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Keywords
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Methods: Our study was primarily designed to determine the effect of consuming two to three servings per day of soy foods, providing ~19 g protein and ~36 mg isoflavones, on iron and zinc status in premenopausal women during a 10-weeks period. As secondary outcomes, we also tested the effect of soy foods on biochemical markers of bone and thyroid hormones. Nonsmoking women (18–28 years) without chronic disease, anemia, pregnancy, or irregular menstrual cycles were randomly assigned to either the soy food (SF, $n=31$) or animal food (AF, $n=32$) group. Blood and urine samples and 3-day dietary records were collected at baseline and postintervention.

Results: At baseline, iron and zinc status, bone markers, and thyroid hormones were not different between groups. After intervention, no significant changes were observed in hemoglobin, transferrin saturation, serum iron, ferritin, or transferrin receptor (TFR) concentrations. Plasma zinc, but not serum alkaline phosphatase, significantly decreased in both groups ($-0.8\,\mu$mol/L). The change in bone-specific alkaline phosphatase was significant between SF (1.5 U/L) and AF (-0.7 U/L) groups. No significant changes were observed in bone resorption, thyroid-stimulating hormone (TSH), or free thyroxine after soy food intake.

Conclusions: Incorporating ~19 g soy protein from soy foods for 10 weeks had no significant effect on iron or zinc status, bone resorption or formation, or thyroid hormone status in premenopausal women.

Introduction

Soy foods have become popular in the western world because of their proposed health benefits. Soy protein has been shown to modestly lower blood low-density lipoprotein cholesterol (LDL-C),[1–3] and proposed to reduce bone loss in postmenopausal women,[4,5] prevent breast cancer if exposure occurs early in life,[6–8] and help control diabetes.[9,10] Despite potential benefits, mineral balance may be impaired with soy consumption because soybeans contain (by weight) ~1%–3% phytic acid,[11] which binds minerals such as zinc, iron, and calcium, thereby decreasing their bioavailability. Although phytic acid is the major inhibitory factor in soy, the protein moiety also has been shown to inhibit iron absorption.[12]

Iron bioavailability has been shown to be extremely poor not only from soy foods[13] but also when soy was mixed with other foods in single-meal feeding studies.[13–15] For example, iron absorption was reduced from 5.5% to 1%, 1.9%, and 0.4%, respectively, when substituting full fat soy flour, textured soy flour, or isolated soy protein (ISP by definition is >90% protein) for egg albumin in a liquid diet.[13] Soy flour has a significant amount of iron, and when it was partially substituted for meat in a meal, the inhibitory effect was shown to be partially offset by improved availability with meat, as well as by an increase in the nonheme iron content of the meal.[16] Data on the effect of soy foods or products on iron status with long-term feeding studies are limited and conflicting. Daily consumption of 40 g/day ISP for 6 months showed no adverse effect on iron status in perimenopausal women with normal or borderline slightly compromised iron status at baseline.[17] In contrast, however, the same amount of protein for 6 weeks significantly lowered iron status in postmenopausal women

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who had moderately elevated iron stores, although values remained within the normal range.[18] Recent data showed that iron is well absorbed from soybean ferritin,[19] possibly because ferritin iron is taken up by a different mechanism than are other nonheme iron sources.[20] Because of this different mechanism, ferritin iron absorption may be protected from dietary inhibitors of iron absorption. Although no data are available, it is possible that ferritin may be degraded during the processing of soy products, and once the iron is released from ferritin, phytate may affect its absorption, thus affecting iron status with soy consumption.

Fewer data are available on the effect of soy foods on zinc absorption or balance. Soy protein-based diets are generally considered to have low zinc bioavailability because of their high phytate/zinc molar ratio.[21] When this ratio exceeds 15, as is the case with soy products, zinc absorption is typically <15% compared to 30%–50% with lower ratios.[21,22] The inhibitory effect of phytate has been shown to be attenuated by dephytinization of soy protein[23–25] and by incorporating milk and meat into soy-containing meals.[26,27] However, there are limited and conflicting data on the long-term effects of soy on zinc balance. A soy protein (69 g/day)-based diet for 3 months was reported to cause a negative zinc balance and a reduction in plasma (25%) and neutrophil (31%) zinc concentrations in a small group ($n=5$) of male subjects.[28] However, no effect of soy protein (0.8 g/kg of body weight) on zinc absorption or balance was found in another long-term (82 days) feeding study involving male subjects ($n=6$).[29]

