



Soy isoflavones modulate immune function in healthy postmenopausal women^{1–3}

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ABSTRACT

Background: The immune system may be compromised after menopause because of the effects of aging and diminishing concentrations of estrogen, an immune-modulating hormone. Isoflavones, plant-derived compounds with estrogenic and antioxidant properties, may offer immunologic benefits to women during this stage of life.

Objective: The objective of this study was to evaluate the effects of soy isoflavones, both in soymilk and in supplement form, on markers of immunity and oxidative stress in postmenopausal women.

Design: Postmenopausal women aged 50–65 y ($n = 52$) enrolled in this 16-wk double-blind, placebo-controlled trial were randomly assigned to 1 of 3 experimental groups: 1) control, 706 mL cow milk/d plus a placebo supplement; 2) soymilk, 71.6 mg isoflavones derived from 706 mL soymilk/d plus a placebo supplement; and 3) supplement, 70 mg isoflavones in a supplement plus 706 mL cow milk/d. Plasma and 24-h urine samples were obtained at baseline and at 16 wk. Immune variables included lymphocyte subsets, cytokine production, and markers of inflammation and oxidative damage.

Results: Isoflavone intervention in postmenopausal women resulted in higher ($P < 0.05$) B cell populations and lower ($P < 0.05$) plasma concentrations of 8-hydroxy-2-deoxy-guanosine, an oxidative marker of DNA damage. Isoflavone treatment did not significantly influence concentrations of interferon γ , interleukin 2, tumor necrosis factor α , or C-reactive protein in plasma or of 8-isoprostanate in urine.

Conclusions: Soymilk and supplemental isoflavones modulate B cell populations and appear to be protective against DNA damage in postmenopausal women. *Am J Clin Nutr* 2006;83:1118–25.

KEY WORDS Isoflavones, immune function, postmenopausal women, DNA damage, soymilk

INTRODUCTION

Isoflavones are a subclass of flavonoids, which are a broad group of polyphenolic compounds widely distributed in foods of plant origin. Biological effects attributed to flavonoids include antiestrogenic and proestrogenic effects, as well as antioxidant and antiproliferative actions (1). The 3 main isoflavone derivatives are daidzein, genistein, and glycitein, which are found predominantly in soybeans and other legumes (2). During digestive and absorptive processes, isoflavones often undergo further metabolic transformations (3). For example, daidzein can be converted to equol by intestinal microflora. The biological activities of this mammalian-derived isoflavone are thought to be stronger than those of its parent compound, daidzein (4).

Isoflavones have been classically defined as phytoestrogens, which are compounds that exert estrogenic effects. The presence of the phenolic ring enables isoflavones to bind to both types of estrogen receptors: Er α and Er β (5). Unlike endogenous estrogen, however, isoflavones bind with a much higher affinity to Er β , which suggests that they may be more accurately defined as selective estrogen receptor modulators capable of both proestrogenic and antiestrogenic effects (6). This hormonal mechanism explains, in part, how isoflavones protect against age-related diseases, including cardiovascular disease (7, 8), osteoporosis (9), and cancers of the breast (10) and prostate (11, 12).

The antioxidant and antiproliferative properties of isoflavones offer additional, important mechanisms through which they may protect against many prevalent chronic diseases (13, 14). Cellular damage resulting from oxidative stress is believed to be a major contributor to the etiology of cardiovascular disease through LDL oxidation and to cancer through DNA strand breaks that may lead to mutations (15–17). In humans, isoflavones have been found to prevent LDL oxidation in vivo (16) and to inhibit DNA damage in vitro (17).

The immune system encompasses an array of defenses that help to guard against the development of age-related diseases. However, like other bodily systems, its functions can be adversely affected by oxidative damage and hormonal changes (18). Therefore, the immune system may benefit from the various biological properties of isoflavones. Enhanced immune responses have been found in animals fed genistein, the isoflavone most abundant in soy foods (19, 20). In humans, consumption of isoflavone-containing soy foods modulates cytokine production (21), and daidzein and genistein glucuronides enhance the activity of natural killer cells in vitro (22).

Postmenopausal women in particular are susceptible to chronic diseases associated with aging and to major shifts in hormonal status. Thus, isoflavones may improve immune

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function in this population. Also, it is important to determine whether dietary or supplement forms of isoflavones affect immunologic variables similarly. Therefore, the purpose of the present study was to evaluate the effects of soy isoflavones, obtained from either soymilk or in a supplement, on immune and oxidative markers in postmenopausal women.

