

# Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women<sup>1–3</sup>

Laura N Hanson, Heather M Engelman, D Lee Alekel, Kevin L Schalinske, Marian L Kohut, and Manju B Reddy

## ABSTRACT

**Background:** Soy protein or its components may protect against the atherosclerotic cardiovascular disease (CVD) risk factors total homocysteine (tHcy), C-reactive protein (CRP), and excess body iron, which generally increase with menopause.

**Objective:** The primary objective of this study was to determine the independent effect of the soy protein components isoflavones and phytate on CVD risk factors in postmenopausal women. The secondary objective was to identify factors [blood lipids, oxidative stress indexes, serum ferritin, plasma folate, plasma vitamin B-12, and body mass index (BMI)] contributing to tHcy and CRP concentrations.

**Design:** In a double-blind, 6-wk study, 55 postmenopausal women aged 47–72 y were randomly assigned to 1 of 4 soy protein (40 g/d) isolate treatments: native phytate and native isoflavone ( $n = 14$ ), native phytate and low isoflavone ( $n = 13$ ), low phytate and native isoflavone ( $n = 14$ ), or low phytate and low isoflavone ( $n = 14$ ). We measured iron indexes, tHcy, CRP, and BMI.

**Results:** Soy protein with native phytate significantly reduced tHcy ( $P = 0.017$ ), transferrin saturation ( $P = 0.027$ ), and ferritin ( $P = 0.029$ ), whereas soy protein with native isoflavones had no effect on any variables. At baseline, BMI was highly correlated with tHcy ( $r = 0.39$ ,  $P = 0.003$ ) and CRP ( $r = 0.55$ ,  $P < 0.0001$ ), whereas HDL cholesterol was correlated with CRP ( $r = -0.30$ ,  $P = 0.02$ ). Multiple regression analysis showed that LDL cholesterol and BMI contributed significantly ( $R^2 = 19.9\%$ ,  $P = 0.003$ ) to the overall variance in tHcy.

**Conclusion:** Consuming phytate-rich foods and maintaining a healthy weight may reduce atherosclerotic CVD risk factors in postmenopausal women. *Am J Clin Nutr* 2006;84:774–80.

**KEY WORDS** Postmenopausal women, homocysteine, C-reactive protein, iron, cardiovascular disease, soy protein, isoflavones, phytate

## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States (1). The incidence of CVD is lower in premenopausal women than in men; however, CVD risk in postmenopausal women is 3.4 times that in premenopausal women (2). These differences in risk may be partially related to increases in total homocysteine [tHcy (3)], C-reactive protein [CRP (4)], and excess body iron (5). Elevated tHcy is an independent, modifiable risk factor for atherosclerotic CVD (6). CVD patients from the Women's Health Study (3) who were in the highest

quartile for tHcy concentrations ( $>13.26 \mu\text{mol/L}$ ) were twice as likely to experience a future cardiovascular event as were those in the lowest quartile ( $<9.54 \mu\text{mol/L}$ ). Whereas premenopausal women typically have lower tHcy concentrations than do men of all ages, the women's values increase by 7–20% after menopause (7, 8) and become comparable to the values in similarly aged men. Although tHcy may affect atherosclerotic CVD risk by several mechanisms, strong evidence exists that hyperhomocysteinemia suppresses the production of nitric oxide (9), an important vasodilator and antioxidant, and that suppression indirectly causes damage to the vasculature (10–12). The mechanism by which tHcy is involved in the initiation and progression of atherosclerotic CVD should be studied further. Elevated CRP, a marker of acute inflammation, is a reliable predictor of CVD and concentrations  $>75$ th percentile (ie,  $>2.11 \text{ mg/dL}$ ) are associated with a 1.5-fold risk of myocardial infarction (4). Studies have shown that hormone therapy results in a short-term rise in CRP (13, 14). CRP may stimulate the incorporation of monocytes into atherosclerotic lesions (15, 16) and thus contribute to endothelial dysfunction (17, 18). Iron stores increase with age in both men and women, paralleling the rise in CVD risk (5). A Finnish study associated excess iron stores ( $>200 \mu\text{g}$  ferritin/L) in men with CVD (19). In women, serum ferritin concentrations are clearly elevated with each decade of life beyond age 40 y, and those higher concentrations increase the risk of coronary artery disease (20). Excess iron may cause oxidative damage (21, 22) and also may adversely alter lipid metabolism (23).