The effect of soy consumption on bone has also been widely studied. Interest in these areas is largely because soy foods are essentially unique dietary sources of isoflavones. Soy isoflavones were shown to attenuate bone loss from the lumbar spine in perimenopausal women,[4] possibly by stimulating bone formation[4,30] or inhibiting bone resorption.[30,31] Other studies, however, have not substantiated these beneficial effects on bone mineral density (BMD)[32,33] or biochemical markers of bone.[33] The effect of soy consumption on thyroid status is also of interest because four decades ago, goiter was reported in infants fed soy formula that was not fortified with iodine.[34–36] Perhaps soy isoflavones may impair thyroid hormone synthesis when iodide intake is inadequate.[37] Additionally, it is possible that soy consumption could affect thyroid hormone synthesis by affecting iron status, as thyroid hormone synthesis involves iron-dependent enzymes (thyroid peroxidase and hepatic 5′-deiodinase).[38]

Overall, single-meal studies clearly report an inhibitory effect of ISP on iron and zinc absorption, but it is not clear if chronic intake of traditional and processed soy foods significantly impacts iron and zinc status in healthy young women. Because young women may have poor dietary habits that place them at risk for micronutrient deficiencies, we investigated the effect of incorporating commonly consumed soy foods for 10 weeks on mineral status in young women. As a secondary outcome of the study, we also assessed the potential effects of soy food intake on biochemical markers of bone and thyroid hormone status.

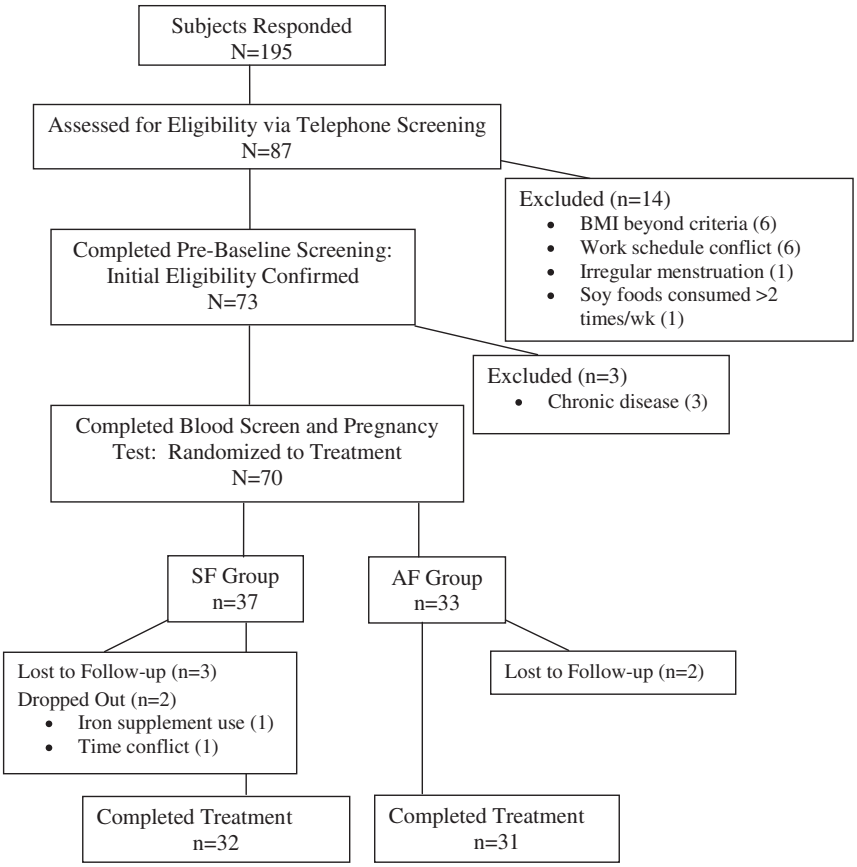

FIG. 1. Subject recruitment. AF, animal food; BMI, body mass index; SF, soy food.

Materials and Methods

Subject selection

We recruited women aged 18–40 years by sending emails to Iowa State University personnel and posting fliers throughout the campus. As shown in Figure 1, 195 subjects initially responded to study postings. Based on our initial expectation and power calculations, we needed 70 subjects; thus, we screened only the first 87 of 195 subjects who responded, anticipating ~20% eligibility. Based on the initial telephone screening of 87 subjects, 74 subjects were eligible to participate. Women who had chronic disease, gastrointestinal problems, or anemia or who were pregnant were excluded, as were those who smoked cigarettes, had irregular menstrual cycles (<24 or >32 days menstrual cycle), a body mass index (BMI) ≤18.5 or ≥30 kg/m^2, or consumed soy products more than twice per week. Women who met these criteria and were willing to be randomly assigned to either the soy food (SF) or animal food (AF) group for 10 weeks were included. Once we confirmed that subjects were not pregnant (using pregnancy tests) and had normal blood chemistry profiles, 70 subjects were invited to participate. Based on our previous study,[19] we designed this study with 35 subjects per group to detect a 20% mean change (log scale) in ferritin (primary outcome) within treatment, with 80% power at $p=0.05$ level of significance. All subjects were instructed to discontinue their vitamin/mineral supplement use. We had a final total sample size of 63 subjects, subsequent to losing 2 subjects because of time conflicts or unwillingness to discontinue mineral supplements, and 5 were lost to follow-up. We provided verbal explanation and obtained written informed consent from each subject before baseline data collection. The study protocol, consent forms, and data collection-related materials were approved by Iowa State University Human Subjects Review Committee (Institutional Review Board ID 07-505).

