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ORIGINAL CONTRIBUTION

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Short-term effects of high soy supplementation on sex hormones, bone markers, and lipid parameters in young female adults

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Summary *Background* High intake of soy products has been suggested to prevent breast cancer, osteoporosis, and cardiovascular diseases. *Aim of the study* To investigate the effects of isoflavone-containing soy on circulating sex hormones, biomarkers of bone turnover, and lipoprotein profiles. *Methods* Fourteen young women received in a randomized crossover design 5 soy cookies (52 mg isoflavones) or 5 soy-free cookies (no isoflavones) per day for one menstrual cycle starting one week before menstruation. Serum and urine analyses were performed on day 3 after onset of menstruation (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), during the midluteal phase (t_4), and again 3 days after onset of the next menstruation (t_5). *Results* With the exception of higher progesterone levels at t_2, soy supplementation did not affect the physiologic fluctuations in circulating sex hormones. The ratio of C-telopeptide (a bone resorption marker) to osteocalcin (a bone formation marker) was slightly higher at t_4 during the soy period compared to t_4 during the control period ($P < 0.05$), indicating an uncoupling of bone resorption and formation processes. Serum levels of total cholesterol, LDL cholesterol, and HDL cholesterol were not influenced by soy intake. *Conclusions* High short-term isoflavone-containing soy intake slightly affects physiologic fluctuations in bone turnover, but has no significant effects on most circulating sex hormones and on lipoprotein parameters in young healthy women.

Key words soy – isoflavones – bone turnover – menstrual cycle

Introduction

High intake of soy products has been suggested to prevent breast cancer [1, 2], osteoporosis [3], and cardiovascular diseases [3]. These diseases are at least in part influenced by estrogen exposure: while life-long exposure to high circulating levels of free endogenous estrogens such as 17-β-estradiol (E_2) is associated with an elevated risk for breast cancer [4], low circulating levels of E_2 are related to bone loss [5] and dyslipoproteinemia [6]. Possible beneficial effects of soy in the prevention of E_2-related diseases have been attributed to its high isoflavone content. Isoflavones such as genistein and daidzein are found in high amounts in the protein fraction of soy.

It has been assumed that isoflavones exert their cancer-protective effects at least in part by influencing circulating E_2 levels during the menstrual cycle and by effecting menstrual cycle length [7]. Data from controlled trials are however inconsistent: no effects [8], a reduction [9, 10] or an increase in serum E_2 levels [11, 12] have

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been observed with isoflavone-containing soy products. Moreover, interventional studies could not demonstrate soy-induced changes in cycle length [13].

Data on the effects of isoflavones on bone health in peri- and postmenopausal women are more consistent: epidemiological studies indicate that a high intake of soy isoflavones can slow the rate of bone loss at the lumbar spine in perimenopausal women [14]. Moreover, controlled clinical trials in perimenopausal and postmenopausal women have demonstrated that isoflavones can significantly increase bone mineral density at the lumbar spine [15, 16]. In a recent study, daily administration of 54 mg genistein reduced postmenopausal bone mineral loss at the femoral neck and lumbar spine as effectively as hormone replacement therapy with 1 mg/day E_2 [17]. However, a study in premenopausal women using daily supplementation of 64 mg or 128 mg isoflavones for three menstrual cycles resulted in a significantly higher bone resorption during the early follicular phase compared to a control period with only 10 mg isoflavones per day [18]. In that study, bone turnover was assessed by the bone resorption marker deoxypyridinoline and the bone formation marker osteocalcin.

In the United States, a soy health claim already exists indicating a hypocholesterolemic effect of the soy protein fraction. Such an effect has been especially observed in patients with hypercholesterolemia. In normocholesterolemic subjects effects on the serum lipoprotein profile are less clear [19]. Therefore, it is interesting that, in a recent study in premenopausal, normocholesterolemic women, intake of 129 mg isoflavones significantly decreased serum LDL cholesterol levels, the ratio of total cholesterol to HDL cholesterol, and the ratio of LDL cholesterol to HDL cholesterol compared to an intake of 64 and 10 mg isoflavones during specific phases of the menstrual cycle, indicating a dose-dependency of isoflavone intake on blood lipid profiles [20].