The FDA approved a food label claim that 25 g soy protein/d may help prevent coronary heart disease (24), based on reductions in lipids and lipoproteins. CVD risk reduction also may be due to soy protein's lowering effect on tHcy (25, 26). Soy isoflavones, saponins,  $\beta$ -conglycinin, and phytate may contribute to the cardioprotective benefit of soy protein, but it is difficult to distinguish their specific effects. Soy protein with isoflavones

<sup>1</sup> From the Departments of Food Science and Human Nutrition (LNH, HME, DLA, KLS, and MBR) and of Health and Human Performance (MLK), Iowa State University, Ames, IA.

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<sup>3</sup> Reprints not available. Address correspondence to MB Reddy, 1127 Human Nutritional Sciences Building, Iowa State University, Ames, IA 50011. E-mail: mbreddy@iastate.edu.

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**TABLE 1**  
Nutrient content of 40 g soy protein isolates<sup>1</sup>

Treatment	Phytate	Aglycone isoflavones	Iron	Calcium
	<i>g</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>
LP/LI ( <i>n</i> = 14)	0.22	1.2	5.9	504
NP/LI ( <i>n</i> = 13)	0.64	1.2	5.7	510
LP/NI ( <i>n</i> = 14)	0.22	85.8	5.9	488
NP/NI ( <i>n</i> = 14)	0.78	84.6	5.7	480

<sup>1</sup> LP/LI, low phytate and low isoflavone; NP/LI, normal phytate and low isoflavone; LP/NI, low phytate and normal isoflavone; NP/NI, normal phytate and normal isoflavone. Information on the nutrient content of soy isolates was provided by the Solae Company (St Louis, MO).

has been shown to reduce total cholesterol (27), but its effect on tHcy (25) and CRP (28) is not conclusive. Phytic acid strongly inhibits iron absorption in humans (29, 30) and may reduce iron-associated oxidative damage (31, 32). Thus, phytate may be beneficial in reducing CVD risk in persons who are prone to excess iron. This study was designed to ascertain the independent effects of the soy protein components isoflavones and phytate on tHcy, CRP, and iron status in postmenopausal women.

## SUBJECTS AND METHODS

### Study design

Fifty-five healthy postmenopausal women aged 47–72 y participated in a randomized, double-blind, 6-wk soy protein study. We randomly assigned subjects to 1 of 4 soy protein isolate (SPI) treatments (40 g/d): low phytate and low isoflavone (LP/LI; *n* = 14), native phytate and low isoflavone (NP/LI; *n* = 13), low phytate and native isoflavone (LP/NI; *n* = 14), or native phytate/native isoflavone (NP/NI; *n* = 14). The SPI powders that the women consumed daily were manufactured by the Solae Company (St Louis, MO). Isoflavones were removed by alcohol extraction, and phytate was removed by enzyme hydrolysis by the manufacturer (Table 1). According to the Solae Company, the isoflavone content of the native isoflavone formula was higher than the normal SPI isoflavone content; hence, the term “native” is used instead of “normal” in this study. Each packet contained 20 g SPI, and women were asked to consume 2 packets/d (40 g/d). We provided subjects with recipes that incorporated the SPI powder into meals and beverages.

### Subject selection

We used a telephone screening questionnaire to recruit non-smoking, postmenopausal women ( $\geq 1$  y from cessation of menses) with no history of chronic disease, cholesterol-lowering medications, or hysterectomy and no use of oral hormonal therapy within the past 1 y or of topical hormones within the past 6 mo. Participants were asked to avoid nutritional supplements and isoflavone- or phytate-rich foods during the treatment period. To enhance compliance, at baseline, we provided the women a list of these foods with specific instructions related to avoiding other soy foods during the study. Fifty-five women completed the study; 2 subjects dropped out because of gastrointestinal discomfort with the protein treatment.

