or LDL cholesterol, suggesting that lean mass per se may exert protective effects. Perhaps the effect of lean mass on lipids has not been reported because fat mass or body mass index rather than lean mass is typically assessed.

In summary, soy protein, regardless of its isoflavone content, did not alter lipid and lipoprotein concentrations, but also did not adversely affect coagulation and fibrinolytic factors in perimenopausal women. Any treatment effect on lipids or lipoproteins may have been overshadowed by hormonal fluctuations during the perimenopausal period. Dietary vitamin E and dietary fat (as % of total energy) favorably affected their lipid profiles, whereas plasma PAI-1 and fibrinogen, body weight and ferritin adversely affected their lipid profiles. The coagulation and fibrinolytic factors that we identified should be examined further as indices of CVD risk in midlife women.

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LITERATURE CITED


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