A Pilot and Feasibility Study on the Effects of Naturopathic Botanical and Dietary Interventions on Sex Steroid Hormone Metabolism in Premenopausal Women

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Abstract

Naturopathic physicians commonly make dietary and/or dietary supplement recommendations for breast cancer prevention. This placebo-controlled, parallel-arm, pilot study tested the effects of two naturopathic interventions over five menstrual cycles on sex steroid hormones and metabolic markers in 40 healthy premenopausal women. The intervention arms were as follows: combination botanical supplement (*Curcuma longa, Cynara scolymus, Rosmarinus officinalis, Schisandra chinensis, Silybum marinum*, and *Taraxacum officinalis*; $n = 15$), dietary changes (3 servings/d crucifers or dark leafy greens, 30 g/d fiber, 1-2 liters/d water, and limiting caffeine and alcohol consumption to 1 serving each/wk; $n = 10$), and placebo ($n = 15$). Early-and late-follicular phase serum samples from cycles 1 and 5 were analyzed for estrogens (estrone, estrone-sulfate, total estradiol, and free estradiol), androgens (dehydroepiandrosterone, dehydroepiandrosterone-sulfate, androstenedione, total testosterone, and free testosterone), sex hormone-binding globulin, and metabolic markers (insulin, insulin-like growth factor-I, insulin-like growth factor binding protein-3, and leptin). Serum samples collected during the mid-luteal phase of cycles 1 and 5 were analyzed for total estradiol, free estradiol, and sex hormone-binding globulin. Urine samples collected during the late follicular phase of cycles 1 and 5 were analyzed for 2-hydroxyestrone and 16α-hydroxyestrone. During the early follicular phase, compared with placebo, the botanical supplement decreased dehydroepiandrosterone (−13.2%; $P = 0.02$), dehydroepiandrosterone-sulfate (−14.6%; $P = 0.07$), androstenedione (−8.6%; $P = 0.05$), and estrone-sulfate (−12.0%; $P = 0.08$). No other trends or statistically significant changes were observed. When comparing dietary changes with placebo, no statistically significant differences were observed. Overall, in this pilot study, the naturopathic interventions had no substantial effects on estrogen measures. Early-follicular phase androgens decreased with the botanical supplement. (Cancer Epidemiol Biomarkers Prev 2007; 16(8):1601–9)

Introduction

Epidemiologic studies suggest that premenopausal women with higher exposure to endogenous sex steroid hormones, especially estrogens and androgens, have increased risk of developing postmenopausal breast cancer (1, 2). The epidemiologic literature is inconsistent about the relationship between premenopausal urinary estrogen metabolites (2-hydroxyestrone, 16α-hydroxyestrone, and their ratio; refs. 3, 4) and metabolic markers [insulin, insulin-like growth factor (IGF)-I, IGF binding protein-3, and leptin; refs. 5, 6] and subsequent breast cancer risk. The incidence of breast cancer is low before menopause. However, the time before menopause may be a critical period for instituting preventive interventions to alter hormonal and metabolic factors that could influence both premenopausal and postmenopausal breast cancer risk.

Effective breast cancer prevention strategies for women to begin while premenopausal are limited. Tamoxifen, a selective estrogen receptor modulator, is the only U.S. Food and Drug Administration–approved chemopreventive agent for premenopausal women at high breast cancer risk (7). The recent Study of Tamoxifen and Raloxifene trial showed that raloxifene is as effective as tamoxifen in reducing the risk of invasive breast cancer (8). However, the Study of Tamoxifen and Raloxifene trial was limited to postmenopausal women. Pilot studies of raloxifene have been conducted in premenopausal women but have shown increases in estradiol concentrations and decreases in bone mineral density (9, 10). Surgical breast cancer prevention options include mastectomy and oophorectomy (11-13), yet these options are undesirable for many women given their side effects (e.g., loss of ovarian estrogen production and increased risk of endometrial cancer) and implications (e.g., disfigurement and inability to conceive). Some women may prefer to take a more proactive role in their breast cancer prevention strategies by adopting lifestyle modifications. Many women are using forms of complementary and alternative medicine for breast cancer prevention approaches and/or options that are more attune with their personal belief systems (14).

Licensed naturopathic physicians (15) are a group of complementary and alternative medicine providers who often counsel women on dietary and lifestyle strategies to reduce their breast cancer risk. Naturopathic physicians commonly recommend dietary changes and botanical supplements to premenopausal women who are concerned about their breast cancer risk, with the aim of decreasing endogenous exposure to sex steroid hormones and the ultimate goal of decreasing premenopausal and postmenopausal breast cancer risk. Naturopathic physicians hypothesize that specific foods and botanical supplements increase hepatic conjugation of sex steroid hormones and aid in the urinary and fecal excretion of