Recently performed receptor studies may explain some of the presumed isoflavone effects on E_2-related diseases: genistein, but not daidzein, has a similar affinity to the estrogen receptor β (ERβ) compared to estradiol (E_2). In contrast, the affinity of genistein (and also of daidzein) to ERα is weak [21]. It is worthy to note that bone contains only ERβ, while ERα and ERβ are located in the vascular system and in the placenta, the uterus, and in breast tissue [22]. Thus, dissimilar affinities of isoflavones to ERα and ERβ, and different tissue distribution of these two estrogen receptors could possibly explain inconsistent effects of isoflavones on the prevalence of various E_2-related diseases.

With the exception of the investigations of Wangen et al. [18] and Merz-Demlow et al. [20] we are not aware of other intervention trials with soy products on biochemical parameters of bone metabolism and lipoprotein profiles across the menstrual cycle of young healthy female adults. These two studies compared soy protein products of different isoflavone concentrations with each other. There is however evidence that some effects of soy protein may not only depend on its isoflavone content [18]. The present study was therefore aimed to compare the effects of a product made from soy flour with an iso-energetic soy-free product.

Materials and methods

Subjects

Seventeen healthy young caucasic women (age: 24.0 ± 0.9 years; body mass index: 24.0± 0.9 kg/m^2) were enrolled in the study. None of the subjects had chronic diseases affecting bone metabolism, eating disorders such as anorexia and bulimia nervosa, or a body mass index < 18 kg/m^2. Moreover, none of the participants was taking oral contraceptives or had amenorrhea. All subjects had had regular menstrual cycles for at least 3 months (mean cycle length: 30.8 ± 2.5 days). Pregnancy was excluded by use of standard tests 1 day before actual examinations. All participants gave written informed consent to the study, which had been approved by the Ethics Committee of the University of Bonn.

Study protocol

Design

With the use of a cross-over design, subjects were randomly assigned to 2 groups. Participants either received a dietary supplement of five soy cookies per day (total of 56 g) made from whole soy flour (soy period; SP) or, isoenergetically, five cookies per day based on white wheat flour (wheat flour period; WP) while they remained on their habitual diet. Isoflavone content of the soy cookies and the white flour cookies was analyzed prior to study begin. One daily portion of five soy cookies contained 19 mg daidzein (range: 17–23 mg) and 33 mg genistein (range 29–39 mg). Isoflavone content of the white flour cookies was < 0.1 mg. Supplementation started one week before the predicted menstruation. Each dietary period lasted for one menstrual cycle. The two periods were separated by a washout period of 2 menstrual cycles. Serum and urine analyses were performed on day 3 after onset of menstruation (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), during the midluteal phase (t_4), and again 3 days after onset of the next menstruation (t_5). The time of ovulation was calculated according to the method of Knaus and Ogino by using the mean duration of the last three menstrual cycles and subtracting 14 days. Midluteal phase was calculated by the day of ovulation plus 7 days. Nutrient intake was as-

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sessed using a prospective standardized food record which had to be completed by the subjects on each day before the actual examinations. Blood was harvested (t_1–t_5) from the antecubital vein into serum monovettes after an overnight fast. One day before blood sampling 24-h urine collections were performed (0800 h to 0800 h). Body mass was measured at t_1 and at t_5 of each study period with a precision of 50 g.

Assessment of nutrient intake and study compliance

The nutrient content of the diets was quantified using the computer program Ebis (Hohenheim, Germany), which is based on the German data collection "Bundeslebensmittelschlüssel II". The reliability of nutrient intake, as estimated by the food record, was determined by measuring 24-h nitrogen excretion assuming that 2 g of nitrogen are excreted via extrarenal routes. Compliance with the study intervention was assessed by the measurement of 24-h renal isoflavone excretion in each urine sample of t_1–t_5. Completeness of urine collection was determined by the analyses of urinary creatinine excretion.

Biochemical analyses

Sex hormones

Estradiol (E_2), follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone binding globulin (SHBG) were analyzed by an Elisa autoanalyzer (Abbott, Wiesbaden, Germany). Progesterone (P_4) was analyzed using a ^{125}I-labelled RIA test kit (Biochem Immunosystems, Dissen, Germany). All coefficients of variation (CVs) were < 8 %.