Written informed consent was obtained from all subjects. The study protocol, the consent forms, and the subject-related materials

were approved by the Iowa State University Human Subjects Review Committee (Institutional Review Board ID# 02–351).

### Compliance

We provided to each subject a fixed number of protein packets and instructions to return the unconsumed packets at the end of the study for ascertainment of compliance. According to the number of packets returned, 51 subjects were 100% compliant and the other 4 were 95% compliant. These 4 subjects returned unused packets (2, 3, 4, or 8 packets), and 2 of the subjects who were 100% compliant requested additional packets (3 or 4 packets), which were needed either because of a counting error when the packets were provided or because the subjects lost packets. These 4 subjects were dispersed across treatment groups, and thus the degree of compliance should not have significantly affected our results. In addition, random urine samples from 4 subjects in each treatment (16 total) were analyzed to measure urinary isoflavone excretion from 4 subjects in each treatment (16 total) at baseline and 6 wk. Results confirmed excellent compliance and indicated marked differences between the native- and low-isoflavone groups: LP/NI and NP/NI, 23 and 29  $\mu\text{mol/L}$ , respectively, and LP/LI and NP/LI, 1.6 and 2.8  $\mu\text{mol/L}$ , respectively.

### Data collection

Typical dietary intake of the subjects was assessed at baseline by using a food-frequency questionnaire from Block Dietary Data Systems (Berkeley, CA). In addition, interviewers administered health and medical history (33), nutrition history (33), and soy food intake (34) questionnaires. At baseline and at 6 wk, we collected fasted blood samples between 0700 and 0800 at baseline and at 6 wk for analysis of CVD risk factors and indexes of iron status. Each woman also collected a 24-h urine sample for urinary isoflavone analysis. We stored aliquots of blood and urine samples at  $-80^\circ\text{C}$  and at  $-20^\circ\text{C}$ , respectively, until they were analyzed.

### Sample analysis

We measured tHcy by using a method adapted from Araki and Sako (35) and Ubbink et al (36). Plasma samples were derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (Sigma Chemical Co, St Louis, MO), and tHcy samples were measured by using HPLC with a  $\mu\text{Bondapak C18}$  Radial-Pak column (Waters Associates, Milford, MA) and a fluorescence detector with excitation at 385 nm and emission at

**TABLE 2**  
Baseline subject characteristics<sup>1</sup>

	LP/LI (n = 14)	NP/LI (n = 13)	LP/NI (n = 14)	NP/NI (n = 14)
Age (y) <sup>2</sup>	56 (49–70)	59 (53–69)	58 (47–72)	60 (50–70)
Height (m)	1.66 ± 0.07 <sup>3</sup>	1.62 ± 0.08	1.65 ± 0.05	1.65 ± 0.05
Weight (kg)	71.0 ± 10.1	72.6 ± 11.9	72.7 ± 9.6	69.2 ± 10.7
BMI (kg/m <sup>2</sup> )	25.9 ± 3.6	27.9 ± 4.2	26.5 ± 3.4	25.3 ± 3.8
Daily dietary intake <sup>4,5</sup>				
Energy (kcal)	1690 (515–2559)	1934 (763–2708)	1796 (877–2827)	1626 (1144–2595)
Protein (g)	60 (22–104)	75 (30–108)	67 (31–122)	58 (39–103)
Carbohydrate (g)	229 (53–412)	228 (119–405)	200 (85–339)	192 (146–328)
Fat (g)	63 (25–96)	64 (22–113)	67 (36–139)	65 (30–105)
Saturated fat (g)	19 (7–37)	18 (6–30)	17 (12–38)	18 (9–26)
Folate (μg)	321 (114–654)	448 (208–617)	364 (234–810)	343 (251–582)
Vitamin B-12 (μg)	3.5 (0.8–6.9)	3.7 (1.6–8.0)	3.8 (1.6–5.0)	2.5 (0.8–5.4)
Vitamin B-6 (mg)	1.8 (0.7–2.8)	1.9 (1.1–2.8)	1.6 (0.8–3.1)	1.4 (1.0–2.5)
Iron (mg)	11.6 (4.5–20.3)	13.9 (7.0–22.1)	12.8 (5.6–22.2)	11.0 (8.5–19.4)

<sup>1</sup> LP/LI, low phytate and low isoflavone; NP/LI, normal phytate and low isoflavone; LP/NI, low phytate and normal isoflavone; NP/NI, normal phytate and normal isoflavone. No significant differences were found between the treatment groups (ANOVA for each variable).