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sex steroid hormones (16, 17). Typical dietary recommendations (and rationale for use) presented by naturopathic physicians include the following: increasing intake of specific vegetables, including cruciferous vegetables (high in indole-3-carbinol, which has been shown to shift estrogen metabolism from the 16α-hydroxyestrone pathway toward the less carcinogenic 2-hydroxyestrone pathway; ref. 18), beets (to decrease intestinal transit time and provide folic acid, a methyl group donor in many hepatic enzyme systems; refs. 16, 17), and dark leafy greens (also high in folic acid; refs. 16, 17); increasing dietary fiber (to decrease intestinal transit time and decrease the bioavailability of endogenous hormones and metabolites; ref. 19); increasing water consumption (to increase overall hormone excretion rates through urine and bowel movements; refs. 16, 17); and decreasing intake of caffeine and alcohol (both metabolized by hepatic enzymes; refs. 16, 17). Recommendations for botanical supplements may include botanicals that increase bile production and excretion, such as *Curcuma longa* (turmeric) root, *Cynara scolymus* (artichoke) leaf, *Rosmarinus officinalis* (rosemary) leaf, *Schisandra chinensis* (schisandra) berry, and *Taraxacum officinalis* (dandelion) root (to increase the rate at which endogenous hormone metabolites are excreted; refs. 17, 20, 21), and botanicals that increase hepatocyte regeneration, such as *Silybum marianum* (milk thistle) seed (to allow for more hepatocytes to be available to engage in enzymatic activities; ref. 21). Studies of individual dietary agents (e.g., cruciferous vegetables and fiber) have shown effects on urinary estrogen metabolites and serum estrogen concentrations (22, 23). However, to date, no randomized placebo-controlled trials have tested the effects of these combined dietary or combined botanical strategies on endogenous sex steroid hormone concentrations and metabolic markers.

We conducted a three-arm pilot and feasibility study to test the separate effects of naturopathic botanical and dietary interventions on sex steroid hormone metabolism and metabolic markers in healthy, premenopausal women over five consecutive menstrual cycles.

Materials and Methods

Participants. Between January and August 2003, we recruited healthy premenopausal women ages 21 to 45 years by advertising in the general community and by contacting women who had participated previously in breast cancer risk counseling studies at our institution and had agreed to be recontacted for future studies.

Women were screened for the following eligibility criteria: age 21 to 45 years, premenopausal with regular menstrual cycles (self-reported as ''regular'' and 25-32 days in length), body mass index 17 to 30 kg/m^2, and willing and able to travel to our institution for fasting morning clinic visits. Women were excluded for the following: use of oral contraceptives, hormones, or hormonally active herbs; use of any medications that influence hepatic metabolism, including antidepressants and antihistamines; pregnant, lactating, or planning to become pregnant in next 6 months; history of hormone-related conditions, including diabetes and thyroid disease; or history of breast cancer, kidney, liver, or gallbladder disease.

Written informed consent was obtained from all participants before enrollment, and the study protocol was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center (Seattle, WA).

Run-in Phase and Menstrual Cycle 1 (Baseline) Visits. This study featured a run-in phase to assess potential participants' willingness and ability to track menstrual cycles and other daily events and to collect biological specimens during three defined phases of their menstrual cycle. At the initial screening visit, women completed a self-administered food frequency questionnaire (developed by the Nutrition Assessment Shared Resource of the Fred Hutchinson Cancer Research Center) and a baseline questionnaire (demographics, medical history, and use of complementary and alternative medicine), and their height and weight were measured. Women were instructed on how to complete a daily menstrual cycle and lifestyle tracking booklet and were asked to call the study phone line when they started their next menstrual cycle (cycle 1, day 1). Women were scheduled for an early-follicular phase (cycle 1, days 3-5) and a late-follicular phase (cycle 1, days 9-11) clinic visit for a fasting morning blood draw and spot urine collection. At the late-follicular phase clinic visit, women were provided with urinary ovulation test kits (Clearblue) and instructed to begin using the kits a few days before their anticipated ovulation date (beginning ~day 11 of their menstrual cycle). Once ovulation was detected, women called the study phone line and were scheduled to attend a mid-luteal phase clinic visit 5 days after ovulation for a fasting morning blood draw and spot urine collection. If the kit did not detect ovulation, the mid-luteal phase clinic visit was scheduled between days 19 and 23 of her menstrual cycle.

Randomization. At the last clinic visit (mid-luteal phase visit) of the run-in phase, eligibility and interest in participating in the study was confirmed, and a total of 40 participants were randomized to three study arms using a block design. Thirty women were evenly randomized into each of three arms (10 into each of botanical supplement, placebo pill, or dietary change arms). Ten women were unwilling to be randomized into the dietary change arm and were randomized into the botanical supplement or placebo pill arms (five into each arm). The botanical supplement and placebo arms were double blinded; the dietary intervention arm was unblinded. Participants were instructed to maintain the study interventions for their subsequent four menstrual cycles (cycles 2-5).