Parameters of mineral and bone metabolism

Serum and urine calcium and phosphate levels were analyzed using atomic absorption spectrometry (calcium) and a colorimetric test kit (phosphate; BioMerieux, Nuertingen, Germany). CVs were < 4 %. Serum intact osteocalcin (bone formation marker), serum C-Telopeptide levels (CTx; bone collagen resorption marker), the isoform 5b of the tartrate-resistent acid phosphatase (BoneTRAP; a bone resorption marker), and intact parathyroid hormone (PTH) were measured by means of Elisa assays using commercial test kits supplied by DRG (Osteocalcin and PTH, Marburg, Germany), Nordic Bioscience Diagnostics (CTx; Hamburg, Germany), and by medac Diagnostika (Bone TRAP; Wedel, Germany). 25-hydroxyvitamin D was measured using a ^{125}I-labelled RIA test kit (Diasorin, Stillwater, MN, USA). The CVs of all test kits were < 10 %. The ratio of serum CTx/osteocalcin was used as an index of the balance of bone turnover [23].

Lipid parameters

Triglycerides, total cholesterol, and HDL cholesterol were measured using commercially available photometric test kits (Roche Diagnostics, Mannheim, Germany). CVs of the test kits were < 4 %. LDL cholesterol levels were calculated using the Friedewald formula [24]: LDL cholesterol (mmol/L) = total cholesterol – HDL cholesterol – triglycerides/2.2. Interleukin 6 (IL-6) was measured by an ultra-sensitive Elisa test kit (R&D Systems, Minneapolis, USA). CV was < 10 %.

Parameters of study compliance

Soy and urine concentrations of the isoflavones genistein and daidzein were analyzed according to the method of Franke et al. [25] with minor modifications. Briefly, 1 g dry soy flour was extracted with acetonitrile/water (2 + 1; v/v). An aliquot of the supernatant was incubated with 2 mg β-glucosidase (12.4 units per mg; Sigma, St. Louis, USA) and 40 µl of a β-glucuronidase/arylsulfatase mixture (100.000 units β-glucuronidase per mL and 5000 units arylsulfatase; Sigma, St. Louis, USA) for 24 h at 37 °C. Additional concentration and purification of the isoflavones was achieved by an extraction procedure with 3 x 2 mL ethyl acetate. The combined organic phases were evaporated under nitrogen to dryness and redissolved in 150 µl mobile phase and 50 µl 0.2 M acetate puffer (pH 4.0). An aliquot of 20 µl was analyzed using HPLC and UV detection by absorbance at 260 nm. Isoflavones from 1 mL urine were purified using a solid phase extraction with C 18 columns. An aliquot was incubated for 24 h at 37 °C with 50 µL β-glucuronidase/arylsulfatase (100,000 units β-glucuronidase per ml and 5000 units arylsulfatase per ml; Sigma, St. Louis, USA), was subsequently extracted with ethyl acetate, and was also analyzed by the HPLC/UV detection method [25]. Total urinary nitrogen was determined in 24-h urine pools using highly sensitive chemiluminescence with an Antek automated nitrogen analyzer (Antek 7000V). CV was 2.8 %. Urinary creatinine was assessed by the Jaffé reaction.

Statistics

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS10/Chicago, IL, USA). Time effects during the placebo and soy period were evaluated using the Friedman test. In the case of significant differences between the sampling time points, Wilcoxon-Wilcox tests were used to further specify these differences [26]. The paired Wilcoxon test was used in order to compare energy and nutrient intakes of the study periods and the effect of soy or placebo supplementation at specific time points. P values < 0.05 were considered significant. Data are presented as mean ± SE.

Results

Basic data

Three out of the 17 subjects were excluded from the study because of anovulatory cycles (increase in serum P_4 values across the menstrual cycle < 1.5 ng/mL) during WP (n = 2) or SP (n = 1). Thus, only 14 subjects could be included in the final data analyses. Their dietary data are given in Table 1. There were no significant differences among the diets in energy or macronutrient consumption. However, soy supplementation was associated with a significantly higher intake of calcium, phosphorus, dietary fiber, and poly-unsaturated fatty acids. Renal nitrogen excretion was 8.00 ± 0.74 g and 8.76 ± 0.72 g per day during WP and SP, respectively. Thus, total daily nitrogen excretion (24-h renal nitrogen excretion plus 2 g extra-renal nitrogen losses) were approximately 4 % and 12 % higher than the estimated daily nitrogen intake of 9.60 ± 0.80 g during WP and 9.60 ± 0.64 g during SP (calculated as g of protein intake/6.25).

