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Estrogen levels in nipple aspirate fluid and serum during a randomized soy trial

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Abstract

Background—Based on the hypothesized protective effect, we examined the effect of soy foods on estrogens in nipple aspirate fluid (NAF) and serum, possible indicators of breast cancer risk.

Methods—In a cross-over design, we randomized 96 women who produced ≥10 μL NAF to a high- or low-soy diet for 6-months. During the high-soy diet, participants consumed 2 soy servings of soy milk, tofu, or soy nuts (approximately 50 mg of isoflavones/day); during the low-soy diet, they maintained their usual diet. Six NAF samples were obtained using a FirstCyte© Aspirator. Estradiol (E_2) and estrone sulfate (E_1S) were assessed in NAF and estrone (E_1) in serum only using highly sensitive radioimmunoassays. Mixed-effects regression models accounting for repeated measures and left-censoring limits were applied.

Results—Mean E_2 and E_1S were lower during the high-soy than the low-soy diet (113 vs. 313 pg/mL and 46 vs. 68 ng/mL, respectively) without reaching significance (p=0.07); the interaction between group and diet and was not significant. There was no effect of the soy treatment on serum E_2 (p=0.76), E_1 (p=0.86), or E_1S (p=0.56). Within individuals, NAF and serum levels of E_2 (r_s=0.37; p<0.001) but not E_1S (r_s=0.004; p=0.97) were correlated. E_2 and E_1S in NAF and serum were strongly associated (r_s=0.78 and r_s=0.48; p<0.001).

Conclusions—Soy foods in amounts consumed by Asians did not significantly modify estrogen levels in NAF and serum.

Impact—The trend towards lower estrogens in NAF during the high-soy diet counters concerns about adverse effects of soy foods on breast cancer risk.

Keywords

Soy foods; isoflavones; breast fluid; serum; breast cancer risk; diet and nutrition

Introduction

Regular soy consumption has been associated with reduced breast cancer risk (1); however, its protective mechanism, if it exists, is not fully understood. One postulated mechanism involves the estrogenic potential of soy isoflavones although a recent meta-analysis suggested no effect of soy or isoflavones on circulating sex hormones in premenopausal and

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Disclosure of Potential Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

postmenopausal women (2). These findings, however, have not invalidated the entire hormonal hypothesis for soy and isoflavones because estrogen levels in breast tissues appear to be quite different from circulating estrogen levels measured in serum (3–7). It has been shown repeatedly that mammary tissue contains enzymes for local estrone (E_1) and estradiol (E_2) production via the aromatase and sulfatase pathways converting androgenic (androstenedione and testosterone) and estrogenic [estrone sulfate (E_1S) and estradiol sulfate] precursors, respectively (7–9). Thus, the possible effects of bioactive steroids within the breast are likely not fully captured by measuring circulating hormone levels (10, 11) while soy or isoflavones may act directly in the breast by modifying local estrogen levels or breast cell growth. Measurements in nipple aspirate fluid (NAF) may be a more appropriate indicator to detect changes in the breast associated with soy intake. The NAF method offers a non-invasive approach to assess information on cellular and non-cellular markers of breast cancer risk. The fluid is in constant contact with the ductal epithelium, the site of development of most breast cancer. In the current analysis, we examine the effects of 2 daily servings of soy foods on E_2 and E_1S levels in NAF and corresponding serum levels among premenopausal women who participated in a 6-month trial.

Materials and Methods

Study design and procedures

We conducted a randomized, crossover soy intervention study with two 6-month diet periods (high-soy and low-soy) separated by a 1-month washout period. Study methods have been described in detail previously (12). Briefly, we excluded women who did not have a uterus, ovaries, or regular menstrual periods, consumed >5 soy servings per week, had breast implants, used estrogen-containing oral contraceptives, were pregnant or breast-feeding, had been diagnosed with cancer, and did not produce at least 10 μL of NAF (64% of 310 women screened). At the initial screening visit, the women completed demographic and soy food frequency questionnaires, weight and height measurements, a 24-hour dietary recall, and a NAF collection. After screening, 96 participants were randomized to begin either the 6-month high-soy (Group A) or low-soy (Group B) diet and crossed over to the other diet after 1-month washout. Subsequently, 14 women (15%) dropped out; the remaining 82 participants completed 5 consecutive visits during which NAF collection was attempted: at midway (months 3 and 11) and at the end (months 6 and 13) of each diet period and after the washout period (month 7).