Study design

This was a partially controlled feeding study: some meals were provided on site, and others were consumed at home. Random numbers were generated using statistical software (GraphPad Statmate), and 70 subjects were assigned to either the AF or SF group based on the randomization scheme and their ID numbers. Each group was provided ~two servings/day of either animal or soy foods during the 10-week study. Foods provided were considered to be those commonly consumed and widely available. Subjects came to the Nutrition and Wellness Research Center at Iowa State 3 days per week (Monday, Wednesday, Friday) to eat their meals and to pick up the frozen meals to consume at home for the remaining days. Milk and yogurt to be consumed at home daily were provided as a part of the intervention meal every day. The weekly menu for subjects in each group is presented in Table 1. The soy protein content in the SF group ranged from 18 to 22 g/day, with an average of 19 g. The isoflavone content (estimated from the United States Department of Agriculture [USDA] database and the manufacturers) of soy foods we provided was approximately 36 mg/day. In the meals we provided, total protein content and energy averaged 27 g and 2240 kJ per day for the AF group and 25 g and 2018 kJ for the SF group, respectively. Because the majority of subjects were students and were not available during their spring break, we provided soy protein (20 g) bars (devoid of whey protein) to the SF group and whey protein (20 g) bars (devoid of soy protein) to the AF group for 7 days to ensure reasonable compliance. Subjects in the SF group were instructed to restrict any additional soy foods and to limit animal products to one serving/day. The AF group was instructed not to consume, in addition to the meals we provided during the study period, more than one serving/day of animal foods (excluding dairy). We were unable to use blinding in this study because it was readily apparent to both the subjects and study staff which foods were being prepared and served to particular subjects.

Table 1. Weekly Menu for Subjects in Animal Food and Soy Food Groups

	AF	*SF*
Monday	Chili without beans	Soy chili
	Crackers/peanut butter	Crackers/soynut butter
	Yogurt	Soy yogurt
Tuesday	Meat balls	Soy meat balls
	Pasta with sauce	Pasta with sauce
	Milk	Soy milk
Wednesday	Taco with beef	Taco with soy
	Yogurt	Soy yogurt
Thursday	Beef	Tofu
	White rice	White rice
	Stir fried peas and carrots	Stir fried peas and carrots
	Milk	Soy milk
Friday	Burger/bun	Soy burger/bun
	Milk	Soy milk
Saturday	Green peas	Edamame
	White rice	White rice
	Carrots	Carrots
	Peanuts	Soy nuts
	Yogurt	Soy yogurt
Sunday	Chicken	Soy chicken
	Pasta/sauce	Soy pasta/sauce
	Milk	Soy milk

Milk and yogurt were always consumed at home; condiments were provided to subjects at each meal in the research unit.

AF, animal food; SF, soy food; Edamame, young green edible soybeans.

Data collection

Questionnaires were administered to subjects at baseline to collect information on demographic characteristics, medical and reproductive history, and soy food intake habits. Prior to and during the last week of intervention, 3-day dietary intake records (2 weekdays and 1 weekend) were obtained. We assessed the nutrient intake of subjects from 3-day dietary records using NutritionistPro (version 2.3.1). At baseline and postintervention, body weight, standing height (measured at baseline only), and waist and hip circumferences were measured by trained staff according to a standard protocol. Overnight fasting blood samples were drawn by phlebotomists, and 24-hour urine samples (instructions provided) were collected from each subject at baseline and postintervention. Aliquots of serum/plasma and urine samples were stored at −80°C until analysis.

Laboratory measurements

A certified clinical laboratory (LabCorp; Kansas City, KS) performed a general blood chemistry profile (complete blood count [CBC] with differential, iron status [serum iron, total iron-binding capacity, Tf saturation]), serum thyroid hormone status (thyroid-stimulating hormone [TSH] and thyroxine), and zinc status indicators (plasma and urinary zinc, serum alkaline phosphatase [ALP]). We measured serum ferritin using an immunoradiometric kit and Tf receptor (TfR) using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's (Ramco Laboratories) guidelines. The TfR-F index was calculated as the ratio of serum TfR to log-transformed serum ferritin to determine nonanemic iron deficiency.[39] Commercial ELISA kits were used to measure serum bone-specific ALP (BAP, bone formation marker) activity (Metra® BAP) and serum cross-linked C-terminal telopeptides of type I collagen (CTx, bone resorption marker) concentration (serum CrossLaps® ELISA) using a microtiter plate reader (ELx808U with KC Junior software, version 1.14) according to the manufacturer's guidelines. Urinary isoflavones were determined using a modified high-performance liquid chromatography (HPLC) procedure.[40] The intra-assay and interassay coefficients of variation (%), respectively, were 5.3% and 6.0% for ferritin, 4.1% and 11.6% for TfR, 2.1% and 9.5% for BAP, and 1.8% and 7.3% for CTx.