<sup>2</sup>  $\bar{x}$ ; range in parentheses.

<sup>3</sup>  $\bar{x} \pm$  SD (all such values).

<sup>4</sup> All values are median; range in parentheses.

<sup>5</sup> Assessed by using the Block Dietary Data Systems Food-Frequency Questionnaire.

515 nm. We assessed plasma folate and vitamin B-12 status by using a radioimmunoassay kit (MP Biomedicals, Irvine, CA). Serum CRP was measured by using a high-sensitivity enzyme immunoassay kit (ALPCO Diagnostics, Windham, NH), and serum ferritin was measured by using an enzyme-linked immunosorbent assay kit (Ramco Laboratories, Houston, TX). The intraassay and interassay CVs were 11% and 4% for folate and vitamin B-12, 6% and 12% for CRP, and 8% and 12% for ferritin, respectively. A certified clinical laboratory (Quest Diagnostics, St Louis, MO) analyzed blood samples for hemoglobin, hematocrit, serum iron, total iron-binding capacity, and transferrin saturation to ascertain iron status.

### Statistical analysis

We performed statistical analyses by using SAS software (version 8.0; SAS Institute, Cary, NC) and considered the results significant at  $P \leq 0.05$ . On the basis of our sample size ( $n = 14$ /group), we had 80% power to detect a tHcy decline of  $0.8 \pm 1.0$  μmol/L with treatment. The descriptive statistics included mean (range) for age; mean ± SD for body mass index (BMI; in kg/m<sup>2</sup>), height, weight, tHcy, CRP, and iron indexes; and median (range) for dietary factors. We compared subject characteristics, dietary intake, and blood indexes in the treatment groups at baseline by using analysis of variance to ascertain potential inherent differences between the groups. To ascertain the effects of treatment on tHcy, CRP, and iron indexes, we compared the change (from baseline to 6 wk) in these variables between the treatment groups by using 2-factor analysis of variance. We performed Pearson correlation analysis on baseline variables to determine the relation between tHcy and CRP and other CVD risk factors (lipids and lipoproteins, oxidative stress indexes, serum ferritin, plasma folate, plasma vitamin B-12, and BMI). We used multiple regression analysis with stepwise selection to ascertain the effect of these factors on baseline tHcy and CRP concentrations.

## RESULTS

### Subject characteristics

The subjects reported that they were postmenopausal and that they had passed a mean of 6.4 y since the last menses. Thirteen subjects reported having been diagnosed with iron deficiency at some point in their lifetime, but only 2 reported current regular use of iron supplements. Women across treatment groups did not differ in age, height, weight, or BMI at baseline (**Table 2**) or at 6 wk. The differences between the treatment groups in dietary intake at baseline were not significant. Although the mean dietary intake of folate, vitamin B-6, and vitamin B-12 met the 1998 Dietary Reference Intakes (37), several women did not meet the Dietary Reference Intakes for these nutrients: 64% for folate, 20% for vitamin B-6, and 25% for vitamin B-12. However, plasma folate and B-12 concentrations were within normal ranges for all women.