The goal during the high-soy diet period was to add 2 daily servings of soy foods to the regular diet by replacing similar food items; one serving was defined as ¾ cup of soy milk, ½ cup of tofu, or ¼ cup of soy nuts and provided approximately 25 mg of isoflavones per serving. During the low-soy diet period, participants were instructed to maintain their usual diet and limit consuming soy-containing products to <3 servings per week. Compliance as assessed by seven 24-hour dietary recalls and 8 urinary isoflavonoid measurements (daidzein, genistein, equol, and O-desmethylangolensin) measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) was excellent and constant over time (12). Because the potential benefits of soy may partly depend on the ability of intestinal bacteria to produce equol (13), a metabolite of the isoflavone daidzein, we classified participants into those who ever produced equol (N=43) and those who did not (N=39) using a relative equol:dadzein ratio cutoff of 0.018 (14) with a dadzein threshold exceeding 2 nmol per mg creatinine as evidence for soy intake (15). The Committee on Human Subjects at the University of Hawaii and the participating clinics approved the study protocol. All participants signed an informed consent form. The trial was registered under NCT00513916 (16).

Sample collection

NAF sample collection was attempted at all study visits planned to occur during the midluteal phase (3–11 days before the next menstruation) based on previous menstruation dates. In a follow-up phone call, the actual date of the next menstruation after the visit was recorded to calculate the number of days between NAF collection and the next menstruation. Subsequently, collections were grouped by menstrual cycle phase counting backwards from the next menstruation: follicular phase (28–17 days, n=28), midcycle (16–12 days, n=51), midluteal (n=181), late-luteal (0–2 days, n=23), and >28 days (n=28).

For NAF collection, a trained staff member demonstrated the collection technique using a FirstCyte© Aspirator, a device similar to a manual breast pump consisting of a 10 or 20 cc syringe attached to a small suction cup (17). After cleansing, warming, and massaging, the subject compressed the breast with both hands while the coordinator applied negative pressure to the cup over the nipple by withdrawing the plunger of the syringe halfway until fluid appeared at the nipple surface. After collecting all droplets, the woman expressed additional fluid through massage and pressure behind the nipple area. A maximum of three attempts per breast were made. The NAF was collected with microcapillary tubes (10, 20, and 50 μL), and the total amount was recorded. The first 20 μL of NAF were pooled in phosphate-buffered saline (PBS) in a dilution of 1:11, well mixed, aliquoted, and stored at −80°C. The next 5–20 μL were used to preserve breast cells stored for cytologic analysis. If more NAF was obtained, it was diluted in PBS again. For the 15% of women who were able to produce >90 μL NAF, the collection was usually terminated at 120 μL.

Three blood samples were collected on the same day as NAF, one at baseline and one at the end of each diet period. After allowing the serum to clot for 30 minutes and centrifuging at 3000 rpm for 15 minutes, it was aliquoted into 1 ml cryovials and frozen at −80°C.

Estrogen Assays in NAF and Serum

For each subject, four diluted NAF specimen equivalent to 4 × 10 μL NAF (baseline, month 3 or 6, month 7, and month 10 or 13) and three 1 mL serum samples (baseline, month 6, and month 13) were sent to the Reproductive Endocrine Research Laboratory at the University of Southern California. Using radioimmunoassays (RIA), E_2, E_1, and E_1S were measured in 0.5 mL serum, but, due to the small volume, NAF samples were analyzed for E_2 and E_1S only (18).

