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Dietary Isoflavones Affect Sex Hormone-Binding Globulin Levels in Postmenopausal Women*

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ABSTRACT

The studies presented in this report were designed to further investigate the causal association between phytoestrogen action and increase in sex hormone-binding globulin (SHBG) levels. Phytoestrogens include isoflavones that bind to estrogen receptors and therefore exert estrogenic action. This study included 20 postmenopausal women that ingested 30 g soy milk daily for 10 weeks. Plasma concentrations of isoflavones and SHBG were measured. Total isoflavones significantly increased from 0.014 ± 0.01 μmol/L (baseline) to 0.53 ± 0.19 μmol/L, and paired responses showed that some subjects clearly increased their SHBG levels. The percent change in SHBG showed a positive correlation with phytoestrogen concentration; all women who had circulating phytoestrogen levels above 0.6 μmol/L increased by at least 30% their SHBG values. Results suggest that phytoestrogens may significantly increase SHBG in subjects whose SHBG concentrations are in the low end of the concentration range. (*J Clin Endocrinol Metab* **85:** 2797–2800, 2000)

PHYTOESTROGENS, found abundantly in grains, fruits, and vegetables, may be responsible in part for the protective effects of vegetarian diets, including their anticarcinogenic and antiosteoporotic influences and their reduction of cardiovascular diseases and menopausal symptoms. Most phytoestrogens in typical human diets contain at least one aromatic ring with a hydroxyl group, similar to those of estrogens, and have been found to bind to estrogen receptors (1).

Like some other estrogen-like molecules, the ability of phytoestrogens to replace or modify estrogenic pathways has been difficult to ascertain. There is considerable individual variability in the intake, absorption, excretion, and metabolism of these compounds (2–6), characteristics that contribute to determine their plasma bioavailability. Little is known about the bioavailability of absorbed phytoestrogens; some plant-derived estrogens bind poorly to sex hormone-binding globulin (SHBG), circumventing the mechanism that limits steroid cell uptake (7–9).

SHBG is a glycoprotein produced by hepatocytes, and its blood levels in humans are influenced by steroidal and peptidic hormones, T_4, and dietary factors (4, 10–18). Among the latter, lignans and isoflavones have been shown to increase the synthesis and secretion of SHBG by human HepG2 hepatoblastoma cells (4, 17, 18). However, studies in individuals consuming mainly vegetarian or soy-based diets did not show a clear association between ingested amounts of phytoestrogens and increases in SHBG concentration (4, 19–23).

The regulation of SHBG production has been studied in HepG2 hepatoblastoma cells (11–15), but little is known about the molecular control of its expression in the liver; the response of these cells to phytoestrogens has been equated to that of estradiol. Northern hybridization showed that both estradiol (E_2) and phytoestrogens increased SHBG messenger ribonucleic acid levels marginally (16, 18), but both compounds either increased the release of the protein (10, 13–18) or had no effect (15).

In this study we further analyze the effects of phytoestrogens on SHBG production by *in vivo* paired tests, with each individual serving as her own control. The study included 20 healthy postmenopausal women who received daily 30 g powdered soy milk for 10 weeks. Measurements included phytoestrogen concentrations in urine and plasma to control compliance with the ingested dose.

Subjects and Methods

Subjects

Twenty ambulatory postmenopausal women (aged 47–65 yr; mean age, 54.2 ± 5.7) were recruited from the Santiago Metropolitan area. All subjects were considered healthy on the basis of their history, physical examination, electrocardiogram, and routine blood and urine analysis. All subjects consumed a mixed diet, were overweight, were generally sedentary, and took neither hormonal nor vitamin supplements in the preceding 6 months. The study was approved by the institutional review board for research on human subjects of the Institute of Nutrition and Food Technology, University of Chile (Santiago, Chile), and written informed consent was obtained from all subjects.

Participants were followed up during 10 consecutive weeks. All subjects consumed their usual mixed, low fiber diet, supplemented with the daily intake of 30 g of a dry, powdered soy milk, freely available in the market (Fuente Natural, Brazil) distributed in three servings during the day. They attended the clinic six times during the study: twice during the period preceding the beginning of the observation, twice during the experimental period (5th and 7th weeks), and twice after its conclusion (10th week). Participants attended the clinic after an overnight fast. At each appointment, they were weighed, and blood was drawn by venipuncture. The blood samples were centrifuged, and serum was stored at −20 C. A first morning urine specimen was collected on the same day.

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Laboratory methods

Commercially available RIA kits (Diagnostic Products, Los Angeles, CA) were used to measure serum concentrations of E_2, estrone (E_1), LH, and FSH. The sex hormone-binding globulin concentration in plasma was measured using an immunoradiometric assay (Farmos Diagnostica, Oulunsalo, Finland). Samples from individual women were assayed in one batch. For all analytes, the intraassay coefficient of variation was less than 5%, and the interassay variabilities were 7.7%, 14.7%, 6.3%, 10.3%, and 1.8% for E_2, E_1, LH, FSH, and SHBG, respectively. The results given are based on duplicate assays.