Statistical analyses

Statistical analyses were performed using SAS version 9.1, with results considered statistically significant at $p \le 0.05$. The normality of data was checked before analysis using the Kolmogorov-Smirnov test and by examining the distribution of data using histograms. Descriptive statistics included mean (range) for age, height, weight, BMI, waist circumference, hip circumference, and waist/hip ratio; mean ± standard deviation (SD) for hemoglobin, serum iron, and serum ALP; and median (range) for serum ferritin, TfR, Tf saturation, plasma and urinary zinc, and all dietary intake variables. For normally distributed data, an unpaired Student's t test was performed for between-group comparisons, and a paired Student's t test was used for within-group comparisons. For data that were not normally distributed, comparisons were conducted using a Mann-Whitney test between groups and a Wilcoxon signed-rank test within groups. Samples with serum ferritin values $< 1\ \mu g/L$ ($n = 5$ of 126 measurements) were assigned a value of $2\ \mu g/L$ for statistical analysis.

Results

Subject characteristics

A total of 63 subjects completed the 10-week dietary intervention. Table 2 includes the baseline characteristics of subjects. At baseline, subjects in the two groups did not differ significantly in age, height, or hip circumference. The significant difference between the two groups in weight, BMI, and waist circumference was because 1 subject in the AF group fell beyond our BMI inclusion criterion ($33\ kg/m^2$). The majority of the 63 subjects were Caucasian; 11 were Asian, 2 were of mixed race, 1 was African American, and 1 was Hispanic/Latina.

Table 2. Baseline Characteristics of Subjects, by Treatment Group

	Treatment group	
	AF (n = *31*)	*SF* (n = *32*)
Age, years	21 (18–27)[a]	22 (18–28)
Height (m)	1.66 (1.54–1.76)	1.65 (1.54–1.77)
Weight (kg)*	64.0 (51.1–96.2)	60.0 (49.0–76.8)
BMI (kg/m^2)*	23.3 (18.5–33.9)	22.0 (18.5–27.2)
Waist circumference (cm)*	72.6 (61.2–98.6)	69.6 (58.7–78.2)
Hip circumference (cm)	100.3 (89.4–123.2)	97.5 (86.4–107.7)
Waist/hip ratio	0.72 (0.63–0.83)	0.71 (0.65–0.77)

Student's t test was used to compare the differences between groups for height, body mass index (BMI), and waist hip ratio because these variables were normally distributed; Mann-Whitney test was used to perform comparison of age, weight, waist circumference, and hip circumference between groups.

[a]Mean (minimum–maximum).

*$p < 0.05$.

Dietary intake

No significant differences between the groups were found in total dietary intake of energy, protein, carbohydrate, fat, iron, zinc, calcium, or vitamin C at baseline and post-

Table 3. Total Daily Dietary Intake of Subjects at Baseline and Postintervention

	Treatment group	
	AF (n = *31*)	*SF* (n = *32*)
Energy (kJ)		
Baseline	6,665 (3,483–10,320)[a]	6,958 (4,023–10,802)
Postintervention[b]	6,866 (4,421–13,083)	7,034 (4,375–9,965)
Protein (g)		
Baseline	66 (42–97)	67 (39–109)
Postintervention	69 (42–189)	68 (42–97)
Carbohydrate (g)		
Baseline	233 (82–321)	226 (158–414)
Postintervention	231 (134–330)	222 (116–391)
Total fat (g)		
Baseline	51 (24–112)	53 (17–100)
Postintervention	57 (31–122)	57 (24–99)
Iron (mg)		
Baseline	13.7 (6.8–36.5)	14.0 (7.7–32.8)
Postintervention	14.4 (7.4–31.3)*	14.2 (8.5–31.9)
Zinc (mg)		
Baseline	7.8 (3.6–15.9)	8.0 (3.5–17.7)
Postintervention	10.0 (5.3–10.0)***	9.3 (4.5–13.8)
Calcium (mg)		
Baseline	725.6 (285.8–1,803)	827.5 (312.2–1,580)
Postintervention	854.5 (446.5–1,942)**	931.2 (466.6–1,330)
Vitamin C (mg)		
Baseline	98.1 (21.4–434.2)	97.0 (10.9–322.1)
Postintervention	51.2 (18.0–129.9)***	54.6 (14.5–229.0)***

Nonparametric tests were used to compare the difference within each group or between groups. No significant difference was found in any dietary factor between groups at baseline and postintervention.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ indicate differences between baseline and postintervention values within each group.