Interventions. The botanical supplement formula was based on current naturopathic clinical practice and was chosen for the herbs' actions on the liver, specifically for their abilities to increase bile production and flow and for hepatocyte regeneration (17, 20, 21). Each capsule of the botanical combination product included 100 mg *C. longa* (turmeric) root extract standardized to 95% curcumin; 100 mg *C. scolymus* (artichoke) leaf 6:1 extract; 100 mg *R. officinalis* (rosemary) leaf 5:1 extract; 100 mg *S. marinum* (milk thistle) seed extract standardized to 80% silybin, silichristin, silidianin, and silymarin; 100 mg *T. officinalis* (dandelion) root 4:1 extract; and 50 mg *S. chinensis* (schisandra) berry 20:1 extract. Rice powder placebo capsules were aesthetically matched to the botanical supplement capsules. Participants in the botanical supplement and placebo arms were instructed to take four capsules two times per day with meals.

The dietary intervention, based on current naturopathic clinical practice, was chosen for the abilities of foods to upregulate estrogen metabolism, increase methylation and sulfation pathways, decrease intestinal transit time, and increase renal output (16, 17). Study participants were asked to meet the following dietary goals: 3 servings (1/2 cup each) per day of cruciferous vegetables, garlic, onions, beets, dark leafy greens; 30 grams of fiber per day; 1 to 2 liters of water per day; 1 cup per week or less of coffee and black tea (green tea was not limited); and 1 serving per week of alcohol. Each week, two grocery bags of fresh, organically grown vegetables were made available for participants to pick up at the research clinic; the vegetables provided were more than sufficient to meet each woman's targeted daily vegetable intake. Nutritionist support included eight 1-h group sessions that provided information on the rationale for the dietary changes, recipes, shopping ideas, and trouble shooting tips. E-mail and phone consultations were available as needed.

Follow-up (Cycle 5) Visits. Menstrual cycle 5 clinic visits were conducted as per cycle 1. Early-follicular phase clinic visits were conducted during days 3 to 5, late-follicular phase clinic visits were conducted during days 9 to 11 of cycle 5, and mid-luteal phase clinic visits were conducted 5 days after ovulation, or on days 19 to 23 if the kit did not detect ovulation. Fasting morning blood samples and spot urine samples were collected at each clinic visit. At the last study visit, women completed a follow-up questionnaire and food frequency questionnaire and participants in the botanical supplement and placebo arms turned in their remaining pills and pill bottles.

Throughout the study period, participants kept a daily record of their menstrual cycle and were asked to notify study staff when they began their menstrual cycles. Using the same daily records, participants in the botanical supplement and placebo arms tracked their daily study pill intake, and participants in the dietary changes arm tracked their daily serving intakes of study-related foods and beverages. Participants were monitored for adverse events and menstrual cycle timing via telephone calls at 1, 2, 4, 6, 8, 10, and 12 weeks after beginning the interventions. Participants had access to study staff via a 24-h study pager if they had questions or needed to report adverse events.

Serum Assays. Serum samples were separated on-site after collection and stored at −70°C at the Fred Hutchinson Cancer Research Center until analysis. Early-follicular phase blood samples from cycles 1 and 5 were analyzed for basic serum chemistry panels, including glucose, blood urea nitrogen, creatinine, blood urea nitrogen/creatinine ratio, sodium, potassium, chloride, carbon dioxide, calcium, and hepatic function panels (total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, aspartate transaminase, and alanine transaminase; Quest Diagnostics).

Early- and late-follicular phase serum samples from cycles 1 and 5 were analyzed (Stanczyk laboratory, University of Southern California, Los Angeles, CA) for estrogens (estrone, estrone-sulfate, total estradiol, and free estradiol), androgens [dehydroepiandrosterone, dehydroepiandrosterone-sulfate (DHEAS), androstenedione, total testosterone, and free testosterone], sex hormone-binding globulin (SHBG), and metabolic markers (insulin, IGF-I, IGF binding protein-3, and leptin). Mid-luteal phase serum sample analyses from cycles 1 and 5 were restricted to those sex steroid hormones that were anticipated to fluctuate the most over the course of the menstrual cycle: total estradiol, free estradiol, and SHBG (used to calculate free estradiol).

Serum concentrations of estradiol, estrone, dehydroepiandrosterone, testosterone, and androstenedione were quantified by validated, previously described RIAs (24, 25). Free testosterone was calculated using total testosterone and SHBG concentrations and an assumed constant for albumin in a validated algorithm (26, 27). Free estradiol was calculated in a similar manner. Estrone-sulfate was quantified by a highly specific RIA (Diagnostic Systems Laboratories). DHEAS, insulin, and SHBG were quantified by direct immunoassays using the Immulite analyzer (Diagnostic Products Corp.). IGF-I and IGF binding protein-3 were analyzed by direct chemiluminescent immunoassays on the Nichols Advantage analyzer (Quest Diagnostics, Nichols Institute). Leptin was measured by direct RIA (Linco). In-house quality control intra-assay and interassay coefficients of variation (CV) ranged from 4% to 8% and 8% to 13%, respectively. Two blinded duplicate quality control samples were included in each batch. Intra-assay blinded CVs had a median value of 5.2% (range, 0.0-25.3%) and the interassay blinded CVs had a median value of 8.6% (range, 1.4-15.9%).