SUBJECTS AND METHODS

Subjects and study design

This study was conducted as part of a larger research project designed to investigate the effects of soy isoflavones on cognitive function. Thus, the sample sizes were determined on the basis of expected changes in cognitive, rather than immunologic, variables. Healthy postmenopausal women between 50 and 65 y of age were recruited regionally from the states of Idaho and Washington. Potential subjects ($n = 300$) were screened by telephone to determine study eligibility. Women free of major health conditions, with a body mass index (in kg/m^2) between 18 and 40, and who had not menstruated for ≥ 1 y were eligible to participate in the study. Additional exclusion criteria included legume allergies, smoking, kidney stones, and antibiotic therapy within the past 6 mo.

Telephone screening resulted in 150 women who were both eligible and interested in enrollment; a total of 117 of these women completed the enrollment process. Of these women, 5 dropped out of the study, all for personal reasons. After the exclusion of women using hormone replacement therapy, the first 52 women completing the cognitive study composed the subset for this secondary investigation of immune and antioxidant variables. All study procedures were approved by the Institutional Review Board of Washington State University.

Before the intervention, the subjects providing informed consent adhered to a 4-wk adjustment diet that minimized the intake of foods containing isoflavones. After this washout period, the 16-wk intervention period began. Subjects were enrolled in groups of 20–28 women; these groups were then considered as treatment blocks. Within each block, subjects were randomly assigned to receive: 1) cow milk and placebo supplement (control group); 2) soymilk (White Wave Inc, Boulder, CO) and placebo supplement (soymilk group, 71.6 ± 3.1 mg isoflavones/d on the basis of 30 samples); or 3) cow milk and isoflavone tablets (supplement group, 70 mg isoflavones/d). Randomization was performed by random number generation. Researchers and subjects remained blinded to group assignment throughout the study.

The nutrient composition and caloric value of the 2 types of milk was nearly identical (Table 1). The isoflavone content and composition of the supplements (30 mg daidzein, 33 mg genistein, and 7 mg glycitein) were formulated to match those of the soymilk, and the values were verified by Archer Daniels Midland Co (Novasoy; Decatur, IL). The cow milk (placebo) was treated with vanilla (flavoring) and caramel (coloring agent) so that it resembled the soymilk in flavor and appearance. The isoflavone content of the soymilk was determined by HPLC (P Murphy, Iowa State University) and is presented in Table 1. Milk products were delivered to each subject weekly. Placebo tablets were composed of maltodextrin with 10% caramel color, with appearance similar to the isoflavone supplement.

TABLE 1

Nutrient and isoflavone composition of cow milk and soymilk

Nutrient	Cow milk ¹	Soymilk ²
Carbohydrate (g)	36	30
Fat (g)	9	10
Protein (g)	24	18
Energy (kcal)	294	282
Calcium (mg)	900	900
Vitamin D (IU)	300	360
Isoflavones (mg/d)		
Daidzein	—	30.9 ± 1.3^3
Genistein	—	37.4 ± 1.3
Glycitein	—	3.6 ± 0.5
Total	—	71.6 ± 3.1^4

¹ Low-fat (1%) cow milk, 706 mL/d.

² Vanilla Silk Soymilk (White Wave, Inc, Boulder, CO); 706 mL/d.

³ $\bar{x} \pm \text{SE}$ (all such values).

⁴ Calculated total daily amount of isoflavones for 706 mL soymilk/d.

Actual analysis of 30 pooled soymilk samples.

Subjects were instructed to consume a total of 706 mL milk/d (soymilk or cow milk) for 16 wk. Milk was consumed in the morning (353 mL) and in the evening (353 mL). Supplements (isoflavone or placebo tablet) were taken with the milk, twice per day, throughout the intervention period. The research team's dietitian provided individual instruction, along with written guidelines, to the women regarding replacement of usual dairy or other similar food items with the study milk to allow for consistent macronutrient intake and prevention of weight gain during the trial. Compliance was assessed via personal communication, dietary records, and the appearance of isoflavone metabolites in plasma and urine. Furthermore, all subjects were instructed to eliminate soy products from their diet throughout the study.