Detailed data on urine volume, creatinine and isoflavone excretion are given in Table 2. Urine and creatinine excretion were similar within the menstrual cycles and between study periods.

As expected, excretions of daidzein and genistein

Table 1 Energy and nutrient intake of 14 young women during daily intake of 56 g soy cookies or a soy-free control period

Mean ± SE	Control period	Soy period	Significance P
Energy (kcal/day)	1743±720	1752±113	NS
Carbohydrates (g/day)	211±11	212±14	NS
Fat (g/day)	64±5	65±6	NS
Saturated fatty acids (g/day)	29±2	31±3	NS
Mono-unsaturated fatty acids (g/day)	19±2	22±2	NS
Poly-unsaturated fatty acids (g/day)	13±1	16±1	< 0.05
Cholesterol (mg/day)	227±28	236±28	NS
Protein (g/day)	60±5	60±4	NS
Dietary fiber (g/day)	14.9±0.7	23.4±1.0	< 0.0001
Calcium (mg/day)	933±88	1168±77	< 0.025
Phosphorus (mg/day)	1096±70	1419±63	< 0.0001
Vitamin D (µg/day)	1.4±0.4	1.2±0.3	NS
Ascorbic acid (mg/day)	133±20	95±12	NS
Tocopherol equivalents (mg/day)	10.8±0.8	12.3±0.8	NS
Caroten (mg/day)	3.2±0.4	4.6±0.8	NS

Table 2 Renal fluid, creatinine, mineral, and isoflavone excretions across the menstrual cycle during daily intake of 56 g soy cookies or a soy-free control period in 14 young women; mean values ± SE on day 3 of the follicular phase (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), at midluteal phase (t_4), and on day 3 of the next follicular phase (t_5). Significant fluctuations across the menstrual cycle were observed during WP for calcium; values in the same row with different superscript letters are significantly different, P < 0.025

	t_1	t_2	t_3	t_4	t_5
Fluid volume (ml/day)					
Control period	1534±224	1399±261	1585±266	2043±336	1517±204
Soy period	1503±164	1535±210	1706±267	1723±238	1553±284
Creatinine (mmo/day)					
Control period	8.3±0.8	8.6±0.8	8.4±0.7	9.3±0.7	8.0±0.8
Soy period	8.1±0.9	8.8±1.0	9.1±0.7	9.4±0.8	8.7±0.8
Genistein (mmol/day)					
Control period	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Soy period	3.44±0.51	2.79±0.46	2.53±0.39	3.33±0.50	3.62±0.63
Daidzein (mg/day)					
Control period	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Soy period	6.56±1.05	5.23±0.70	4.82±0.74	6.63±0.72	7.29±1.10
Calcium (mg/day)					
Control period	3.15±0.50[b]	3.20±0.59	3.65±0.48	4.02±0.64[a]	3.06±0.58[b]
Soy period	2.62±0.49	2.70±0.45	3.86±0.66	3.87±0.78	3.45±0.66
Phosphorus (mmol/day)					
Control period	21.0±1.9	19.7±1.9	18.7±2.2	20.5±3.1	19.7±2.0
Soy period	17.7±2.4	19.1±2.9	21.3±1.9	22.0±2.0	22.6±2.2

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were markedly higher during SP compared to WP. Renal excretion of daidzein during soy supplementation varied between 25.3 % and 38.3 % (mean: 32.1 %) of daily daidzein intake. Renal genistein excretion during soy supplementation varied between 7.7 % and 11 % (mean: 9.5 %) of daily genistein intake.

Body mass did not differ within each study period or between t_1 of SP and WP or t_5 of SP and WP ($P > 0.05$; data not shown).