Urine Assays. At the time of collection, an aliquot of urine was stored for creatinine analysis, and the remaining urine was supplemented with 1 part ascorbic acid solution (100 mmol/L solution of USP ascorbic acid using 17.6 g/liter deionized water) to 30 parts urine to prevent oxidation of labile estrogen metabolites. Samples were stored at −70°C until analysis at the Fred Hutchinson Cancer Research Center. Analyses were done on late-follicular phase samples, when estrogen levels are at their peak during the menstrual cycle, from cycles 1 and 5. Urine samples were analyzed for 2-hydroxyestrone and 16α-hydroxyestrone using the commercially available Estramet 2/16 enzyme immunoassay (Immuna Care Corp.; ref. 28). Two in-house duplicate quality control samples and one kit control sample were included in each batch. Intra-assay CVs for 2-hydroxyestrone measured in the in-house control samples ranged from 0.1% to 6.0% and the interassay CV was 8.6%; the interassay CV for the kit control sample was 4.6%. Intra-assay CVs for 16α-hydroxyestrone measured in the in-house control samples ranged from 6.8% to 24.0% and the interassay CV was 7.8%; the interassay CV for the kit control sample was 6.0%.

Urinary creatinine concentrations were measured based on a kinetic modification of the Jaffe reaction using the Roche Reagent for Creatinine (Roche Diagnostic Systems) on a Roche Cobas Mira Plus chemistry analyzer.

Botanical Supplement Quality Assurance. The botanical supplement and placebo capsules were provided by Vital Nutrients. Quality assurance measures were conducted by outside laboratories on each of the six botanicals used in the supplement. Each botanical was screened for microbiology (Eastern Analytical Laboratory), heavy metals (Chemical Solutions Ltd.), and pesticides and herbicides (Environmental Micro Analysis, Inc.). High-performance liquid chromatography assays were conducted for total curcuminoids in the *C. longa*; caffeoylquinic acids as chlorogenic acid in the *C. scolymus*; silybins in the *S. marianum*; and carnosol, carnosic acid, ursolic acid, and rosmarinic acid in the *R. officinalis* (Analytical Laboratories in Anaheim, Inc.). Solvent residue screens were conducted for propanol in the *C. longa* and acetone in the *S. marianum* (Central Analytical Laboratories). TLC identification was conducted on the *C. scolymus, R. officinalis, T. officinalis*, and *S. chinensis* (Alkemists Pharmaceuticals; Flora Research).

Nutrient Data. Nutrient calculations were conducted using the Nutrient Data System for Research software version 4.04, developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN) Food and Nutrient Database 32 (December 2001).

Statistical Analyses. We compared serum and urinary biomarker values between each active intervention arm (botanical supplement and dietary changes) and the placebo control using intention-to-treat analyses. In analyses of the dietary change arm, the comparison group included only the women within the placebo arm who had initially been willing to be randomized to all three study arms ($n = 10$ per arm). In analyses of the botanical supplement arm, the comparison group included all women who were randomized to the placebo arm ($n = 15$). Only participants with complete data for a given biomarker were included in that biomarker's analyses.

Serum and urinary biomarker data were not normally distributed; therefore, all analyses were conducted on the log scale. Cycle 1 (baseline) and cycle 5 biomarker data are presented as geometric means with corresponding 95% confidence intervals. Urinary estrogen metabolite data are expressed as per milligram creatinine (Cr) to adjust for variability in urine output. Percentage changes between cycle 1 (baseline) and cycle 5 (follow-up) were calculated as follows: $[(\text{cycle 5} / \text{cycle 1}) - 1] \times 100$. Two-sided t tests with unequal variances were used to test changes in biomarker levels, and a P value of ≤ 0.05 was considered statistically significant. Due to the pilot and exploratory nature of the analyses, there was no attempt to