Blood and urine samples were obtained at baseline (0 wk) and after the intervention (16 wk). Blood was drawn into evacuated collection tubes (10 mL) coated with EDTA. An aliquot of whole blood from each subject was used for flow cytometry analysis immediately after the collection. The remainder was centrifuged for 30 min ($1000 \times g$, 30 min, 4 °C), and aliquots of the plasma were frozen at -80 °C until analyzed for interleukin 2 (IL-2), interferon γ (IFN- γ), tumor necrosis factor α (TNF- α), C-reactive protein (CRP), 8-hydroxy-2-deoxy-guanosine (8-OHdg), and isoflavone concentrations. Two 24-h urine collections (0 wk and 16 wk) were obtained from participants. Total urine volume was recorded, and aliquots were frozen at -20 °C until analyzed for concentrations of the oxidative markers, 8-isoprostanate, and urinary isoflavones.

Analytic procedures

Isoflavone concentrations

Isoflavone concentrations in plasma and urine were measured by HPLC as previously described (23, 24). Briefly, β -glucuronidase-sulfatase H-2 (EC 3.2.1.31, from *Helix pomatia*; Sigma Chemical Co, St Louis, MO) was added to plasma (1 mL) and urine (5 mL), and the mixtures were incubated at 37 °C for 20 h to release isoflavone aglycones. Samples were then loaded onto Extrelut QE columns (EM Science, Gibbstown, NJ) and extracted with 8 mL (plasma) or 12 mL (urine) ethyl acetate. The eluent was collected and dried under nitrogen gas. Extracted isoflavones were dissolved in 80% methanol in water for HPLC

analysis. An HPLC system (Waters, Milford, MA) equipped with a photodiode array detector (PDA 996) was used to distinguish the ultraviolet spectra of the specific isoflavone compounds: daidzein, equol, and genistein. Isoflavones were separated in a μ -Bondapak C₁₈ reversed-phase column (Waters; 3.9 mm \times 30 cm). The samples were eluted by using a linear gradient of 40–70% methanol in 30 min at a flow rate of 1 mL/min. Retention times for the isoflavones were as follows: daidzein at 14.9 min, equol at 16.8 min, and genistein at 19.5 min. Daidzein, equol, and genistein were detected at 248, 280, and 260 nm, respectively. The identity of each isoflavone was confirmed by comparing its absorption spectrum with that of a corresponding standard. Plasma and urine isoflavone concentrations were calculated on the basis of standard curves constructed for daidzein (Valeant Pharmaceuticals, Costa Mesa, CA), genistein (Calbiochem, San Diego, CA), and equol (Sigma-Aldrich, St Louis, MO).

Lymphocyte subsets

Specific populations of lymphocytes were measured by using dual-color flow cytometric analysis (FACScalibur equipped with Cell Quest software; BD Biosciences, San Diego, CA): total T cells (CD3⁺CD19⁻), T cytotoxic cells (Tc; C120D3⁺CD8⁺), T helper cells (Th; CD3⁺CD4⁺), ratio of Th to Tc cells (Th:TC; CD3⁺CD4⁺/CD3⁺CD8⁺), B cells (CD3⁻CD19⁺), and natural killer cells (CD3⁻CD16⁺56⁺). Whole blood aliquots obtained from subjects were treated with a lysis buffer to avoid contamination by erythrocytes (25). After centrifugation, the resultant cell pellet was washed, and cells were labeled with monoclonal antibodies conjugated with fluorescein isothiocyanate or phycoerythrin: anti-CD3 conjugated to fluorescein isothiocyanate, and anti-CD8, anti-CD4, or anti-CD19 conjugated to phycoerythrin (Caltag Laboratories, Burlingame, CA). Cell suspensions were fixed with paraformaldehyde and analyzed by using a leucogate, or automatically established lymphocyte analysis gate. The leucogate helps to define and distinguish the lymphocytes from other blood cell types. A total of 2000 gated events were counted for each sample.

Cytokine production

Plasma samples were analyzed by enzyme-linked immunosorbent assays (ELISA) for IFN- γ (human IFN- γ BD OptEIA ELISA set; BD Biosciences, San Diego, CA), TNF- α (BD OptEIA set human TNF; BD Biosciences), and IL-2 (BD OptEIA set human IL-2; BD Biosciences). In these assays, wells were coated with capture antibody, diluted in coating buffer, and incubated overnight at 4 °C. Samples were pipetted into appropriate wells and incubated for 2 h at room temperature. Detection antibody with avidin–horseradish peroxidase was added to plates for color development, and the plates were incubated for 30 min. A stop solution was then added, and absorbance was read on a plate reader (Bio-Tek Instruments Inc, Winooski, VT) at 450 nm.

C-reactive protein

Plasma samples were analyzed for CRP by ELISA (human CRP ELISA kit; Alpha Diagnostic International, San Antonio, TX). The human CRP kit is a sandwich-type ELISA that is based

on the CRP in the samples binding simultaneously to 2 antibodies, one fixed to the microtiter well plates and the other conjugated to horseradish peroxidase. After color development, absorbance was read at 450 nm. Concentrations of CRP in the samples were measured on the basis of a standard curve.