Sex hormones

During both menstrual cycles circulating sex hormones showed the typical physiologic fluctuations (Table 3). In detail, a cyclic pattern was observed for serum E_2, FSH, LH, and P_4 levels. Serum SHBG levels remained constant between examinations t_1 and t_5 of both periods. At t_2, serum levels of SHBG, FSH, E_2, P_4, and LH were 10 %, 33 %, 35 %, 71 %, and 73 % higher during WP compared to SP. However, due to the large standard error, the level of statistical significance was only detected for P_4 ($P < 0.05$). The mean duration of the menstrual cycle was 30.4 ± 2.6 days during WP and 30.4 ± 3.5 days during SP and did not significantly differ between study periods ($P > 0.05$).

Bone markers

The bone collagen resorption marker CTx showed significant variations during WP with decreased levels at t_4 (Table 4). Serum levels of intact osteocalcin did not change significantly across the menstrual cycle of WP. However, the CTx/osteocalcin ratio showed a nadir at t_3 and t_4 during WP. During SP such a physiologic fluctuation in serum CTx levels and in the CTx/osteocalcin ratio was not observed (Fig. 1). Moreover, the CTx/osteocalcin ratio was significantly higher at t_4 of SP compared to WP. Serum levels of bone TRAP showed no significant variations, neither during WP nor during SP. However, bone TRAP levels were significantly higher at t_5 of SP compared to t_5 of WP (Fig. 2). Renal calcium excretion showed significant variations during WP with the high-

Table 3 Serum levels of sex hormones and lipid parameters across the menstrual cycle during daily intake of 56 g soy cookies or a soy-free control period in 14 young women; mean values ± SE on day 3 of the follicular phase (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), at midluteal phase (t_4), and on day 3 of the next follicular phase (t_5). Significant fluctuations across the menstrual cycle were observed during SP and WP for estradiol ($P < 0.001$), FSH ($P < 0.01$), LH ($P < 0.01$), progesterone ($P < 0.001$), and HDL cholesterol ($P < 0.05$); values in the same column with identical superscript letters are significantly different, $P < 0.05$; values in the same row with different superscript letters are significantly different, $P < 0.05$

	t_1	t_2	t_3	t_4	t_5
Estradiol (pg/mL)					
Control period	90±5	185±16	195±21	214±14	87±6
Soy period	100±6	249±41	181±21	224±27	98±10
FSH (mIE/mL)					
Control period	5.33±0.34	4.49±0.37	5.07±0.61	2.69±0.23	5.59±0.31
Soy period	5.23±0.38	5.96±0.88	3.87±0.33	2.74±0.29	5.11±0.50
LH (mIE/mL)					
Control period	4.49±0.44	7.56±1.85	12.27±3.59	4.79±0.62	4.33±0.39
Soy period	4.58±0.49	13.07±3.56	7.09±1.01	6.01±1.35	4.12±0.59
Progesteron (ng/mL)					
Control period	0.38±0.03	0.34±0.04[a]	6.86±1.52	13.79±1.29	0.41±0.04
Soy period	0.78±0.39	0.58±0.12[a]	8.16±2.13	11.96±2.51	2.00±0.93
SHBG (nmol/L)					
Control period	48.8±3.6	47.2±4.7	50.2±4.7	50.4±3.8	52.7±4.4
Soy period	52.7±5.2	52.0±5.8	56.1±5.3	57.5±6.1	54.0±4.7
Total cholesterol (mmol/L)					
Control period	4.06±0.17	4.17±0.19	4.07±0.20	4.12±0.21	4.03±0.17
Soy period	4.01±0.18	4.18±0.16	4.11±0.20	4.03±0.19	4.17±0.17
HDL cholesterol (mmol/L)					
Control period	1.37±0.05	1.39±0.05	1.42±0.06[a]	1.42±0.06[a]	1.32±0.07[b]
Soy period	1.31±0.06[a]	1.42±0.07[b]	1.40±0.09	1.33±0.07	1.35±0.09
LDL cholesterol (mmol/L)					
Control period	2.31±0.15	2.35±0.16	2.25±0.17	2.31±0.18	2.30±0.15
Soy period	2.29±0.14	2.35±0.15	2.30±0.17	2.34±0.16	2.43±0.15
Triglycerides (mmol/L)					
Control period	0.838±0.056	0.951±0.123	0.913±0.630	0.862±0.064	0.922±0.109
Soy period	0.904±0.067	0.878±0.058	0.912±0.638	0.826±0.066	0.836±0.060
Interleukin-6 (pg/mL)					
Control period	2.40±0.43	1.89±0.21	2.15±0.23	1.99±0.33	2.01±0.19
Soy period	2.01±0.31	2.34±0.34	2.23±0.39	2.46±0.45	2.30±0.49