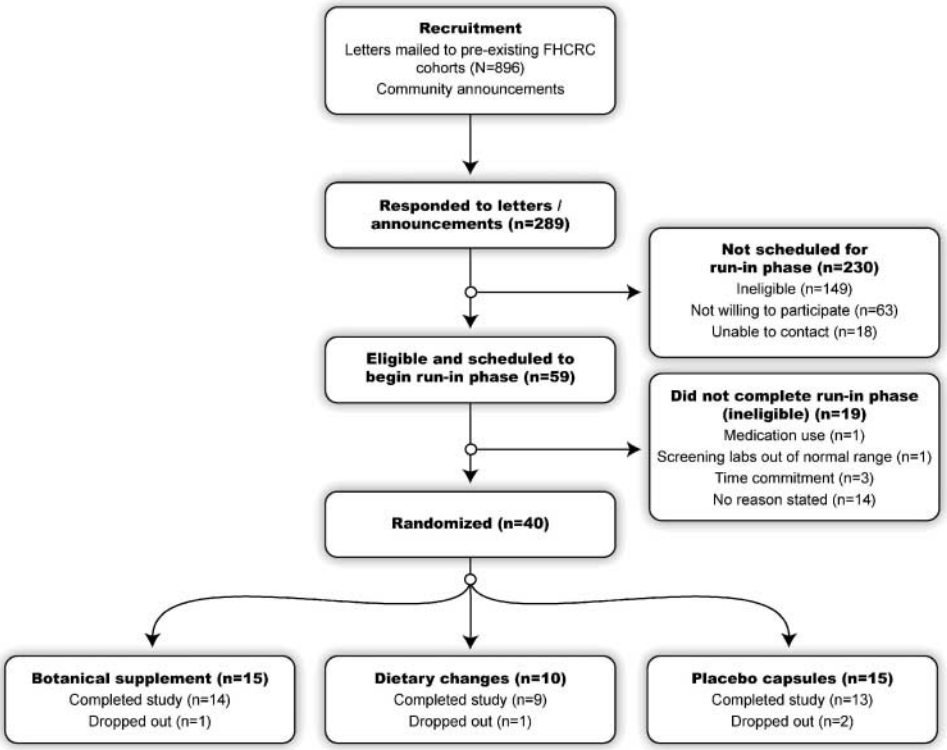

Figure 1. Study participant recruitment and randomization.

adjust *P* values for multiple testing to control the overall type I error. Analyses were conducted using Stata SE 9.2.

Results

Recruitment and Study Participants. We contacted 896 women who had participated previously in breast cancer risk counseling studies at our institution and an additional 101 women responded to our community announcements. A total of 289 women expressed interest in participating in this study, and 40 (13 recruited via the list of prior study participants and 27 recruited via community announcements) were randomized into one of the three study arms after screening for eligibility and undergoing the run-in phase

Table 1. Baseline characteristics of randomized study participants (*N* = 40)

Characteristics	Botanical supplement (*n* = 15)	Dietary changes (*n* = 10)	Placebo		All (*N* = 40)
			n = 15*	*n* = 10†	
Age (y)	34.7 (6.7)	35.1 (7.2)	37.3 (6.1)	35.8 (6.9)	35.8 (6.5)
Race, *n* (%)					
White‡	14 (93.3)	9 (90.0)	12 (80.0)	8 (80.0)	35 (87.5)
American Indian/Alaska Native	0	0	1 (6.7)	0	1 (2.5)
Asian	1 (6.7)	0	1 (6.7)	1 (10.0)	2 (5.0)
Native Hawaiian/other Pacific Islander	0	0	1 (6.7)	1 (10.0)	1 (2.5)
Other	0	1 (10.0)	0	0	1 (2.5)
BMI (kg/m^2)	23.0 (2.8)	22.5 (2.9)	23.0 (2.3)	22.4 (2.4)	22.9 (2.6)
Annual household income >$50K, *n* (%)	10 (66.7)	5 (50.0)	11 (73.3)	7 (70.0)	26 (65.0)
Ever used vitamins, herbs, or nutritional supplements, *n* (%)	12 (85.7)	8 (80.0)	9 (60.0)	5 (50.0)	29 (74.4)
Ever seen CAM provider, *n* (%)	13 (86.7)	9 (90.0)	13 (86.7)	9 (90.0)	35 (87.5)
Daily dietary intake					
Calories (kcal)	1,866.4 (885.4)	1,686.5 (505.5)	1,787.7 (729.6)	1,696.7 (491.8)	1,790.0 (727.9)
Protein (g)	78.2 (38.8)	67.2 (21.9)	73.9 (35.1)	67.4 (23.8)	73.7 (33.2)
Carbohydrate (g)	237.7 (112.3)	226.3 (90.2)	227.2 (86.7)	209.1 (48.2)	230.7 (95.1)
Fat (g)	65.8 (36.5)	59.5 (18.0)	63.4 (28.7)	63.8 (21.8)	63.3 (29.0)
Fiber (g)	21.7 (10.3)	25.2 (16.7)	21.3 (7.4)	20.1 (5.3)	22.4 (11.2)
Vegetables (1/2 cup servings)	3.1 (1.3)	3.3 (2.2)	3.2 (1.7)	2.9 (1.3)	3.2 (1.7)
Water (cups)	5.2 (2.6)	6.3 (3.1)	5.6 (2.8)	5.2 (2.5)	5.6 (2.8)
Caffeine (mg)	122.4 (88.8)	233.1 (372.5)	170.8 (131.8)	147.1 (142.7)	169.4 (209.4)
Alcohol (g)	9.1 (7.5)	5.3 (5.8)	9.3 (10.4)	9.2 (7.2)	8.2 (8.4)
Cooked greens (1/2 cup servings)	0.3 (0.4)	0.4 (0.7)	0.3 (0.6)	0.4 (0.7)	0.4 (0.5)
Duration of menstrual cycle 1 (days)	31.1 (8.2)	29.1 (4.6)	27.4 (3.4)	26.6 (3.8)	29.3 (6.1)

NOTE: Participant characteristics did not differ significantly between groups. Data shown are mean (SD) unless otherwise indicated.
Abbreviations: CAM, complementary and alternative medicine; BMI, body mass index.
*Includes all women randomized to placebo (five of these women were unwilling to be randomized to the dietary changes arm).
† Includes women who were willing to be randomized to all three arms.
‡ Includes one Hispanic woman in the placebo arm.