For the E_2 assay, aliquots (0.1 ml) of previously diluted (1:11) NAF samples were transferred to RIA tubes (12×75 mm tubes). Similarly, aliquots of quality control (QC) serum samples were transferred to RIA tubes for monitoring the reliability of the E_2 RIA. In order to monitor procedural losses, approximately 200 c.p.m. of ^3H-E_2 were added to the QC samples and to 3 counting vials used to determine the total amount of ^3H-E_2 added. E_2 was then extracted by adding 2.5 mL of ethyl acetate: hexane (3:2) to each tube and vortexing the tubes for 1 min. The organic layer in each tube was transferred to a second set of RIA tubes and the solvents were evaporated under nitrogen at 37°C. The extraction procedure was repeated one more time. The residue was then redissolved in 0.2 ml assay buffer (0.1 M phosphate buffer, pH 7.4). An iodinated E_2 derivative and rabbit anti-E_2 serum were added to all sample and QC tubes, as well as duplicate tubes containing various concentrations of E_2 used for generating the standard curve, the contents were then mixed by brief vortexing. After an overnight incubation period, a second antibody (goat anti-rabbit) was added to all RIA tubes. Following centrifugation, the supernatant was removed and discarded, and the remaining pellet was counted; the counts were used to calculate the E_2 concentration in each NAF sample. The sensitivity of the E_2 RIA is 2 pg/mL. An aliquot from each of the 3 QC tubes was taken for counting, and based on the amount of radioactivity in these tubes and the total ^{3}H-E_2 counts added, the procedural loss was calculated and used to correct the E_2 values obtained by RIA. The QC results in serum based on standards indicated coefficient of variations (CV) of 0.10–0.18 for E_1 and E_2 and 0.16–0.17 for NAF, whereas CVs for 14 blinded serum and 16 blinded NAF samples were 0.15 and 0.20

For the E_1S assay, the water phase left from the NAF E_2 assay after ethyl acetate:hexane extraction was evaporated under nitrogen, and the residue was redissolved in 0.1 mL assay buffer to measure E_1S by direct RIA with a commercial kit (Beckman Coulter, Brea, CA) that provides reagents that generate an assay which measures E_1S with high accuracy in serum. Values obtained with this assay are similar to corresponding values obtained by GC-MS/MS. In the RIA, the iodinated E_1S radioligand and antibody against E_1S were added to the RIA tubes containing the redissolved extracts, as well as to tubes containing different concentrations of E_1S standards and QC samples. After an incubation period of 3 hours at room temperature, a second antibody was added. Following a 15-minute incubation period, the tubes were centrifuged, the supernatant discarded, the pellets counted, and the E_1S values were determined. The E_1S RIA sensitivity is 0.01 ng/mL. The CVs for the standards were 0.07 in serum and 0.06–0.08 in NAF, whereas the respective CVs for 14 blinded serum and 16 blinded NAF samples were 0.07 and 0.21.

Statistical Analysis

The statistical analysis was performed using the SAS software package version 9.2. (SAS Institute, Inc., Cary, NC). To assess differences in baseline characteristics between the two randomization groups, Student's t tests were performed for continuous variables and $\chi 2$ tests for categorical variables; an α level of 0.05 was considered significant. Due to non-normal distributions, log-transformed values were used for serum estrogens and Wilcoxon rank-sum tests for NAF estrogens. Within-subject correlations between NAF and serum estrogen concentrations at baseline were assessed using the Spearman's rank correlation coefficient. Mixed-effects regression models as described below examined the association across the entire study period and adjusted for menstrual phase, group, and dietary treatment.

Log transformed values of serum estrogen levels were used in mixed-effects regression (PROC MIXED) models incorporating a random intercept term to examine the effect of soy. For estrogen levels in NAF, mixed-effects regression models accounting for repeated measures and left-censoring limits as described by Thiébaut and Jacqmin-Gadda (19) were implemented using the SAS procedure PROC NLMIXED. Log-transformed values for NAF E_2 and NAF E_1S were modeled by a normal distribution that incorporated the correlation from repeated measures using a random-intercept term and the left censoring of values below the detection level for NAF E_2 and E_1S, respectively. The model included variables indicating group membership and the diet sequence to test for differences in randomization and for a possible carry-over effect. Additional models included an interaction term between diet and group as well as age and menstrual phase at time of NAF collection to examine the possible influence of imbalance between randomized groups. Furthermore, we excluded women with Asian ancestry, nulliparous women, and women who did not adhere to the study protocol (<40 mg of isoflavones per day during the high-soy diet and >10 mg/day during the low-soy diet based on the 24-hour recalls).