[a]Median (minimum–maximum).

[b]Postintervention values reflect total daily intake based on usual food intake plus the meals provided by the study.

intervention (Table 3). Although the data are not shown in Table 3, the soy foods provided to the SF compared to the AF group apparently contributed to the higher daily intake of iron (5.2 mg vs. 4.0 mg), calcium (395 mg vs. 319 mg), and vitamin C (6.2 mg vs. 5.2 mg) but lower intake of zinc (3.2 mg vs 4.1 mg). Considering overall dietary intake, however, total iron ($p = 0.044$), zinc $p = 0.0035$), and calcium $p = 0.005$) increased significantly from baseline to postintervention in the AF group. Dietary vitamin C decreased significantly ($p < 0.001$) from baseline to postintervention in both groups.

Most subjects met the current Dietary Reference Intake (DRI)[41] for protein (46 g/day) and carbohydrate (130 g/day) at baseline and postintervention. However, more than half of the subjects fell below the DRI values for iron (18 mg/day), zinc (8 mg/day), and calcium (1000 mg/day) at baseline.[42,43] In addition, the number of subjects who did not meet the current DRI for vitamin C (75 mg/day) increased from baseline to postintervention: 12 to 22 in the AF group and 9 to 20 in the SF group.

Compliance

Because subjects consumed only 3 of 14 meals per week under our surveillance, compliance for soy food intake was checked using urinary isoflavone analysis for 6 subjects randomly selected in each group at baseline and postintervention. There was no significant difference between the two groups at baseline. As expected, the SF group had a higher urinary isoflavone excretion than the AF group postintervention, indicating compliance to soy food intake. Total urinary isoflavones increased significantly ($p = 0.016$) by 16-fold (from 0.36 ± 0.26 to 5.83 ± 4.48 mg/L urine) in the SF group, whereas it decreased by 1.4-fold (not significant, from 2.59 ± 4.16 to 1.75 ± 3.52 mg/L urine) in the AF group. Although our study was a partially controlled feeding study and given the approximately 36 mg/day of isoflavones consumed, the increase in isoflavone excretion was comparable to that of previous studies[19,31] in which subjects were exposed to 80 mg/day of isoflavones.

Iron status

Iron status at baseline and postintervention is presented in Table 4. Average hemoglobin concentration was normal for both groups, and no change was observed after intervention. The median values for serum iron, ferritin, TfR, and Tf saturation were within the normal range at both time points. No significant changes were observed in serum iron, Tf saturation, or TfR concentration. The posttreatment values for the AF and SF groups, respectively (18.8 and 22.5 μg/L for ferritin and 4.94 and 4.47 for the Tfr-F index), were not significantly different from their respective baseline values. After intervention, the number of subjects classified as iron deficient based on a serum ferritin cutoff value of < 15 μg/L[44] increased from 10 to 11 in the SF group and remained at 11 in the AF group. In contrast, based on a TfR cutoff value (> 8.5 mg/L) to

Table 4. Iron Status of Subjects by Treatment at Baseline and Postintervention

	Treatment group		
	AF (n = *31*)	*SF* (n = *32*)	*Normal range*
Hemoglobin (g/L)[a]			115–150
Baseline	131.9 ± 7.6	132.8 ± 5.8	
Postintervention	133.9 ± 6.2	134.5 ± 5.8	
Change	2.3 ± 6.4	1.7 ± 4.7	
Serum iron (μmol/L)[a]			35–155
Baseline	17.8 ± 7.1	17.0 ± 9.2	
Postintervention	18.3 ± 6.1	16.1 ± 7.8	
Change	0.6 ± 7.9	− 0.9 ± 11.7	
Transferrin saturation (%)[b]			15–55
Baseline	27 (10–53)	23 (10–82)	
Postintervention	28 (13–48)	24 (10–54)	
Change	3 (− 24–23)	2 (− 52–41)	
Transferrin receptor (mg/L)[b]			2.9–8.3
Baseline	6.39 (2.82–10.84)	6.16 (4.27–10.86)	
Postintervention	6.18 (3.41–14.81)	6.19 (3.71–14.20)	
Change	− 0.11 (− 4.37–4.85)	− 0.16 (− 2.77–3.62)	
Serum ferritin (μg/L)[b]			12–150
Baseline	21.2 (2–110.8)	23.6 (2.4–77.8)	
Postintervention	18.8 (2.0–91.1)	22.5 (2.0–65.7)	
Change	− 1.4 (− 35.2–26.8)	− 2.5 (− 46.7–27.4)	
TfR-F index[b]			< 4.5
Baseline	5.15 (1.84–22.82)	4.86 (2.68–26.65)	
Postintervention	4.94 (2.19–20.33)	4.47 (2.92–45.81)	
Change	− 0.18 (− 17.10–12.55)	− 0.13 (− 4.17–19.16)	

Student's *t* test was used for comparison of hemoglobin because it was normally distributed; nonparametric tests were used for comparison of other iron indices because they were not normally distributed. No significant difference was found between treatment groups for any variable at baseline or postintervention.