Table 2. Self-reported side effects rated as moderate or severe during menstrual cycles 2 to 5

Symptom	Botanical supplement, *n* (%)	Dietary changes, *n* (%)	Placebo, *n* (%)
	n = 15	*n* = 10	*n* = 15
Gastrointestinal symptoms			
Gas/bloating	6 (40.0)	5 (50.0)	5 (33.3)
Increased bowel movements/diarrhea	2 (13.3)	5 (50.0)	6 (40.0)
Indigestion	5 (33.3)*	1 (10.0)	0 (0)
Loss of appetite	1 (6.7)	1 (10.0)	1 (6.7)
Nausea	0 (0)	0 (0)	0 (0)
Stomach pain	1 (6.7)	1 (10.0)	2 (13.3)
Neurologic symptoms			
Changes in sleep	4 (26.7)	5 (50.0)	5 (33.3)
Dizziness	1 (6.7)	0 (0)	0 (0)
Headache	1 (6.7)	2 (20.0)	3 (20.0)
Dermatologic symptoms			
Hives/itching/skin rash	2 (13.3)	0 (0)	5 (33.3)

*P = 0.014 when compared with placebo.

(Fig. 1). Participant demographics across treatment arms are shown in Table 1. There were no statistically significant differences in baseline demographic, dietary, and menstrual cycle characteristics between active and placebo arms nor were there differences between placebo participants who were or were not willing to be randomized to the dietary intervention. At baseline, geometric mean concentrations of SHBG and total testosterone in the early follicular phase were lower among women in the placebo arm (n = 15) compared with women in the botanical supplement arm (n = 15; P = 0.01 and 0.01, respectively). No other baseline differences were statistically significant (see Tables 3 and 4).

Table 3. Concentrations [geometric mean (95% confidence intervals)] and percentage changes from cycle 1 (baseline) to cycle 5 in serum estrogens, serum SHBG, and urinary estrogen metabolites in the early follicular, late follicular, and mid-luteal phases of the menstrual cycle, by study arm

	Early follicular			Late follicular			Mid-luteal		
	Cycle 1, mean (95% CI)	Cycle 5, mean (95% CI)	% Change	Cycle 1, mean (95% CI)	Cycle 5, mean (95% CI)	% Change	Cycle 1, mean (95% CI)	Cycle 5, mean (95% CI)	% Change
Serum									
Estrone (pg/mL)									
Botanical (n = 13)	52 (46-59)	49 (41-58)	−5.77	99 (73-134)	86 (63-118)	−13.1			
Diet (n = 10)	47 (38-58)	56 (42-75)	19.15	87 (61-123)	79 (59-105)	−9.20			
Placebo (n = 12)	47 (41-55)	52 (37-73)	10.64	92 (74-115)	74 (55-99)	−19.6			
Estrone-sulfate (ng/mL)									
Botanical (n = 13)	1.25 (1.08-1.45)	1.10 (0.94-1.28)	−12.0*	1.89 (1.46-2.45)	1.65 (1.24-2.20)	−12.7			
Diet (n = 10)	1.27 (1.15-1.42)	1.36 (1.09-1.69)	7.09	2.05 (1.58-2.66)	1.97 (1.53-2.53)	−3.90			
Placebo (n = 12)	1.36 (1.15-1.62)	1.46 (1.17-1.81)	7.35	2.22 (1.77-2.79)	1.80 (1.41-2.31)	−18.9			
Total estradiol (pg/mL)									
Botanical (n = 12)	53 (43-64)	57 (47-70)	7.55	142 (93-216)	149 (100-223)	4.93	154 (120-197)	166 (140-195)	7.79
Diet (n = 9)	43 (33-55)	58 (46-73)	34.88	127 (75-216)	113 (78-163)	−11.0	138 (99-193)	159 (121-209)	15.22
Placebo (n = 12)	47 (36-63)	57 (34-95)	21.28	143 (111-185)	103 (65-164)	−28.0	141 (112-176)	136 (112-166)	−3.55
Free estradiol (pg/mL)									
Botanical (n = 13)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	0.00	3.3 (2.2-4.9)	3.1 (2.2-4.5)	−6.06	2.9 (2.3-3.7)	3.2 (2.6-3.8)	10.34
Diet (n = 9)	1.0 (0.8-1.3)	1.3 (1.1-1.6)	30.00	3.0 (1.8-5.2)	2.6 (1.8-3.7)	−13.3	3.1 (2.2-4.3)	3.6 (2.7-4.9)	16.13
Placebo (n = 12)	1.1 (0.8-1.5)	1.3 (0.8-2.0)	18.18	3.4 (2.6-4.4)	2.4 (1.6-3.5)	−29.4	3.3 (2.6-4.2)	3.0 (2.5-3.7)	−9.09
SHBG (nmol/L)									
Botanical (n = 13)	72 (56-92) †	72 (55-95)	0.00	66 (51-86)	72 (56-94)	9.09	75 (54-104)	78 (61-101)	4.00
Diet (n = 9)	49 (38-62)	56 (42-74)	14.29	47 (37-61)	52 (39-69)	10.64	58 (46-73)	53 (39-70)	−8.62
Placebo (n = 12)	48 (35-66)	52 (39-69)	8.33	46 (33-63)	50 (36-69)	8.70	49 (36-65)	54 (40-74)	10.20
Urine									
2-Hydroxyestrone (ng/mg Cr)									
Botanical (n = 14)				24.2 (17.7-33.0)	23.1 (17.5-30.6)	−4.55			
Diet (n = 10)				20.8 (13.1-33.0)	20.4 (11.1-37.2)	−1.92			
Placebo (n = 13)				16.2 (6.7-39.1)	12.9 (7.7-21.9)	−20.4			
16α-Hydroxyestrone (ng/mg Cr)									
Botanical (n = 14)				12.9 (11.0-15.2)	11.5 (9.2-14.3)	−10.9			
Diet (n = 10)				12.0 (8.6-16.7)	13.1 (8.8-19.6)	9.17			
Placebo (n = 13)				12.3 (5.1-29.4)	10.5 (7.7-14.4)	−14.6			
2α-Hydroxyestrone/16α-hydroxyestrone ratio									
Botanical (n = 14)				1.9 (1.5-2.4)	2.0 (1.7-2.5)	5.26			
Diet (n = 10)				1.7 (1.3-2.4)	1.6 (0.9-2.7)	−5.88			
Placebo (n = 13)				1.3 (0.9-2.0)	1.2 (0.9-1.7)	−7.69			