Lipid peroxidation

8-Isoprostane was measured in purified urine by an enzyme immunoassay method (8-isoprostane EIA kit; Cayman Chemical Company, Ann Arbor, MI). This assay is based on the competition between 8-isoprostane and an 8-iso-acetylcholinesterase tracer. The concentration of the tracer is held constant, while the amount of 8-isoprostane in the urine samples varies. The 8-isoprostane antibody binds to the IgG mouse monoclonal antibody that was previously attached to the wells. After washing, color is developed with Ellman's reagent, and absorbance is measured at 410 nm.

DNA damage

Plasma concentrations of 8-OHdg were measured by ELISA (Bioxytech 8-OHdg-EIA kit; OXIS Health Products Inc, Portland, OR). Samples and standards were added to microtiter plate wells that had been precoated with 8-OHdg. The 8-OHdg present in both the wells and the samples competes for binding with the monoclonal antibody to 8-OHdg. Plates were washed, leaving only the antibody that had bound to the 8-OHdg coating the wells. Secondary antibody conjugated to horseradish peroxidase was then added and bound to the monoclonal antibody remaining on the plate. Chromogen was added after another wash step, color was developed, and absorbance was measured at 450 nm.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) or analysis of covariance (ANCOVA) by using the general linear model procedure of SAS statistical software (version 8; 26). Normality was tested by using the Shapiro-Wilk test. Due to skewness, logarithmic transformation was performed only on the raw data for B cell subsets, plasma IFN- γ , and 8-OHdg. The initial statistical model included the effects of block, treatment, week, and the interaction of treatment by week. On preliminary statistical analyses, it was determined that there were no effects of block; this variable was removed from the statistical model before further analyses. The change in percentage B cell populations between baseline and 16 wk was further analyzed by ANCOVA with control for the baseline value. Treatment means were compared by using the Tukey-Kramer test. *P* values < 0.05 were considered statistically significant.

RESULTS

Subjects

The demographic characteristics of the subjects are presented in **Table 2**. A total of 52 (95%) postmenopausal women completed the study. The mean age of the study participants did not differ significantly by group. There was no significant group \times week interaction in body mass index. Ninety-four percent of the study participants were white, and most (88%) had received education beyond the high school level. Study treatments, including cow milk, soymilk, and supplemental tablets, were well tolerated overall. Compliance with the study protocol was very

TABLE 2

Demographic characteristics of the women by group

	Control group (n = 19)	Soymilk group (n = 18)	Supplement group (n = 15)
Age (y) ¹	55.4 ± 3.9 ²	56.1 ± 4.4	55.9 ± 3.5
BMI (kg/m ²) ¹			
0 wk	27.5 ± 4.9	27.4 ± 6.2	28.8 ± 5.4
16 wk	27.3 ± 4.5	27.6 ± 6.3	28.8 ± 5.4
Ethnicity (n)			
White	16	18	15
Asian	2	0	0
American Indian	1	0	0
Education level (n)			
High school	3	3	—
College: 1–4 y	11	8	6
Postbaccalaureate	5	7	9

¹ There were no significant group, week, or group × week interactions for age or BMI as analyzed by ANOVA.

² $\bar{x} \pm \text{SE}$ (all such values).

good as indicated by verbal reports and written dietary records from the women and by the absence (control) or presence (isoflavone interventions) of isoflavone metabolites in plasma and urine samples.

Isoflavone concentrations

At baseline, plasma and urinary concentrations of daidzein, equol, and genistein were generally very low to nondetectable among the women in all experimental groups, thus confirming dietary compliance during the adjustment period. One woman had a detectable concentration (0.20 $\mu\text{mol/L}$) of plasma equol. There was a significant ($P < 0.05$) group × week effect for plasma genistein, whereas this was only marginally significant ($P < 0.09$) for plasma equol and nonsignificant for plasma daidzein. After the 16-wk intervention, plasma concentrations of genistein were significantly higher in both isoflavone treatment groups, but remained undetectable in the control group (Figure 1). Plasma equol was significantly higher in the soymilk group than in the control group.

Urinary concentrations of daidzein, genistein, and equol were significantly higher in the soymilk and supplement groups than in the control group at 16 wk (Figure 2). There was a significant ($P < 0.001$) group × week interaction for all urinary isoflavones. The relative concentration of each isoflavone in the urine generally reflected that observed in the plasma (Figures 1 and 2).