[a]Mean ± standard deviation (SD).

[b]Median (minimum–maximum).

TfR-F index, transferria receptor-ferritin index.

Table 5. Zinc Status of Subjects at Baseline and Postintervention

	Treatment group		
	AF (n = *31*)	*SF* (n = *32*)	*Normal range*
Plasma zinc (μmol/L)[a]			70–100
Baseline	12.2 (9.2–21.7)	12.7 (9.5–22.3)	
Postintervention	11.2 (8.0–17.6)	11.7 (8.1–19.7)	
Change	−0.8 (−7.5–7.0)*	−0.8 (−11.2–5.8)*	
Urine zinc (μmol/g creatinine)[a]			100–900
Baseline	405 (77–1282)	427 (77–1068)	
Postintervention	396 (57–1316)	480 (174–1394)	
Change	−28 (−886–745)	8 (−718–629)	
Serum alkaline phosphatase (U/L)[b]			25–150
Baseline	55.19 ± 10.8	59.78 ± 10.86	
Postintervention**	53.97 ± 11.89	61.09 ± 11.17	
Change	−1.23 ± 7.24	1.13 ± 5.63	

Student's *t* test was used for comparison of serum alkaline phosphatase because it was normally distributed; nonparametric tests were used for comparison of serum and urinary zinc because they were not normally distributed.
*Differences between baseline and postintervention within each group: $p < 0.05$.
**Differences between groups: $p < 0.02$.
[a]Median (minimum–maximum).
[b]Mean ± SD.

identify tissue iron deficiency, the number declined from 7 to 2 in the AF group and 6 to 3 in the SF group, suggesting improved iron status in both groups. Similarly, using the TfR-F index ≥4.5 to identify nonanemic iron deficiency,[39] the number of iron-deficient subjects declined from 18 to 14 in the SF group and from 20 to 18 in the AF group.

Zinc status

The median plasma and urinary zinc concentrations were within the normal range for both groups before and at the end of the intervention. As shown in Table 5, plasma zinc decreased significantly by 0.8 μmol/L in both groups. The change in urinary zinc excretion was not significant between the SF and AF groups. Moreover, the change from baseline to postintervention in serum ALP was not significant for either group. Although the postintervention values were significantly different ($p = 0.017$), the change between the groups was not significant.

Biochemical markers of bone and thyroid hormones

Based on the purported effect of soy isoflavones on bone, we assessed BAP and CTx (Fig. 2). From baseline to 10 weeks, BAP increased in the SF group (1.5 U/L) but decreased in the AF group (−0.7 U/L), and the change between the two groups was significantly different ($p = 0.024$). However, we found no significant effect of treatment on CTx. Thyroxine concentrations were not significantly affected by either treatment (1.23–1.22 ng/dL in the SF group and 1.22–1.20 ng/dL in the AF group). The TSH concentration declined by 13% in the AF group ($p = 0.018$), whereas no significant change was observed in the SF group (Fig. 3). The change in TSH (−0.39 vs. 0.11 uIU/dL) was significantly different between the two groups.

Discussion

Although single-meal studies suggest soy protein inhibits iron and zinc bioavailability, data from long-term studies are

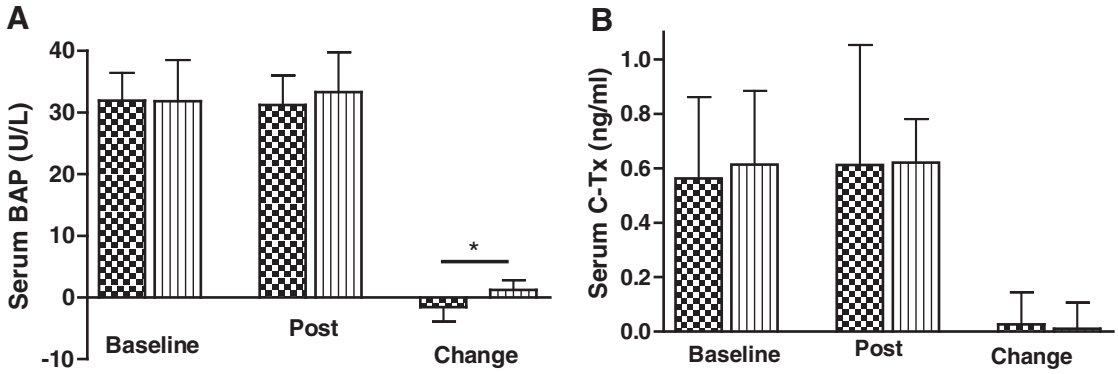

FIG. 2. Effect of soy food consumption on biochemical markers of bone. AF, SF. Bone formation and resorption markers (median + interquartile range) at baseline and postintervention were reflected by **(A)** bone-specific alkaline phosphatase (BAP) and **(B)** C-terminal telopeptides of type-I-collagen (C-Tx), respectively. Nonparametric tests were performed for comparison; $n = 31$ for AF group and $n = 32$ for SF group. *$p < 0.05$.