Total phytoestrogen measurements

Plasma, urine, and soy milk samples for isoflavone analysis were treated as described by Lundh (24); samples were treated with β-glucuronidase sulfatase (Sigma, St. Louis, MO), and unconjugated isoflavones were extracted by liquid-solid extraction. Chromatographic analysis followed the conditions described by Xu (5); samples were injected and analyzed in a high performance liquid chromatography system that included an intelligent pump (model L-6200, Merck-Hitachi), a microprocessor-regulated solvent flow controller (model D-6000, Merck-Hitachi, Tokyo, Japan), and a variable wavelength detector set at 254 nm (model L-6200, Merck-Hitachi). Total areas of daidzein and genistein were determined for each sample; values were added and expressed as total isoflavones. The efficiency of each extraction was calculated by the addition of 1 μg 5α-androstane-3α,17α-diol as an internal standard.

Statistical analysis

Results are expressed as the mean ± SD. Significance was considered at $P < 0.05$. The Mann-Whitney test was used to investigate differences between baseline and final plasma levels. Correlations between SHBG levels and plasma parameters were studied by linear regression. All statistical calculations were performed using SigmaStat software (Sigma, St. Louis, MO).

Results

Total isoflavones, measured in soy milk samples collected throughout the study, showed daidzein and genistein contents of 1.2 ± 0.33 and 1.1 ± 0.34 mg/g, respectively; thus, women ingested roughly 69 mg isoflavones daily. Subject characteristics and plasma hormone levels before and after 10 weeks of drinking the soy milk are shown in Table 1. Body weight, body mass index, and plasma levels of FSH, E_2, and E_1 did not change significantly during the study. However, as the treatment increased SHBG levels moderately, a significant decrease in the mean E_2/SHBG ratio resulted. The total content of phytoestrogens in plasma increased during treatment, indicating appropriate consumption and acceptance of the soy product, although there was some individual variation (range, 0.19–0.88 μmol/L). Circulating phytoestrogen levels remained constant for each woman throughout treatment, whereas urinary phytoestrogens did not show much variation between subjects.

TABLE 1. Subjects characteristics and plasma hormone concentrations

	Baseline values	Final values
Age (yr)	54.3 ± 5.7	54.3 ± 5.7
Wt (kg)	64.4 ± 8.8	65.2 ± 7
BMI (kg/m^2)	27.3 ± 3.4	27.6 ± 3.1
E_2 (pmol/L)	35.84 ± 6.7	35.9 ± 3.5
E_2/SHBG × 10^3	0.64 ± 0.09	0.56 ± 0.07[a]
E_1 (pmol/L)	75.45 ± 14.1	68.97 ± 7.2
SHBG (nmol/L)	55.5 ± 9.6	64.2 ± 5.6[a]
FSH (IU/L)	66.4 ± 23.2	59.6 ± 21
Total isoflavones (μmol/L)	0.014 ± 0.019	0.55 ± 0.19[b]

Values are the mean ± SD.
[a] $P < 0.05$.
[b] $P < 0.001$.

The individual response to soy milk consumption, measured as the plasma SHBG level, is shown in Fig. 1. Individuals who clearly increased their SHBG levels after treatment were those who had low SHBG levels initially, whereas those who did not change or even decreased their circulating SHBG levels, had SHBG values above 55 nmol/L at the beginning of the study.

To better appreciate the influence of treatment on SHBG individual responses, each value was expressed as the percent change in SHBG concentration, and this response was correlated with plasma concentrations of hormones and isoflavones. As shown in Fig. 2, the percent change in SHBG showed a clear positive correlation with respect to plasma phytoestrogen concentration at the end of treatment. All women whose circulating phytoestrogen levels were above 0.6 μmol/L showed an increase of at least 30% in their circulating SHBG levels. Table 2 summarizes linear regression data; SHBG levels before soy milk consumption showed a significant, positive correlation with contemporaneous circulating E_2 levels, but this relationship was not sustained for values obtained at the end of the follow-up. However, the percent change in SHBG exhibited a negative correlation with E_2 levels. On the other hand, SHBG values at the end of the study showed no correlation with circulating total isoflavones. As mentioned above, the percent change in SHBG showed a significant negative correlation with initial SHBG levels and no correlation with E_1 levels.