Lymphocyte subsets

There was a marginal interaction ($P < 0.09$) of group × week for the percentage of B cells. Data were further analyzed by ANCOVA by using the change between baseline and 16 wk and controlling for the baseline value. Statistical analysis showed a significant ($P < 0.03$) effect of group. At 16 wk, B cell populations remained unchanged from baseline among women in the control group (8.5% from 8.6%), but were higher among women in both the soymilk (11.3% from 8.1%) and supplement (12.2% from 8.5%) groups than in the control group (Figure 3). Baseline and 16-wk values of other lymphocyte populations, including total T cells, Th and Tc cells, Th:Tc, and natural killer cells, were

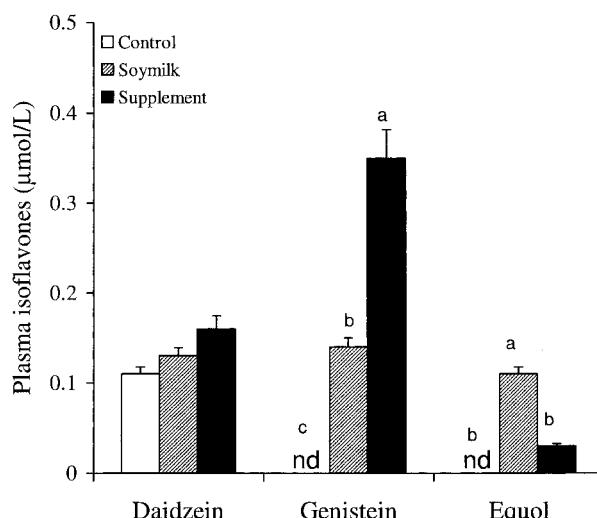


FIGURE 1. Mean ($\pm \text{SEM}$) plasma daidzein, genistein, and equol concentrations at 16 wk in the control ($n = 19$), soymilk ($n = 18$), and supplement ($n = 15$) groups. nd, not detectable. There was a significant ($P < 0.05$) group × week effect for plasma genistein, whereas this was marginally significant ($P < 0.09$) for plasma equol and nonsignificant ($P < 0.1$) for plasma daidzein as analyzed by ANOVA followed by the Tukey-Kramer test. The effects of group and week for genistein ($P < 0.001$) and that of week for equol ($P < 0.001$) were significant; all other comparisons were nonsignificant. Different letters above each bar denote significant differences within each isoflavone, $P < 0.05$.

not significantly different among the 3 experimental groups (Table 3); the interaction of group × week was also not significant for these lymphocyte subpopulations.

Cytokine production

Isoflavone intervention did not significantly influence concentrations of IFN- γ , IL-2, or TNF- α (Table 4), and overall concentrations averaged $15.4 \pm 1.9 \text{ pg/mL}$, $11.4 \pm 0.05 \text{ pg/mL}$,

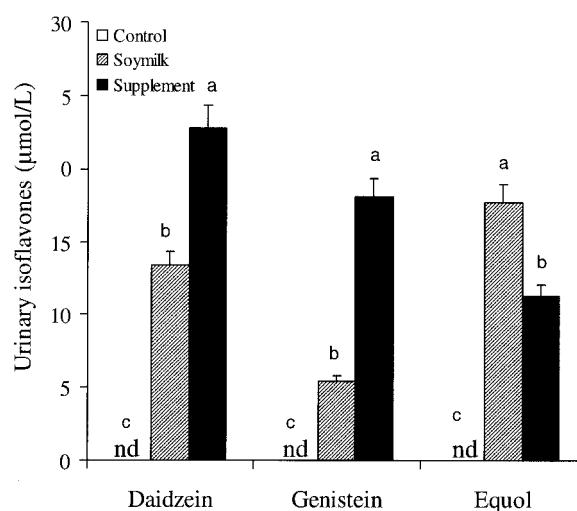


FIGURE 2. Mean ($\pm \text{SEM}$) urinary concentrations of daidzein, genistein, and equol at 16 wk in the control ($n = 19$), soymilk ($n = 18$) and supplement ($n = 15$) groups. There were significant group, week, and group × week effects for daidzein, genistein ($P < 0.001$), and equol ($P < 0.05$) as analyzed by ANOVA followed by the Tukey-Kramer test. Isoflavones were not detectable at baseline in any of the groups. Different letters above each bar denote significant differences within each isoflavone group, $P < 0.05$.