Although healthy postmenopausal women were recruited for this project, these women were mildly hypercholesterolemic ( $\bar{x} \pm$  SD total cholesterol:  $228 \pm 34$  mg/dL; LDL cholesterol:  $143 \pm 32$  mg/dL). The lipid profile and oxidative stress data for these women were published previously (38). At baseline, no subjects had elevated tHcy (ie,  $>12$  μmol/L), and only 3 had elevated CRP (ie,  $>3$  mg/L). Although postmenopausal women typically have adequate or high iron status, at baseline 2 subjects had low serum iron (ie,  $<35$  μg/dL), 2 had low total iron-binding capacity (ie,  $<250$  μg/dL), 6 had low transferrin saturation (ie,  $<15\%$ ), 8 had low iron stores (ie,  $<12$  μg ferritin/L), and 1 had marginally low hemoglobin (ie,  $<11.5$  g/dL). One subject had elevated total iron-binding capacity (ie,  $>450$  μg/dL), 4 had elevated serum ferritin (ie,  $>200$  μg/L), 1 had marginally elevated hemoglobin (ie,  $>15.0$  g/dL), and 3 had elevated hematocrit (ie,  $>44\%$ ). Nevertheless, group mean concentrations of tHcy and CRP and iron indexes were within normal



**TABLE 3**  
Effect of treatment on cardiovascular disease (CVD) risk factors and iron status<sup>1</sup>

	Treatment group				<i>P</i> <sup>2</sup>		
	LP/LI ( <i>n</i> = 14)	NP/LI ( <i>n</i> = 13)	LP/NI ( <i>n</i> = 14)	NP/NI ( <i>n</i> = 14)	Phytate	Isoflavone	Interaction
<b>CVD risk factors</b>							
Homocysteine (μmol/L)							
Week 0	5.8 ± 1.5 <sup>3</sup>	6.9 ± 1.2	6.2 ± 1.8	6.3 ± 1.1			
Week 6	5.6 ± 1.4	5.3 ± 1.9	5.7 ± 1.5	5.5 ± 1.3			
Change	-0.2 ± 1.3	-1.3 ± 1.2	-0.5 ± 0.5	-0.8 ± 1.0	0.02	0.77	0.13
C-reactive protein (mg/L)							
Week 0	1.9 ± 1.4	1.7 ± 1.3	1.4 ± 0.9	1.2 ± 0.8			
Week 6	2.4 ± 2.1	1.7 ± 1.7	1.6 ± 1.3	1.5 ± 1.3			
Change	0.4 ± 1.2	0.0 ± 0.7	0.1 ± 1.0	0.3 ± 0.8	0.61	0.99	0.22
<b>Iron indexes</b>							
Serum iron (μmol/L)							
Week 0	13.8 ± 4.9	17.1 ± 5.7	16.2 ± 3.6	14.9 ± 5.8			
Week 6	15.4 ± 7.5	14.0 ± 3.7	16.0 ± 4.0	13.4 ± 4.3			
Change	1.6 ± 6.6	-3.0 ± 5.7	-0.2 ± 4.1	-1.4 ± 4.4	0.04	0.91	0.23
Transferrin saturation (%)							
Week 0	23.4 ± 9.5	29.7 ± 11.1	28.6 ± 6.6	25.7 ± 11.9			
Week 6	23.6 ± 11.8	21.3 ± 6.2	25.9 ± 6.0	21.5 ± 9.4			
Change	0.3 ± 9.0	-8.4 ± 10.0	-2.7 ± 5.6	-4.2 ± 7.4	0.03	0.82	0.11
Serum ferritin (pmol/L)							
Week 0	142.2 ± 189.2	174.1 ± 148.5	138.6 ± 108.8	133.2 ± 143.6			
Week 6	141.1 ± 201.8	121.3 ± 105.4	135.9 ± 124.0	115.5 ± 102.0			
Change	-1.1 ± 18.6	52.1 ± 67.4	-2.7 ± 64.0	-17.8 ± 51.0	0.03	0.27	0.22

<sup>1</sup> LP/LI, low phytate and low isoflavone; NP/LI, normal phytate and low isoflavone; LP/NI, low phytate and normal isoflavone; NP/NI, normal phytate and normal isoflavone. No significant differences were found at baseline between the treatment groups (ANOVA for each variable).

<sup>2</sup> Two-factor ANOVA.

<sup>3</sup>  $\bar{x} \pm$  SD (all such values).

ranges and did not differ significantly between the treatment groups.