Results

Of the 82 women who completed the study, 40 were in Group A and 42 were in Group B. At baseline, the two randomization groups did not differ by ethnicity, BMI, reproductive characteristics, drop-out rate, and NAF concentrations of E_2 and E_1S (Table 1). However, women in group A were 4 years older than Group B ($p<0.01$), excreted more isoflavonoids in urine (*p*=0.03), and had lower concentrations of serum E_2 (*p*=0.02), E_1 (*p*=0.03), and E_1S (*p*=0.02) before treatment started. Due to scheduling problems, only 58% of the NAF samples were collected during the midluteal phase. This proportion was non-significantly lower for group A than group B (53% vs. 62%; *p*=0.39). After adjustment for menstrual phase, the significant differences in serum estrogen levels persisted.

E_2 and E_1S levels were below the minimum detection limit in 116 (37%) and 82 (26%) NAF samples, respectively; of these, 71 samples had both non-detectable E_2 and E_1S levels. The proportion of non-detectable values were similar by study period, but higher during the follicular phase and for women with cycles >28 days than for midcycle and luteal samples. The 45 women with at least one non-detectable E_2 were more likely to be of Asian or Other ethnicity (*p*=0.07), reported a higher isoflavone intake before the start of the trial (*p*=0.09), were younger by 2.7 years (*p*=0.05), and had a lower BMI by 2.1 m/kg^2 (*p*=0.10) than the 37 participants with 4 detectable values.

Based on baseline samples, the within-woman correlation between NAF and serum was modest for E_2 (r_s=0.37; *p*<0.001) and non-existent for E_1S (r_s=0.004; *p*=0.97) (Figure 1A and B). The stronger association between NAF and serum for E_2 was confirmed in models incorporating all samples and adjusted for menstrual cycle and group and diet effects (β=0.94, *p*<0.001 for E_2 and β=0.25, *p*=0.60 for E_1S). E_2 and E_1S levels were highly correlated within individuals (0.78 for NAF and 0.48 for serum; *p*<0.001 for both) (Figure 1C and D). Whereas the mean E_2 concentration was lower in NAF than in serum (128 vs. 160 pg/mL, *p*=0.01), E_1S was 30-fold higher in NAF than in serum (60.6 vs. 2.2 ng/mL, *p*<0.001). Estrogen concentrations varied across the menstrual cycle for both NAF and serum (Figure 2). Significantly higher values at the midcycle and midluteal phase compared to the follicular phase were observed for NAF E_2, serum E_2, and serum E_1S (*p*<0.05 for all in models adjusted for group and diet effects).

For estrogen concentrations in serum, no effect of the high-soy diet on serum estrogen concentrations was detected in mixed-effect regression models (Table 2). For E_2 and E_1S in NAF, mean values were lowest during the high-soy diet; however, this difference did not reach statistical significance. The respective parameter estimates in the mixed-effect regression models were −0.63 pg/mL (*p*=0.07) and −0.58 ng/mL (*p*=0.07). The effect for group in the NAF E_1S but not in the E_2 model was significant (*p*=0.01 vs. 0.07), whereas diet sequence was not significant in either model. An interaction term between diet and group was not significant for both analytes (*p*=0.58 and *p*=0.11). Similar results for the intervention effect on NAF levels were obtained when we adjusted for age at screening or menstrual phase to control for the imbalance in randomization; the parameter estimates and p-values changed minimally. The exclusion of Asians or nulliparous women made no substantial difference, but the restriction to adherent women strengthened the association for E_1S (*p*=0.03) but not E_2. After stratification by equol producer status, the dietary treatment was not significant in either group. The respective parameter estimates for the 43 producers and 39 non-producers were −1.46 and −0.24 pg/mL for E_2 and −0.91 and −0.41 ng/mL for E_1S.

Discussion

The current analysis among premenopausal women with various ethnic backgrounds found no significant effect of a 6-month soy diet on NAF or serum concentrations of E_2 and E_1S although there was a modest decline of estrogens during the high-soy diet that did not reach statistical significance. Equol producer status did not modify the intervention effect. E_2 and E_1S levels were strongly correlated in both NAF and serum, but their levels in NAF and serum showed only a weak relation. When the observations were grouped by menstrual cycle phase, serum E_2 and E_1S and NAF E_2 showed similar patterns throughout the cycle, whereas varying patterns were observed for E_1S with NAF levels being much higher than serum levels. These findings suggest that, although no significant influence of soy intake on NAF E_2 and E_1S was detected, estrogens in NAF provide different information than serum levels and additional clues to understanding local estrogen synthesis and its possible effect on breast tissue activity.