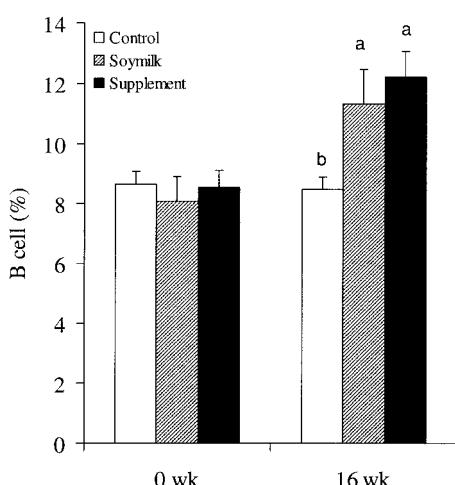


FIGURE 3. Mean (\pm SEM) B cell percentages for the control ($n = 19$), soymilk ($n = 18$), and supplement ($n = 15$) groups at 0 and 16 wk. There was a marginally significant group \times week effect ($P < 0.09$) and a significant week effect ($P < 0.001$) as analyzed by ANOVA and a significant group effect ($P < 0.03$) as analyzed by analysis of covariance by using the change between baseline and 16 wk and controlling for baseline values. Different letters above each bar denote significant group differences within each week, $P < 0.05$ (Tukey-Kramer test).

and 2.08 ± 0.49 ng/mL, respectively. There was no significant group \times week interaction for IFN- γ , IL-2, or TNF- α .

C-reactive protein

Concentrations of CRP did not change significantly in response to isoflavones provided in either soymilk or supplemental form (Table 5). Overall average concentrations were 2590 and 2737 ng/mL at baseline and 16 wk, respectively. There was no significant group \times week interaction.

Lipid peroxidation

Concentrations of urinary 8-isoprostane at 16 wk were not significantly different from those measured at baseline in the 3 experimental groups (Table 5). Overall, average concentrations were 0.778 ng/mL at baseline and 0.780 ng/mL at 16 wk. The group \times week interaction was not significant.

DNA damage

At baseline, concentrations of 8-OHdg among the groups did not differ significantly (control, 14.3 ng/mL; soymilk, 15.3 ng/mL; supplement, 14.4 ng/mL). At 16 wk, however, subjects receiving isoflavones in soymilk or supplemental tablet form had lower ($P < 0.01$) plasma 8-OHdg concentrations (10.9 and 10.4

ng/mL, respectively) than did women in the control group (13.9 ng/mL; Figure 4). There was a significant ($P < 0.001$) group \times week interaction for plasma 8-OHdg.

DISCUSSION

This is the first study evaluating specific antioxidant and immune-modulating actions of soy isoflavone consumption among postmenopausal women. Whether the isoflavones were derived from soymilk or tablets, the immune and antioxidant effects did not differ significantly.

Concentrations of all 3 isoflavones—daidzein, equol, and genistein—were significantly higher in the urine in response to both interventions, which showed that the subjects were compliant with the study protocol. These values were similar to those measured in other isoflavone-consuming populations (27). Genistein was the most prominent isoflavone in plasma at 16 wk. In the urine, concentrations of daidzein and genistein were higher and concentrations of equol were lower in the soymilk group than in the supplement group at 16 wk. Differences between plasma and urinary isoflavone profiles have been reported in other human studies (28–30). Equol production occurred in less than one-half of the women in both isoflavone interventions, which parallels the overall percentage of the human population believed capable of this metabolic transformation (13). The presence of daidzein in the plasma of the control group is puzzling because the subjects were compliant with the diet (as evidenced by the lack of detectable isoflavones in the urine).

Interestingly, the nonhormonal properties of isoflavones appear to play a substantial role in disease prevention for women in this stage of life. Isoflavones can behave as antioxidants and therefore protect against diseases resulting from oxidative damage (2). Two biomarkers, plasma 8-OHdg and 8-isoprostanate, were measured. Plasma concentrations of 8-OHdg reflect oxidative damage to DNA, and concentrations increase during repair of DNA. In vitro studies have shown isoflavones, at human physiologic concentrations, to be protective against oxidatively induced DNA damage (17, 31). Postmenopausal women in both isoflavone treatment groups in the present study had significantly lower concentrations of 8-OHdg than did the control group. These findings suggest a protective effect of soy isoflavones regarding oxidative stress in postmenopausal women.