### Effect of treatment

Concentrations of tHcy and CRP and iron status indexes at baseline and 6 wk are shown in **Table 3**. At 6 wk, tHcy declined from baseline by 19% in the NP/LI group, 8% in the LP/NI group, 12% in the NP/NI group, and 3% in the LP/LI group. The 19% decrease in the NP/LI group paralleled a 28% and 30% decrease in transferrin saturation and serum ferritin, respectively, and a modest 7% decrease in serum iron. The 12% decrease in the NP/NI group paralleled a 16% decrease in transferrin saturation. Two-factor analysis of variance indicated that soy protein with native phytate significantly reduced tHcy ( $P = 0.017$ ), transferrin saturation ( $P = 0.027$ ), and ferritin ( $P = 0.029$ ), whereas soy protein with native isoflavones had no significant effect on any variables. We expected that the effects of isoflavone and phytate were independent of each other. These results showing no interaction between phytate and isoflavones confirmed this hypothesis.

### Factors contributing to total homocysteine and C-reactive protein

Given that tHcy and CRP are atherosclerotic CVD risk factors, we investigated the factors contributing to their circulating concentrations at baseline, including oxidative stress indicators and circulating lipids, which were reported earlier (38). Pearson correlation coefficients between tHcy or CRP and CVD risk factors are shown in **Table 4**. Circulating tHcy and CRP were correlated

positively with each other ( $P = 0.01$ ) and with other CVD risk factors. We expected that plasma folate and vitamin B-12 concentrations would be inversely related to tHcy, but only vitamin

**TABLE 4**  
Relation between baseline total homocysteine (tHcy) and C-reactive protein (CRP) and cardiovascular disease (CVD) risk factors

CVD risk factors	Homocysteine	CRP
tHcy		0.35 <sup>2</sup>
Ferritin	-0.04	0.14
BMI	0.39 <sup>2</sup>	0.55 <sup>3</sup>
Total cholesterol	0.21	0.05
HDL cholesterol	0.20	-0.30 <sup>4</sup>
LDL cholesterol	0.28 <sup>4</sup>	0.07
Triacylglycerol	0.07	0.24
Oxidized LDL	0.31 <sup>4</sup>	0.08
8-iso-Prostaglandin F <sub>2α</sub>	0.16	0.16
Protein carbonyls	0.07	-0.04
Folate	-0.22	-0.01
Vitamin B-12	-0.23	-0.05

<sup>1</sup> *n* = 55. Values are Pearson correlation coefficients (*r*). Mean ( $\pm$  SD) lipid or lipoprotein indexes: HDL cholesterol, 228  $\pm$  34 mg/dL; LDL cholesterol, 143  $\pm$  32 mg/dL; triacylglycerols, 120  $\pm$  70 mg/dL. Mean ( $\pm$  SD) oxidative stress indexes: 8-iso-prostaglandin F<sub>2α</sub>, 4621  $\pm$  1328 pg/mL; protein carbonyls, 0.19  $\pm$  0.05 nmol/mg; oxidized LDL, 75  $\pm$  23 U/L. Indexes published previously (38).

<sup>2</sup>  $P < 0.01$ .

<sup>3</sup>  $P < 0.0001$ .

<sup>4</sup>  $P < 0.05$ .

**TABLE 5**  
Regression analysis of contributors to plasma homocysteine at baseline<sup>1</sup>

Independent variable	Variable estimate	Percentage variance	P
Intercept	1.3569		
BMI	0.1349	13.9	0.006
LDL cholesterol	0.0096	4.9	0.097

<sup>1</sup> *n* = 55. Multiple regression analysis with stepwise selection to determine the contribution of lipids and lipoproteins, oxidative stress indexes, serum ferritin, plasma folate, plasma vitamin B-12, and BMI to baseline total homocysteine concentrations. Overall model,  $R^2 = 19.9$ ; adjusted,  $R^2 = 18.8$  ( $F = 6.48$ ,  $P = 0.003$ ).