Discussion

Twenty women completed the short-term dietary intervention trial, during which each of them acted as her own control. After the beginning of the treatment, circulating phytoestrogen levels increased, and this increase was sustained throughout the study. However, there was some variation between subjects. Differences among individuals could arise from the action of the intestinal microflora on dietary lignans and isoflavone glycosides (2–6), because appropriate

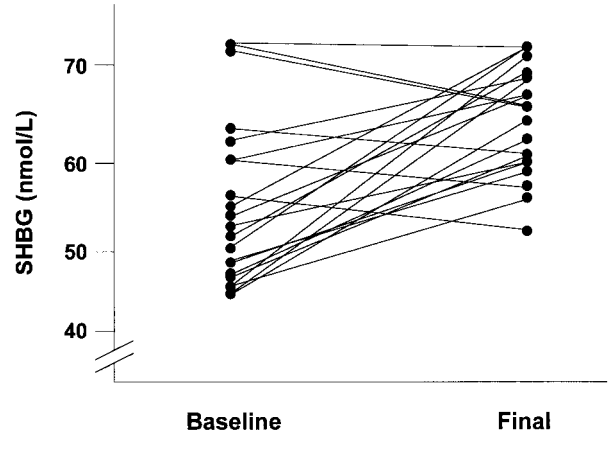

FIG. 1. Individual responses to soy milk treatment, measured as plasma SHBG concentrations before and after soy milk consumption.

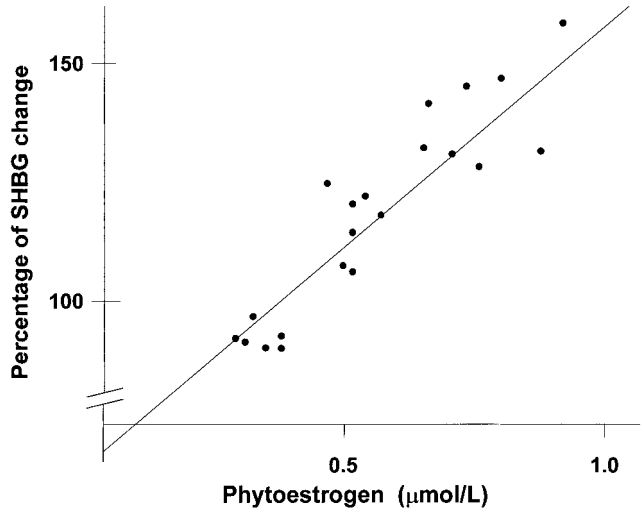

FIG. 2. Relationship between percent change in SHBG levels and plasma total isoflavone concentrations in 20 postmenopausal women.

TABLE 2. Linear regression between baseline, final, or change in SHBG (%) concentration, and plasma concentrations of 17β-estradiol (E_2), estrone (E_1), total isoflavones (tot.iso), or baseline levels of SHBG

	r	*P*
Baseline SHBG *vs.* baseline E_2	0.625	0.003[a]
Final SHBG *vs.* final E_2	−0.129	0.525
Final SHBG *vs.* final tot.iso.	0.0847	0.723
% Change in SHBG *vs.* final tot.iso.	0.848	<0.001[a]
% Change SHBG *vs.* final E_2	−0.433	0.056
% Change SHBG *vs.* final E_1	0.0314	0.895
% Change SHBG *vs.* baseline SHBG	−0.852	<0.001[a]

[a] Statistically significant.

ingestion of the dietary supplement was corroborated by periodical measurements of phytoestrogen concentration in both plasma and urine samples (results not shown). As blood samples were withdrawn after 12–18 h of the last soy milk intake, we infer that these plasma values exemplify the low end of phytoestrogen concentrations attained after daily ingestion of soy milk. Our data agree well with pharmacokinetic data from women who received 50 mg individual isoflavones orally (2).

We observed a moderate increase in mean SHBG values as a consequence of phytoestrogen treatment. Several previous controlled intervention studies in soybean-consuming women (either pre- or postmenopausal) as well as in men (19–21, 23, 25) and in premenopausal women receiving lignans (22) did not report this response. Recently, Duncan *et al.* (26) observed a modest effect on SHBG levels in postmenopausal women consuming isoflavone-enriched diets. None of these studies measured plasma phytoestrogen concentrations or considered individual responses. Our observations made it evident that some subjects significantly increased their SHBG plasma concentrations after treatment, whereas others did not show this response. The first group had the lowest plasma SHBG levels at the beginning of treatment and attained high circulating phytoestrogen levels during treatment. Those women who did not experience changes in their circulating SHBG had SHBG concentrations higher than 55 nmol/L at the beginning of the study.

Linear regression analysis suggests an association between estrogenic action and SHBG levels: the positive relationship observed between baseline SHBG and E_2 levels was disrupted after phytoestrogen treatment. Notwithstanding that the total E_2 levels remained unchanged, its bioavailability would be diminished (9, 27), because of the increased SHBG levels as a consequence of treatment. These observations lead to conclude that estrogenic action, promoted by either E_2 or phytoestrogens, participates in SHBG level regulation. Thus, the estrogenic effect may be noticeable mainly at the low end of normal SHBG levels. Therefore, the basal production of SHBG in adult women seems to be related to general metabolic factors and nutritional status (11, 12, 28) as well as to fluctuations in estrogenic hormone levels.

In conclusion, this study points to a causal association between phytoestrogen action and increased SHBG levels. Our observations suggest that phytoestrogens may significantly increase circulating SHBG levels in those subjects whose SHBG values are at the low end of the concentration range.

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