NOTE: All P values >0.10 unless otherwise indicated. Comparing change between cycles 1 and 5 in botanical or diet arms versus change in placebo using paired t tests with unequal variances.
Abbreviation: 95% CI, 95% confidence interval.
*P = 0.083 when compared with placebo.
† Baseline SHBG significantly higher in botanical arm versus placebo arm (P = 0.01).

Participant Retention, Adherence, and Data Collection. Ninety percent (n = 36) of the participants were retained throughout the study. Two participants withdrew due to medical issues unrelated to the study interventions, and two participants were lost to follow-up. Of the 36 participants retained in the study, the number completing clinic visits during cycle 5 were as follows: early-follicular phase visit, n = 35; late-follicular phase visit, n = 35; and mid-luteal phase visit, n = 33. Daily tracking records kept during cycles 2 to 5 were returned by 34 participants.

On average, women participated in the study intervention for 111 (±8) days, with no statistically significant differences between arms. Based on self-reported pill diaries, women on the botanical supplement took an average of 6.3 (±2.0) capsules per day, whereas women on the placebo took 7.1 (±1.1) capsules per day, which was close to the goal of 8 capsules per day. On average, women in the dietary changes arm exceeded their recommended dietary goals; they ate 3.8 (±1.0) servings of vegetables per day (goal, 3+ servings), drank 2.0 (±0.6) liters of water per day (goal, 1+ liters), consumed 0.5 (±0.4) servings of caffeine per week (goal, ≤1 serving), and drank 0.8 (±0.5) servings of alcohol per week (goal, ≤1 serving).

Safety and Side Effects. All participants had normal basic metabolic function and hepatic function chemistries at cycle 1 (baseline) and cycle 5 (follow-up; data not shown). Self-reported side effects rated as moderate or severe during menstrual cycles 2 to 5 are shown in Table 2. Women taking the botanical supplement reported more indigestion than women taking the placebo, but no other differences between treatment arms were statistically significant. There were no statistically significant differences or changes in menstrual cycle length between groups (data not shown).

Body Weight. Between menstrual cycles 1 and 5, women in the dietary intervention arm lost an average of 0.5 (±0.5) kg body weight compared with women in the placebo arm who gained an average of 1.6 (±0.8) kg (P = 0.05). There was no statistically significant difference in change in body weight when comparing women in the botanical supplement intervention with women in the placebo arm.

Hormones and Metabolic Markers. When comparing botanical supplement with placebo, several changes in early-follicular phase serum hormone concentrations were statistically significant or of borderline significance (Tables 3 and 4). These included decreases in serum dehydroepiandrosterone (−13.2%; P = 0.02), DHEAS (−14.6%; P = 0.07), androstenedione (−8.6%; P = 0.05), and estrone-sulfate (−12.0%; P = 0.08). No other trends or statistically significant changes were observed for other serum sex steroid hormones, serum metabolic markers (data not shown), or urinary estrogen metabolites and their ratio, as measured at different phases of the menstrual cycle. When comparing dietary changes with placebo, no statistically significant differences were observed in serum or urinary measures. Figure 2 shows percentage changes between cycles 1 and 5 in serum estrogens, SHBG, and androgens in the three study arms.