Table 4 Serum parameters of calcium and bone metabolism across the menstrual cycle during daily intake of 56 g soy cookies or a soy-free control period in 14 young women; mean values ± SE on day 3 of the follicular phase (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), at midluteal phase (t_4), and on day 3 of the next follicular phase (t_5). Significant fluctuations across the menstrual cycle were observed during SP for C-telopeptide ($P < 0.05$); values in the same row with different superscript letters are significantly different, $P < 0.05$

	t_1	t_2	t_3	t_4	t_5
25OHD (nmol/L)					
Control period	50.7±9.6	56.6±13.7	44.9±6.1	56.2±11.6	50.4±10.8
Soy period	46.5±6.1	45.6±6.0	47.7±7.3	53.3±12.0	47.9±7.6
PTH (pg/mL)					
Control period	23.2±3.8	23.4±4.9	28.4±5.1	23.2±4.1	30.4±4.6
Soy period	25.8±4.8	28.1±4.3	25.6±5.0	26.0±4.3	25.9±4.7
Calcium (mmol/L)					
Control period	2.27±0.05	2.34±0.05	2.39±0.06	2.33±0.08	2.34±0.08
Soy period	2.30±0.06	2.41±0.06	2.49±0.06	2.45±0.07	2.33±0.08
Phosphorus (mmol/L)					
Control period	1.29±0.04	1.29±0.03	1.31±0.06	1.33±0.05	1.31±0.04
Soy period	1.29±0.04	1.32±0.06	1.35±0.03	1.37±0.03	1.31±0.04
Osteocalcin (ng/mL)					
Control period	16.9±1.8	18.8±2.8	17.4±2.1	17.9±2.3	16.2±1.7
Soy period	16.3±2.1	19.6±4.5	18.0±2.1	17.6±1.7	17.1±2.0
C-telopeptide (pg/mL)					
Control period	656±63[b]	697±103	583±69	551±60[a]	676±71
Soy period	658±83	712±113	693±103	630±68	667±79
C-telopeptide/osteocalcin ratio					
Control period	0.0396±0.0019[b]	0.0379±0.0031[b]	0.0339±0.0021[a]	0.0314±0.0017[a]	0.0423±0.0030[b]
Soy period	0.0442±0.0059	0.0393±0.0030	0.0385±0.0025	0.0358±0.0022[a]	0.0399±0.0022

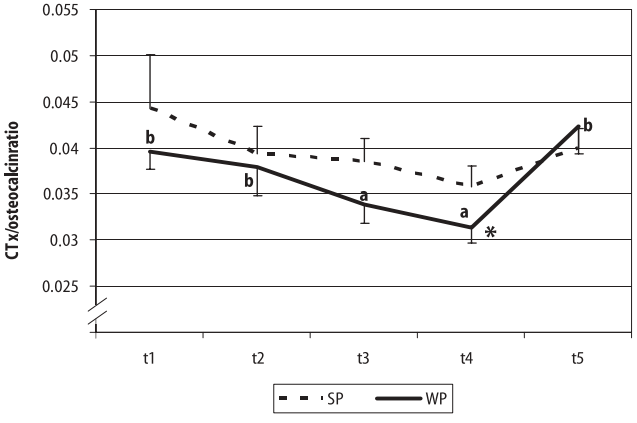

Fig. 1 C-Telopeptide/osteocalcin ratio across the menstrual cycle during daily intake of 56 g soy cookies containing 52 mg isoflavones and a soy-free control period in 14 young women; mean values ± SE on day 3 of the follicular phase (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), at midluteal phase (t_4), and on day 3 of the next follicular phase (t_5). Significant fluctuations across the menstrual cycle were observed during WP for estradiol ($P < 0.05$); values in the same row with different superscript letters are significantly different, $P < 0.05$; * $P < 0.05$ versus t_4 of the soy period