B-12 trended toward significance ( $P = 0.09$ ). BMI was highly correlated (positively) with both tHcy ( $P = 0.003$ ) and CRP ( $P < 0.0001$ ) at baseline, whereas HDL cholesterol was correlated (negatively) with CRP at baseline ( $P = 0.02$ ). Multiple regression analysis indicated that LDL cholesterol and BMI were the only contributors to baseline tHcy concentrations (**Table 5**); together, they accounted for 19.9% ( $P = 0.003$ ) of the variance in tHcy. The only factor that contributed to baseline CRP was BMI ( $P < 0.0001$ ; data not shown).

## DISCUSSION

Soy protein has gained attention because of its potential to influence CVD risk factors. However, detecting the physiologic effects of dietary soy is a complicated matter because soy protein contains many components, such as isoflavones, phytate, saponins, and  $\beta$ -conglycinin, each of which may be responsible for the beneficial effect. Careful study design is necessary to clearly elucidate the effects of specific components of soy. For instance, soy protein and isoflavone intake are negatively related to serum tHcy in premenopausal women (39), but it is difficult to ascertain which component is responsible. Although the finding by Tonstad et al (26) is controversial, they reported that soy protein with isoflavones exerted a tHcy-lowering effect (26). Other investigators suggested that, whereas soy protein reduces tHcy, this effect is independent of isoflavone content (25, 40) and thus may be due to another component of soy. Our study supports these results, documenting a similar change in tHcy between NI and LI treatments (mean declines of 0.65 and 0.84  $\mu\text{mol/L}$ , respectively), which indicates that factors in soy protein other than isoflavones may exert an effect. It may be that other studies have noted a significant effect of isoflavones because they included a casein control. The strength of the current study is that we used an SPI control with low phytate and low isoflavone (LP/LI) content to provide an apt comparison of the independent effects of phytate and isoflavones. Studies that used a casein control noted an increase in tHcy in the control and a decrease in the experimental (25, 26) group. Casein has a higher ratio of methionine to cysteine than does soy protein, which has been associated with an increase in tHcy (41). Thus, the tHcy-lowering effect of soy protein may be greater than that of a casein control.

Although we noted a decline in tHcy with all treatments, the change was significant only with soy protein with native phytate, which suggests that phytate-rich foods may be beneficial in reducing tHcy. The mean tHcy concentration ( $6.3 \pm 1.4 \mu\text{mol/L}$ ) in the women in the current study was well within the normal range, but phytate may have a more dramatic effect in those with

hyperhomocysteinemia. This relation has not previously been reported in the literature; hence, no mechanism has been proposed. However, on the basis of the relation between tHcy and folate, we speculate that the effects on tHcy may be due to the greater availability of intracellular folate. Regrettably, we did not measure intracellular folate, which is a better indicator of long-term status than is plasma folate. Yet other studies have found that plasma folate concentrations, which reflect recent dietary intake, are inversely related to tHcy (42) and deficiency (43) results in persons with hyperhomocysteinemia. However, we did not note this relation and found no change in plasma folate with treatment.

Iron status may not be directly related to tHcy, but it may have an indirect effect through folate catabolism. Increased intracellular ferritin parallels an increase in folate catabolism in cell culture (44), thereby limiting the folate needed for the metabolism of excess homocysteine. In Chinese hamster ovarian cells, insertion and overexpression of the rat gene for heavy-chain ferritin stimulated folate turnover and decreased the intracellular folate by 40% (44). Elevations in heavy-chain ferritin may reflect alterations in the iron regulatory pool (45), which would influence the activity of regulatory enzymes for folate, thereby altering the availability of folate for remethylation of homocysteine (46, 47). Slightly elevated ferritin in postmenopausal women may not cause folate deficiency; however, it is possible that, despite normal plasma folate, ferritin may affect intracellular folate. In the current study, soy protein with native phytate reduced iron stores, as assessed by serum ferritin concentrations, and perhaps spared folate for the remethylation of homocysteine, thereby lowering plasma tHcy. The fact that our treatment had no effect on plasma folate does not rule out an effect on intracellular folate. Further investigation is required to understand the effects of phytate on red cell folate and tHcy.