The lack of an effect on serum estrogens agrees with our previous report (20) and with a meta-analysis (2) based on 11 studies in premenopausal women including our own results (20) and did not detect any effect of soy and isoflavones on E_2 and E_1. Given the difficulties of obtaining breast tissue, only 3 studies so far have investigated the effects of soy on breast cells in humans (21–23). The only previous soy intervention that collected NAF did not assess estrogen levels in NAF but noted a moderate increase in volume, gross cystic disease fluid protein concentration and hyperplastic epithelial cells (21). Two studies among women scheduled to receive a breast biopsy indicated an increase in progesterone expression and proliferation rate of breast lobular epithelium (22) and a decrease of apolipoprotein D levels and an increase of pS2 levels in breast fluid (23).

In comparison to previous reports of estrogens in NAF and serum, the levels in the current study are considerably lower than in early studies that reported E_2 levels of 800 pg/mL and higher (3–6). However, more recent publications presented levels between 100–400 pg/mL (7, 24, 25), which are in the range of our findings. Improved methodology, in particular extraction and higher antibody-specificity may explain the trend towards lower levels. The exception is one report with NAF E_2 levels of 800–2,000 pg/mL in 40 premenopausal women (10), which were described as unusually high (Dr. Robert Chatterton, personal communication). Given the wide range in published NAF E_2 levels, disagreement persists whether or not NAF levels are equal or higher than serum levels. Whereas serum and NAF E_2 levels were measured in the same range in the current study and in one other report from premenopausal women (24), 5–30 fold differences have also been reported (5–7, 10). Over time, NAF E_2 levels appear to be relatively stable; the correlation between two measurements 15 months apart was reported as 0.65 (26). The poor correlation between estrogen levels in NAF and serum observed in our study agrees with others (5, 6, 10, 24, 25); significant associations have only been reported for progesterone (10, 25). The high E_1S levels in NAF as compared to serum and the strong association between E_2 and E_1S concur with previous reports (10, 25) supporting the idea that E_1S in breast tissue provides a reservoir for E_2 production via the sulfatase pathway (7–9). Our findings in relation to the menstrual cycle are in contrast to Chatterton et al. (10); we observed higher NAF E_2 concentrations during the midcycle and midluteal phase than during the follicular phase corresponding to serum level changes during the menstrual cycle.

Limitations of the current study include the large number of potential participants who were ineligible because of insufficient NAF production, the fact that randomization did not lead to completely balanced groups (12), the small amounts of NAF fluid collected limiting the number of analytes, the relatively large proportion of non-detectable estrogen values in NAF, and the difficulty to collect samples from premenopausal women at the same time within the menstrual cycle. We cannot exclude the possibility that imbalance in the randomized groups biased the results although there was no significant interaction between diet and group and adjustment for age and menstrual phase (Table 1) did not explain the differences in serum estrogens or the non-significantly lower NAF estrogens after the high-soy diet (Table 2). It is also difficult to explain why E_2 levels in NAF were higher at the end of the low-soy diet than at baseline; a similar trend was apparent for serum levels. One possibility is that the small amounts of soy in the regular diet of participants had a similar effect as the high-soy intervention. From experimental research it is known that enzymes involved in steroidogenesis may be affected by isoflavones and influence the balance of E_1 and E_2 (27).

Despite these weaknesses, this study had considerable strengths. In comparison to the literature, its sample size was quite large; NAF was obtained repeatedly for 82 women from different ethnic backgrounds. The adherence to the high-soy diet was excellent and the dropout rate was low (12). The estrogen assay used a purification step, i.e., organic solvent extraction prior to the RIA, a method that has been shown to be important for accurate results (28). Because NAF is in constant contact with the ductal epithelium, the site of development of most breast cancer (29), it provides non-invasive insight into breast tissue activity and is more likely to reflect actual estrogen levels in the breast than those measured in serum (10, 11). In particular, the high E_1S concentration in NAF as compared to serum points to local estrogen production.