Isoprostanates are eicosanoid compounds produced by the peroxidation of phospholipids in tissues, and elevated concentrations have been found in plasma and urine during periods of oxidative stress (32). In our study, isoflavones did not

TABLE 3
Population changes in lymphocyte subsets by group¹

Group	Total T cells		Th cells		Tc cells		Th:Tc		NK cells	
	0 wk	16 wk	0 wk	16 wk	0 wk	16 wk	0 wk	16 wk	0 wk	16 wk
Control ($n = 19$)	73.1 ± 1.2	73.8 ± 1.2	48.9 ± 1.6	46.7 ± 1.5	19.1 ± 1.1	19.1 ± 1.1	3.3 ± 0.3	2.9 ± 0.2	11.7 ± 0.6	10.3 ± 0.6
Soymilk ($n = 18$)	76.2 ± 1.2	75.2 ± 1.2	50.2 ± 1.6	47.3 ± 1.6	17.7 ± 1.1	16.7 ± 1.0	3.3 ± 0.2	3.3 ± 0.2	10.6 ± 0.5	9.2 ± 0.5
Supplement ($n = 15$)	73.9 ± 1.3	72.4 ± 1.2	50.6 ± 1.7	44.4 ± 1.5	17.8 ± 1.2	21.1 ± 1.2	3.0 ± 0.3	2.3 ± 0.2	10.4 ± 0.5	9.7 ± 0.5

¹ All values are $\bar{x} \pm$ SEM. Th, Thelper; Tc, T cytotoxic; NK, natural killer. There were no significant ($P > 0.1$) group, week, or group \times week interactions for the percentage of total T cells, Th cells, Tc cells, Th:Tc, or NK cells as assessed by ANOVA.

TABLE 4Concentrations of plasma cytokines by group¹

Group	IFN- γ		IL-2		TNF- α	
	0 wk	16 wk	0 wk	16 wk	0 wk	16 wk
<i>pg/mL</i>						
Control	8.4 ± 4.4	13.5 ± 4.4	11.4 ± 0.1	11.5 ± 0.1	2.26 ± 1.07	1.98 ± 1.31
Soymilk	10.1 ± 4.6	25.7 ± 4.6	11.3 ± 0.1	11.4 ± 0.1	2.45 ± 1.07	2.46 ± 1.20
Supplement	11.4 ± 5.4	24.7 ± 5.2	11.3 ± 0.1	11.4 ± 0.1	1.28 ± 1.20	2.09 ± 1.31

¹ All values are $\bar{x} \pm$ SEM. IFN- γ , interferon γ ; IL-2, interleukin 2; TNF- α , tumor necrosis factor α . There were no significant differences ($P > 0.1$) in group, week, or group \times week interactions for plasma concentrations of IFN- γ , IL-2, or TNF- α as analyzed by ANOVA.

significantly influence urinary concentrations of 8-isoprostane. However, in another human study, Wiseman et al (16) found significantly lower concentrations of 8-isoprostane in response to a high-isoflavone diet. They compared the effects of high- (21.2 mg daidzein, 34.8 mg genistein) and low- (0.9 mg daidzein, 1.0 mg genistein) isoflavone diets on plasma 8-isoprostane concentrations in a randomized crossover trial. Plasma 8-isoprostane concentrations were significantly lower after consumption of the high-isoflavone diet than after the low-isoflavone treatment (16). However, their study used a highly specific and sensitive method (gas chromatography and mass spectrometry) to measure plasma concentrations of 8-isoprostane. The immunoassay method used in our study is more prone to interference by other substances present in biological fluids, including urine (32).

We also measured the plasma concentrations of specific cytokines: IL-2, TNF- α , and IFN- γ . The secretion of IL-2 stimulates T cell proliferation, TNF- α mediates local inflammatory reactions, and IFN- γ activates macrophages. In the present study, concentrations of plasma IFN- γ , IL-2, and TNF- α were not significantly affected by soy isoflavones. A recent study found decreased IFN- γ concentrations in bacteria-infected, estrogen receptor- α -deficient mice fed soy-based diets (33); also, estrogen has been shown to up-regulate IFN- γ concentrations in mice (34). However, the estrogenic effects of isoflavones are complex; they may behave either as estrogen agonists or as antagonists (2).

Serum concentrations of CRP, a multipronged acute phase protein, rise within hours of infection and thereby serve as an indicator of inflammation. Despite this crucial immune role, high concentrations of CRP are strongly associated with increased risk of coronary artery disease (35, 36). In the present study, CRP concentrations were not significantly influenced

by treatment, which suggests that isoflavones may not influence this particular inflammatory response in postmenopausal women. Our findings are similar to the results of a recent study by Jenkins et al (21). Those authors studied the effects of both high- (73 mg/d) and low- (10 mg/d) isoflavone diets on biomarkers of inflammation, including plasma IL-6 and CRP, in middle-aged men and women. Although plasma concentrations of IL-6 were significantly higher in the subjects who received the high-isoflavone diet, no significant changes in CRP concentrations among subjects were observed (21). The effect of isoflavones on IL-6 was not evaluated in our study.