Previous research found no effect of soy protein or isoflavones on CRP (28). The current study confirms these results by showing no effect of soy protein components on CRP concentrations in postmenopausal women. However, these findings may be partially related to the fact that only 3 subjects had elevated CRP ( $>3 \text{ mg/dL}$ ), and it is possible that soy protein may have an effect only in persons with elevated CRP. Future studies are needed to ascertain the effects of soy protein on CRP in persons with elevated inflammation.

Phytate plays a well-documented role in reducing iron absorption in humans enrolled in feeding studies (29, 30). Reducing iron stores is undesirable for those at risk of iron deficiency, but phytate may be beneficial in postmenopausal women who are at risk of excess iron. As was found in previous studies, soy protein with native phytate modestly reduced iron stores in the subjects in the current study after only 6 wk, and similar declines in serum iron and transferrin saturation were also found. The small decline in ferritin noted in the LP treatments may have been due to the residual phytate content. A previous study by our group (29) found that phytate must be reduced to  $< 0.3 \text{ mg/g}$  protein to remove the inhibition on iron absorption. Our LP treatments still contained 5.5 mg phytate/g protein, an amount that is likely sufficient to reduce iron absorption and thus iron status, although not to the same extent as the NP treatments do. As expected, there was no effect of isoflavones on iron indexes.

Because we recruited healthy women without chronic diseases, it was not surprising that only 3 subjects had elevated CRP, and none had elevated tHcy. Plasma tHcy concentrations in our



study ( $6.3 \pm 1.4 \mu\text{mol/L}$ ) were comparable to the  $6.8 \pm 0.1 \mu\text{mol/L}$  concentration recently reported for women from the 2001–2002 National Health and Nutrition Examination Survey (48).

In support of previous findings (49, 50), we observed positive correlations between BMI, tHcy, and CRP, which emphasized the effect of weight on CVD risk factors. Our findings indicate that tHcy and CRP are affected by BMI; thus, maintaining a healthy BMI may reduce atherosclerotic CVD risk through its relation with tHcy and CRP.

Given that tHcy and CRP are atherosclerotic CVD risk factors, we also examined their relation with well-established risk factors at baseline. It was not surprising that LDL cholesterol was correlated positively with baseline tHcy, given that, in hyperlipidemic persons, the risk of CVD is higher with elevated than with normal CRP and tHcy (51). Similarly, the subjects in the current study whose LDL cholesterol was  $>140 \text{ mg/dL}$  had BMIs 2.1 higher, tHcy concentrations  $0.7 \mu\text{mol/L}$  higher, and CRP concentrations  $0.6 \text{ mg/L}$  higher than did those subjects whose LDL cholesterol was  $\leq 140 \text{ mg/dL}$ . Although previous studies showed that excess iron and elevated total (20) and LDL (52) cholesterol have an additive effect on increasing CVD risk, we found no difference in ferritin between subjects with LDL cholesterol  $>140 \text{ mg/dL}$  and those with LDL cholesterol  $\leq 140 \text{ mg/dL}$ . However, those studies included subjects with very high iron stores (ie,  $>100 \mu\text{g/L}$ ), whereas the ferritin values in the current study ranged from 2 to  $322 \mu\text{g/L}$ , and group means were within the normal range (ie,  $59\text{--}78 \mu\text{g/L}$ ).

In conclusion, isoflavones did not protect against CVD risk factors in our study, whereas soy protein with native phytate significantly reduced tHcy and iron stores. Consuming soy protein or other phytate-rich foods may prevent menopause-associated rises in tHcy and iron excess, thereby reducing atherosclerotic CVD risk factors in postmenopausal women. We noted that BMI is a strong predictor of CVD risk factors, particularly tHcy and CRP. Thus, maintaining a healthy BMI through sensible dietary habits and physical activity may help control CVD risk.

MBR and DLA designed the study and secured funding. LNH and HME recruited and interviewed subjects and collected samples and data. MBR and LNH analyzed the data, and MBR, LNH, and DLA reviewed the analyses. LNH prepared the first draft of the manuscript; MBR and DLA refined the subsequent drafts; and KLS and MLK provided consultation on the final draft. None of the authors had a personal or financial conflict of interest.

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