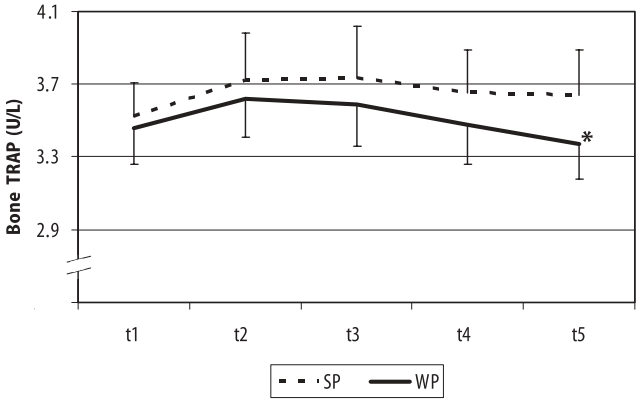

Fig. 2 Serum concentrations of bone TRAP across the menstrual cycle during daily intake of 56 g soy cookies containing 52 mg isoflavones and a soy-free control period in 14 young women; mean values ± SE on day 3 of the follicular phase (t_1), 3 days before ovulation (t_2), 3 days after ovulation (t_3), at midluteal phase (t_4), and on day 3 of the next follicular phase (t_5). No significant fluctuations across the menstrual cycle were observed during WP or SP ($P > 0.05$); * $P < 0.05$ versus t_5 of the soy period

est values at t_4 (Table 2). In contrast, renal calcium excretion did not differ across the menstrual cycle during SP. Moreover, calcium excretion did not significantly differ between specific time points of SP and WP. Serum calcium, phosphate, 25-hydroxyvitamin D, and PTH levels, and renal phosphorus excretion remained constant during SP and WP (Tables 2 and 3).

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Lipid parameters

Neither total cholesterol nor LDL cholesterol nor triglycerides differed within the study periods or between specific time points of study periods. In contrast, HDL cholesterol levels showed monthly fluctuations during both study periods with a peak at t_2 during the soy period and a peak at t_4 during placebo period. However, differences at these time points between study periods were too small to reach statistical significance. Serum IL-6 concentrations did not differ within study periods or between identical time points of the study periods.

Discussion

The present study demonstrates that soy intake significantly influenced bone turnover markers. The effects on circulating sex hormones and lipoprotein parameters were only modest and in most cases the level of statistical significance was not achieved.

Normally, isoflavone intake is 1–2 mg/day in omnivores and approximately 7 mg/day in vegetarians in Europe [27]. The ingested amount of 52 mg soy isoflavones per day is comparable to those amounts which have been used in some earlier studies in order to investigate possible estrogenic effects of isoflavones in young female adults [3, 28, 29] but some others have also used amounts of approximately 100 mg/day [18, 20, 30]. In our study, the percentage of orally ingested genistein and daidzein during SP that was excreted in the urine (9.5 % and 32.1 %) was in the same range as shown by other groups [31, 32]. Moreover, data of 24-h renal creatinine excretion and the relation of 24-h renal nitrogen excretion to the documented nitrogen intake indicate that compliance with the study protocol was achieved.

Soy is a rich source of several essential and non-essential bioactive substances. Theoretically, not only the markedly higher dietary intake of isoflavones but also the higher intake of the bone minerals calcium and phosphorus during SP compared to WP could have led to the physiologic alterations in bone turnover. It should be mentioned, however, that the phosphorus content of soy mainly derives from phytate, which is insoluble and not absorbed from the intestine. Moreover, phytate can bind significant amounts of calcium ions [33]. Since 24-h urinary calcium and phosphate excretion did not differ between WP and SP, it can be assumed that the amount of absorbed calcium and phosphate did not generally differ between study periods.

Sex hormones

With the exception of a higher P_4 level at t_2 we could not find an effect of soy supplementation on circulating concentrations of female sex hormones. We cannot definitively rule out that the statistical power may not have been sufficient to detect small alterations. However, earlier studies also indicate that there is no clear effect of soy supplementation on sex hormone levels across the menstrual cycle [7]. Circulating sex hormone levels do not necessarily reflect their tissue availability. Therefore, others have performed endometrial biopsies in the luteal phase. They found no effect of 64 mg or 129 mg isoflavone intake daily on histological data [7]. Thus, an effect of isoflavones on the reproductive system seems to be rather unlikely. Our data of a similar cycle length during SP and WP support this assumption.