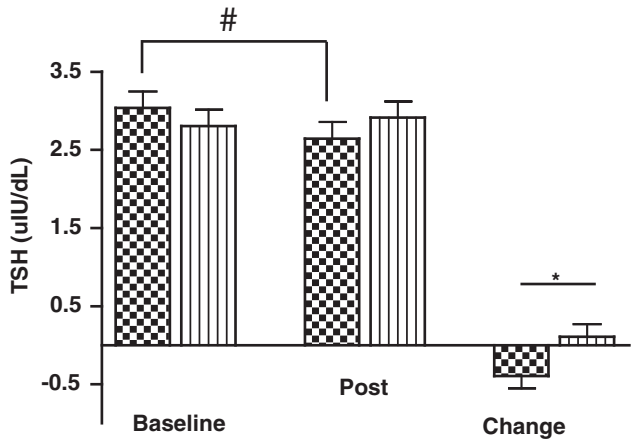

FIG. 3. Effect of soy food consumption on thyroid-stimulating hormone (TSH). AF, SF. TSH at baseline and postintervention (mean ± SEM). Comparisons determined using Student's *t* test to examine the difference within group and between groups; $n = 31$ for AF group and $n = 32$ for SF group. $^*p < 0.05$, $\#p < 0.02$.

limited. This 10-week feeding study demonstrated that incorporating soy foods as part of the habitual diet had no detrimental effect on iron or zinc status in young women of childbearing age. At the end of the study, no significant changes were observed in macronutrient intake, indicating that subjects adjusted their diets in response to the test meals. Total iron intake significantly increased only in the AF group, despite the higher iron content of the soy foods vs. animal foods (5.2 vs. 4.0 mg) that were provided. Subjects in the AF group likely consumed fewer animal products before intervention than during the intervention, thereby contributing to their higher iron intake. Zinc intake in both groups increased after intervention, but this was significant only in the AF group. Similarly, the significant increase in calcium intake in the AF group may have been due to the increased dairy product intake during the study. Although vitamin C intake was reduced in both groups, we did not expect to document an effect on iron status, as the vitamin C enhancing effect on nonheme iron absorption is dampened with a complete diet.[45] Because the study began in February and ended in April, we cannot attribute any differences in dietary intake to seasonal variation.

It is important to investigate the effect of soy consumption on iron status among women of childbearing age, who are at risk for iron deficiency because of substantial menstrual losses and poor dietary habits.[46] Based on a hemoglobin cutoff value of 120 g/L,[44] none of the subjects in our study were anemic either at baseline or at the end of the intervention. Although serum iron and Tf saturation are widely used in iron deficiency screening, their use is limited by the daily fluctuation of serum iron.[47,48] Recently, the TfR-F index was suggested as a useful indicator of subclinical iron deficiency in healthy subjects.[49,50] If the TfR-F index was used to classify subjects with nonanemic iron deficiency,[39] 2 subjects in the AF group and 4 subjects in the SF group who had been iron deficient at baseline became iron sufficient, showing an improvement in iron status with either intervention. Overall, we found no negative effect of soy food intake on iron indices.

Our iron results are consistent with findings from several but not all studies. Our sample size of 63 could have detected a 16% difference (significant) in ferritin between baseline and posttreatment. However, the 5%–6% decrease we observed in both groups was not significant. To detect a 5%–6% change in ferritin, we would have needed more than 232 subjects per treatment, calling into question the biologic relevance of such a small change. A large scale (1308 men and 1541 women) cross-sectional study in Chinese adults[51] found no significant difference in mean serum ferritin concentrations across quartiles of tofu intake. In addition, our previous study also demonstrated that 40 g/day ISP for 24 weeks did not affect iron status in perimenopausal women ($n = 69$).[17] These results differ, however, from our subsequent 6-week study involving postmenopausal women ($n = 15$), which showed a significant decrease in Tf saturation (28%) and serum ferritin (30%), as well as a moderate reduction in serum iron (7%), in response to the consumption of 40 g/day ISP (40 g/d).[18] The discrepancy among these studies may be attributed to differences in iron status of subjects at baseline, in addition to the amount of soy protein intake and the duration of intervention. Iron absorption is typically inversely related to iron stores,[52] and postmenopausal women generally have higher iron stores than premenopausal or perimenopausal women.[53] For example, the average serum ferritin concentration in the postmenopausal women was 65.4 ng/mL,[18] compared to 27.3 ng/mL in our premenopausal women. Our current results from premenopausal women agree with those from the perimenopausal women,[17] but not from those of the postmenopausal[18] women, strongly supporting the hypothesis that subjects with low iron status have higher absorption than those with adequate iron status, particularly from foods with low iron bioavailability.