At this time, it is not known whether estrogen levels in NAF are related to breast cancer, but there is evidence that women who produce NAF, in particular those with cellular NAF, are at higher risk (30, 31). When NAF production was added to the Gail model, the relative risk for women with NAF containing normal cells was 1.5, and the population attributable risk fraction for NAF production was 18% (32). Although this trial detected no significant effect of 6-month daily soy consumption on NAF estrogen levels, the trend points towards lower levels during the high-soy diet, an observation that counters concerns about adverse effects of soy foods and isoflavones on breast cancer risk (33). In addition to estrogen levels, a future analysis will examine breast epithelial cells in those participants who provided sufficient NAF.

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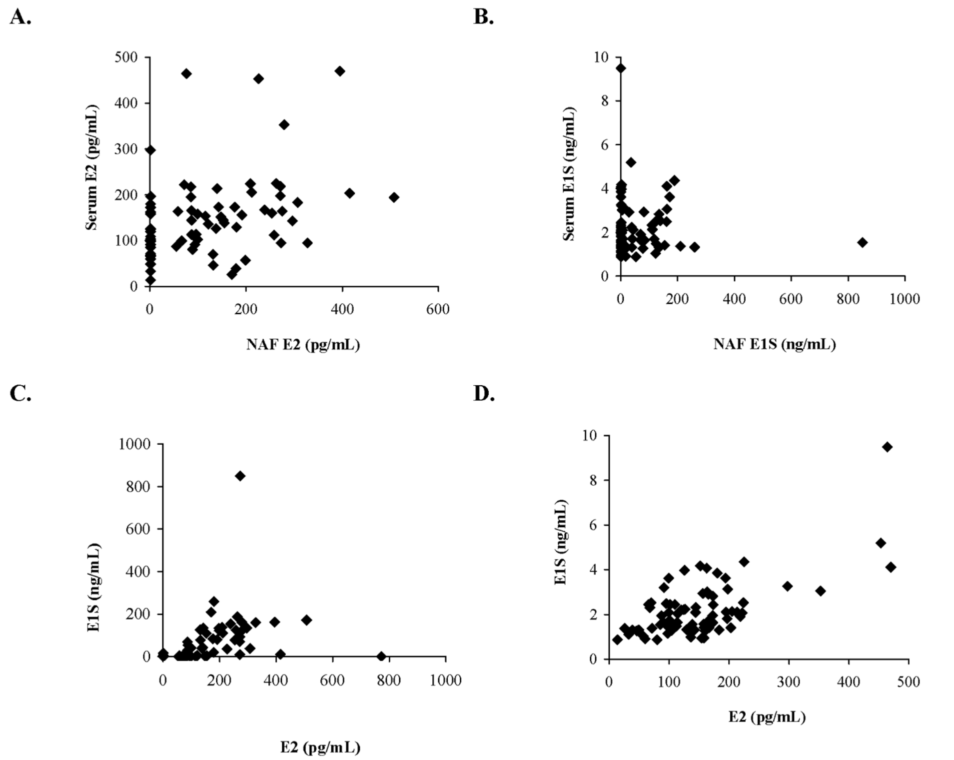

Figure 1.

Within-subject Spearman correlations between baseline serum and NAF concentrations of **[A]** estradiol (E_2) (r_s=0.37; p<0.001); **[B]** estrone sulfate (E_1S) (r_s=0.004; p=0.97); **[C]** between NAF E_2 and E_1S (r_s=0.78; p<0.001); and **[D]** serum E_2 and E_1S (r_s=0.48; p<0.001).

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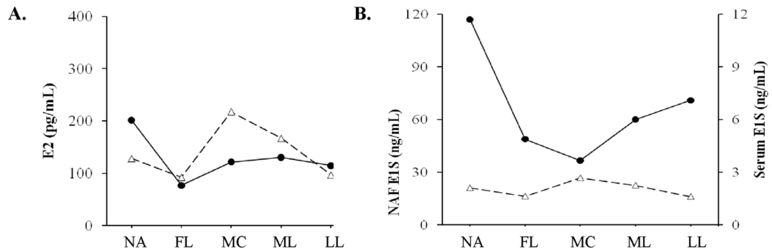

Figure 2.

Mean serum (△) and NAF (●) concentrations of estradiol (E_2) and estrone sulfate (E_1S) from subjects retrospectively grouped by phase of the menstrual cycle. Menopausal phases include >28 days (NA), follicular (FL), midcycle (MC), midluteal (ML), and late-luteal (LL). MC and ML are significantly different from FL adjusting for group and diet effects (p<0.05).