Bone markers

We could confirm earlier results of our study group of physiologic fluctuations in markers of bone collagen resorption across the menstrual cycle during WP [34]. Changes were paralleled by a significantly increased 24-h renal calcium excretion at t_4. Since it is known that estrogens can enhance intestinal calcium absorption [35], these data may reflect an improved calcium supply leading to decreased bone resorption processes. These variations in calcium and bone metabolism were however blunted by soy supplementation. Our data are in general agreement with an earlier investigation demonstrating that daily consumption of 64 mg and 129 mg of soy isoflavones increased the bone collagen resorption marker deoxypyridinoline during specific time points of the menstrual cycle [18]. In our study, the soy-induced changes of the CTx/osteocalcin ratio occurred during the luteal phase when endogenous estrogen levels are high. This was in contrast to the increase of the bone resorption marker bone TRAP at t_5 (low E_2 levels) of SP compared to t_5 of WP (Fig. 2). However, it may well be that bone TRAP responds less quickly to physiologic stimuli compared to CTx and osteocalcin. This assumption is confirmed by the finding that bone TRAP does not show a circadian rhythm [36], while there is a diurnal variation in serum CTx and osteocalcin levels [37, 38]. Moreover, the unchanged CTx/osteocalcin ratio at t_5 of SP compared to t_5 of WP (Fig. 1) indicates that the effect of soy intake on bone turnover during the luteal phase is a transient phenomenon.

The mechanism as to how soy may lead to an uncoupling of bone resorption and formation processes in young females is still obscure. Moreover, data do not confirm results of postmenopausal women where administration of 54 mg genistein decreased concentrations of the bone collagen resorption marker deoxypyridinoline and increased serum osteocalcin levels compared to baseline levels without genistein administration [17]. Obviously, genistein can exert beneficial effects on bone turnover markers when E_2 levels are low,

but can adversely affect the ratio of bone collagen resorption to bone formation when E_2 levels are high (Fig. 1). Epidemiological data on phytoestrogen intake and bone mineral densities in Japanese women support the assumption that bone turnover is influenced differently during low and high E_2 levels: a positive association was found between phytoestrogen intake and spinal bone mineral density in postmenopausal but not in premenopausal women [39]. The clinical relevance of the soy-induced alterations we observed in bone turnover markers during specific time points of the menstrual cycle (Figs. 1 and 2) is unclear at present. However, those earlier epidemiological data indicate that the relatively moderate effects of isoflavone-containing soy on bone metabolism are probably too weak to adversely influence premenopausal bone health in the long run.

Lipid parameters

During both study periods, there was a significant variation in HDL cholesterol levels. Data are in line with earlier results from healthy premenopausal women [20, 40, 41]. Moreover, our results support these earlier data of a HDL cholesterol peak around ovulation. These fluctuations are probably mediated by the cyclic variations in circulating E_2 and P_4 concentrations [41]. Higher intakes of isoflavones, dietary fiber and poly-unsaturated fatty acids during SP compared to WP had no effect on total serum cholesterol, LDL cholesterol, and triglyceride levels (Table 3). The lipid-lowering effect of isoflavone-containing soy proteins obviously depends on the initial serum cholesterol level [21], the amount of isoflavone consumption [20] and on additional components contained in the soy protein fraction [16]. Our results of an unchanged lipoprotein profile during daily intake of 52 mg isoflavones compared to a control period is in line with a recent investigation demonstrating that the consumption of 64 mg isoflavones daily has no effect on the blood lipid profile across the menstrual cycle [22].

Evidence is increasing that dyslipoproteinemia such as low HDL cholesterol and high triglyceride levels are the result of a low-grade systemic inflammation [42]. The cytokine IL-6 has pro-inflammatory properties and promotes atherosclerosis [43]. Although in vitro studies have demonstrated that genistein is able to suppress IL-6 levels [44], our data indicate that daily supplementation with 33 mg genistein does not influence serum IL-6 levels in young females with relatively low IL-6 and triglyceride levels, and relatively high HDL cholesterol levels (Table 3). Thus, the comparable IL-6 levels and the similar lipoprotein profiles during WP and SP fit well together.

In summary, short-term supplementation with high-dose isoflavone-containing soy products has only modest effects on estrogen-related physiological pathways in young healthy women. Nevertheless, the soy-induced moderate uncoupling of bone resorption and formation processes deserves further investigation.

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