Animal and human studies have shown that dietary isoflavones can modulate lymphocyte subsets. Enhanced host resistance in the B16F10 tumor model was found in genistein-treated mice. This was associated with increased Tc and natural killer cell activities (19). Enhanced natural killer cell activity also occurred in adult Sprague-Dawley female rats fed genistein, as well as in human cells exposed to daidzein and genistein (20, 22). One proposed mechanism for immunoregulation by isoflavones is their estrogenic actions, which are mediated through binding to the estrogen receptors. Our finding of increased B cell populations in the isoflavone intervention groups correlates with earlier studies regarding estrogen's enhancement of humoral immunity (37, 38). The normal B cell (CD3/CD19) population in healthy adults is 7–23% (39); therefore, the observed increase of 3–6% in the B cell population suggests a potential heightened humoral response in subjects fed isoflavone and does not suggest autoimmunity.

Advances in molecular biology techniques allow researchers to continually uncover physiologic targets of isoflavones (40). There appear to be a myriad of mechanisms by which isoflavones exert their effects, with even biphasic responses observed in

TABLE 5Concentrations of C-reactive protein (CRP) in plasma and 8-isoprostane in urine by group¹

Group	CRP		8-Isoprostane	
	0 wk	16 wk	0 wk	16 wk
<i>ng/mL</i>				
Control (<i>n</i> = 19)	2789 ± 311	2735 ± 373	0.774 ± 0.01	0.780 ± 0.01
Soymilk (<i>n</i> = 18)	2712 ± 391	2604 ± 378	0.781 ± 0.01	0.782 ± 0.02
Supplement (<i>n</i> = 15)	2269 ± 332	2872 ± 356	0.779 ± 0.02	0.779 ± 0.01

¹ All values are $\bar{x} \pm$ SEM. There were no significant differences ($P > 0.1$) in group, week, or group \times week interactions for concentrations of plasma CRP or urinary 8-isoprostane as analyzed by ANOVA.

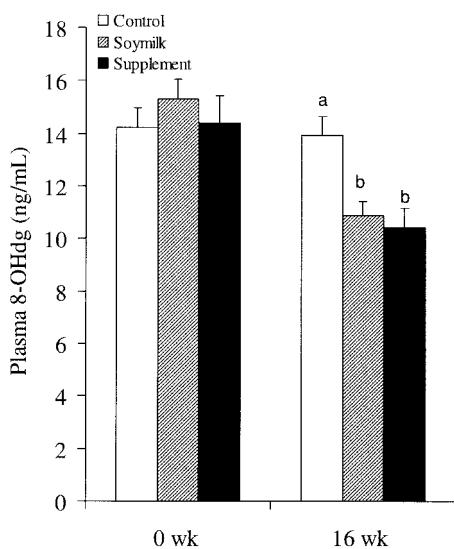


FIGURE 4. Mean (\pm SEM) plasma concentrations of 8-hydroxy-2-deoxy-guanosine (8-OHdg) in the control ($n = 19$), soymilk ($n = 18$), and supplement ($n = 15$) groups before and after 16 wk of intervention. There were significant group, week, and group \times week effects ($P < 0.001$) as analyzed by ANOVA. Different letters above each bar denote significant group differences within each week, $P < 0.05$ (Tukey-Kramer test).

certain biochemical targets (41). It will also be important to ascertain ways in which isoflavones behave under different physiologic conditions. For example, recommended isoflavone intakes may vary depending on desired clinical outcome, such as increased tumor surveillance, compared with when down-regulation of the immune system is advantageous, as in autoimmune disorders.

In conclusion, soy isoflavones stimulated B cells and inhibited DNA oxidative damage in postmenopausal women. Further research should explore the effects of soy isoflavones in immunologically challenged populations.

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TAR-B assisted in planning the study and recruiting the subjects, provided dietary instruction to the participants, performed all laboratory procedures, analyzed the study results, and drafted the manuscript. JSP and BPC coordinated the immune variables and statistical analyses. MKM and LRF assisted with study planning and dietary treatment procedures. KAB coordinated and managed the subject and dietary treatment procedures. None of the authors had any conflicts of interest.

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