Table 1

Characteristics of the study participants by randomization group at baseline[1]

Characteristic	All	Group A (high-soy to low-soy)	Group B (low-soy to high-soy)	*p* value[1]
N	82	40	42	
Race/Ethnicity				
White	42 (51%)	20 (50%)	22 (52%)	0.98
Asian	22 (27%)	11 (23%)	11 (22%)	
Other	18 (22%)	9 (27%)	9 (26%)	
Age at screening, *y*	39.2±6.1	41.3±5.6	37.3±6.0	<0.01
Body mass index, kg/m^2	25.8±5.6	25.8±5.2	25.9±6.0	0.91
Age at menarche, *y*	12.4±1.4	12.4±1.2	12.4±1.5	0.99
Number of children	1.5±1.3	1.6±1.1	1.4±1.5	0.40
Age at first live birth[2], *y*	27.8±6.7	27.7±7.1	28.0±6.4	0.87
Equol producer status[3]	43 (52%)	23 (58%)	20 (48%)	0.37
Isoflavone intake, *mg/d*	21.2±39.7	16.3±38.8	25.8±40.5	0.28
Urinary isoflavones, *nmol/mg creatinine*	4.99± 9.25	7.28±11.33	2.81±6.07	0.03
Midluteal menstrual phase at NAF collection	47 (57%)	21 (53%)	26 (62%)	0.39
NAF estradiol[4], *pg/mL*	130±140	130±132	129±148	0.84
Below detection limit, *N (%)*	27 (34%)	14 (36%)	13 (33%)	0.75
NAF estrone sulfate[4], *ng/mL*	56±110	68±142	46±68	0.32
Below detection limit, *N (%)*	19 (24%)	6 (15%)	13 (32%)	0.09
Serum estradiol[4], *pg/mL*	152±90	124±55	178±107	0.02
Serum estrone[4], *pg/mL*	106±52	91±34	119±62	0.03
Serum estrone sulfate[4], *ng/mL*	2.22±1.28	1.89±0.79	2.54±1.55	0.02

[1]Data are *N* (%) or mean±SD; *p* values are from *t* test for continuous variables and χ^2 test for categorical variables; log transformed values were used for serum estrogens; Wilcoxon rank-sum test was used for NAF estrogens.

[2]N = 59 for parous women: 31 in group A and 28 in group B

[3]Equol producer status is based on detecting urinary daidzein excretion of ≥ 2 nmol/mg and urinary equol to daidzein ratio of ≥ 0.018 in at least one of the 8 urine samples collected throughout the study.

[4]N=79 for NAF estradiol and serum estradiol and estrone; N=80 for NAF estrone sulfate; N=78 for serum estrone sulfate.

Table 2

Estrogen concentrations in nipple aspirate fluid (NAF) and serum

Outcome	Mean±SD			Effect of high-soy diet[2]		
	Baseline[1]	**Low-soy**	**High-soy**	**Parameter estimate**	**Standard error**	***p* value**
NAF						
Estradiol, *pg/mL*	135±152	313±131	113±123	−0.63	0.35	0.07
Above minimum detection limit, *N* (%)	98 (66%)	51 (63%)	48 (58%)			
Estrone sulfate, *ng/mL*	64±128	68±115	46±69	−0.58	0.31	0.07
Above minimum detection limit, *N* (%)	115 (77%)	63 (76%)	56 (67%)			
Serum						
Estradiol, *pg/mL*	152±90	175±172	152±89	−1.03	1.09	0.76
Estrone, *pg/mL*	106±52	116±94	107±53	−1.01	1.06	0.86
Estrone sulfate, *ng/mL*	2.22±1.28	2.21±1.28	2.16±1.02	−1.03	1.05	0.56

Notes: Participants were randomized to either a 6 month high-soy (2 servings/day) or low-soy (<3 servings/week) diet and crossed over after a 1 month washout period. Baseline, low-soy, and high-soy estrogen concentrations reflect raw values.

[1] Samples collected at baseline and after the 1-month washout period prior to crossover.

[2] Reflect the predicted mean difference for the effect of the high-soy diet on log-transformed estrogen concentrations from mixed-effects models.