Zinc deficiency is not common in North America,[43] but the third National Health and Nutrition Examination Survey (NHANES III) data showed that only 39% of female adolescents met the DRI for zinc.[54] In the present study, no subjects were zinc deficient (based on published values for plasma zinc $< 10.71\ \mu$mol/L and urinary zinc $< 153\ \mu$mol/g creatinine)[55] at baseline, and only 2 subjects, both in the AF group, fell below these cutoffs after intervention. An 8-week crossover study reported a 35% reduction in zinc absorption and a 5% decrease in plasma zinc in women ($n = 22$) who consumed lacto-ovovegetarian diets.[56] Our study also showed a 6% reduction ($p = 0.037$) in plasma zinc concentration, but the reduction was not specific to soy feeding, as a similar reduction was noted also in the AF group. We expected parallel changes in both groups because the zinc content of the test meals we provided was similar for both the AF (5.8 mg/day) and SF (5.6 mg/days) groups. However, it was surprising that plasma zinc declined by 7% ($p = 0.048$) in the AF group despite a significant increase in total zinc intake (7.8–10.0 mg/day). Plasma zinc is a relatively insensitive indicator of zinc status, except with severe zinc deficiency, although it is widely used as a screening test. Plasma zinc is generally stable but decreases with several weeks of severe dietary restriction, after tissue zinc has been depleted. Thus, plasma zinc poorly reflects tissue zinc status and should not be used alone to identify marginal zinc deficiency.[57] More importantly, this modest reduction was noted in both groups, despite improvement in dietary zinc intake. In contrast to the current results, in a small feeding study involving males ($n = 5$), 69 g/day soy protein for 3 months caused a more marked reduction (25%) in plasma zinc and caused negative zinc balance.[28]

Further, unlike plasma zinc, we did not observe a reduction in urinary zinc excretion, likely due to the wide variability in this measure. Moreover, the minor change in serum ALP activity from base to postintervention was not different between the groups and would not raise any concern clinically.

Because of the high phytate content of soy foods, we expected to document a negative effect of soy food intake on iron and zinc status, but the results of this study did not support our hypothesis. However, we cannot rule out the potential confounding effect of other phytate-containing foods on mineral status. This illustrates a limitation of this partially controlled feeding study, in that it would have been nearly impossible to control for phytate intake because phytate is ubiquitous in the food supply. Based on our dietary analysis (Table 3), however, there was no difference in macronutrient intake between treatment groups either at baseline or at posttreatment, suggesting that confounding with phytate intake should not have been an issue. In addition, macronutrient intakes were not significantly altered from baseline within each treatment group. This study was realistic in that we determined whether habitual soy-based diets would exert any effect on mineral status rather than controlling 100% of the subjects' dietary intake.

We examined the effect of soy foods on biochemical markers of bone turnover because of speculation that soy isoflavones, similar to estrogen, might inhibit bone resorption[30,31] and enhance calcium absorption[58] and, hence, might maintain BMD.[4,5] Moreover, calcium intake is less in young adults compared to adolescents because of their lower milk consumption.[59] Our study was not of sufficient duration to examine BMD, nor could we link the biochemical markers to osteoporotic risk. Some[4,60,61] but not all[31,33] human studies have reported that soy isoflavones decreased bone resorption or increased bone formation markers, yet these changes were very modest and not likely to be clinically relevant. We did not find significance within-group changes in bone formation or resorption markers, perhaps because these women were not estrogen deficient. Nevertheless, we did note a significant difference in the change in serum BAP between groups, suggesting that soy foods might exert either a modest beneficial effect or at least not a detrimental effect on bone formation.[4] In addition, we found no evidence that soy foods negatively affected serum TSH or thyroxine concentrations, consistent with essentially all data on this subject.[62] The significant decline in TSH in the AF group may have indicated an improvement in thyroid hormone status, perhaps because of the increased consumption of dairy products, a good source of dietary iodine in the United States.[63]

Conclusion

We found no detrimental effect in iron or zinc status in premenopausal women when moderate amounts of soy foods are incorporated into their regular diet. Further, we noted no treatment effect on the biochemical markers of bone or on thyroid hormone status in this study. Given that this was a partially controlled feeding study, we designed it to address the effect of a realistic intake of soy foods on mineral status. Our results will help provide dietary recommendations for soy food intake in the age group that is vulnerable to micronutrient deficiency.

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Disclosure Statement

M.M. reports a conflict of interest with Iowa Soybean Association. Y.Z., D.L.A., P.M.D., and M.B.R. report no conflicts of interest.

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