Discussion

We conducted a pilot and feasibility study to test the effects of commonly prescribed naturopathic botanical supplement and dietary modifications hypothesized to affect estrogen metabolism in premenopausal women. The botanical intervention resulted in a decrease in early-follicular phase estrone-sulfate that was of borderline statistical significance, but no other effects on serum and urinary estrogen measures could be detected in the context of this pilot study. We observed changes in androgen concentrations with the botanical supplement. Compared with placebo, early-follicular phase dehydroepiandrosterone and androstenedione decreased significantly, and there was a trend toward decreasing DHEAS with the botanical supplement. The dietary intervention was associated with weight loss but not with changes in any of the sex steroid hormone or metabolic markers.

A major component of this study was to determine the feasibility of recruiting for and implementing the study interventions. We showed that it is feasible to conduct such a study with intensive recruitment efforts. In addition to community announcements, we initiated contact with ~900 women before we were able to recruit 40 study subjects, 10 of

Table 4. Concentrations [geometric mean (95% confidence intervals)] and percentage changes from cycle 1 (baseline) to cycle 5 in serum androgens in the early follicular and late follicular phases of the menstrual cycle, by study arm

	Early follicular				Late follicular			
	Cycle 1, mean (95% CI)	Cycle 5, mean (95% CI)	% Change	P*	Cycle 1, mean (95% CI)	Cycle 5, mean (95% CI)	% Change	P*
DHEA (ng/mL)								
Botanical (n = 13)	5.22 (3.99-6.83)	4.53 (3.62-6.14)	−13.22	0.016	4.81 (3.82-6.07)	4.81 (3.96-5.84)	0.00	0.981
Diet (n = 10)	5.38 (3.60-8.04)	4.41 (2.75-7.08)	−18.03	0.111	4.60 (3.21-6.60)	4.78 (3.27-6.99)	3.91	0.521
Placebo (n = 12)	3.81 (2.46-5.89)	4.14 (2.84-6.02)	8.66		3.75 (2.65-5.29)	3.76 (2.73-5.17)	0.27	
DHEAS (μg/dL)								
Botanical (n = 13)	144 (123-170)	123 (106-143)	−14.58	0.065	148 (122-180)	122 (102-145)	−17.57	0.161
Diet (n = 10)	133 (105-168)	130 (96-176)	−2.26	0.947	135 (109-167)	134 (101-178)	−0.74	0.564
Placebo (n = 12)	121 (69-213)	121 (73-202)	0.00		129 (78-213)	121 (75-196)	−6.20	
Androstenedione (ng/mL)								
Botanical (n = 13)	1.28 (1.02-1.60)	1.17 (0.94-1.45)	−8.59	0.053	1.59 (1.33-1.90)	1.47 (1.23-1.76)	−7.55	0.492
Diet (n = 10)	1.28 (0.99-1.67)	1.19 (0.85-1.68)	−7.03	0.240	1.40 (1.08-1.83)	1.45 (1.16-1.80)	3.57	0.848
Placebo (n = 12)	1.03 (0.81-1.31)	1.14 (0.88-1.46)	10.68		1.26 (1.06-1.50)	1.26 (1.10-1.57)	0.00	
Total testosterone (ng/dL)								
Botanical (n = 13)	31.6 (27.3-36.6) †	28.3 (23.2-34.5)	−10.44	0.145	36.2 (32.3-40.5)	37.2 (32.1-43.0)	2.76	0.871
Diet (n = 10)	28.3 (22.5-35.6)	28.1 (1.90-4.17)	−0.71	0.826	31.7 (25.5-39.5)	32.1 (25.4-40.5)	1.26	0.806
Placebo (n = 12)	25.1 (20.5-30.6)	26.2 (20.4-33.7)	4.38		29.4 (24.6-35.1)	30.7 (25.0-37.6)	4.42	
Free testosterone (pg/mL)								
Botanical (n = 13)	4.51 (3.77-5.39)	4.05 (3.22-5.10)	−10.20	0.189	5.60 (4.67-6.70)	5.23 (4.38-6.25)	−6.61	0.438
Diet (n = 10)	5.21 (4.13-6.58)	4.84 (3.60-6.49)	−7.10	0.594	5.83 (4.64-7.32)	5.71 (4.69-6.95)	−2.06	0.885
Placebo (n = 12)	4.38 (3.37-5.71)	4.63 (3.64-5.89)	5.71		5.50 (4.52-6.70)	5.51 (4.40-6.89)	0.18	

Abbreviation: DHEA, dehydroepiandrosterone.

*Comparing change between cycles 1 and 5 in botanical or diet arms versus change in placebo using paired t tests with unequal variances.

† Baseline total testosterone significantly higher in botanical arm versus placebo arm (P = 0.01).

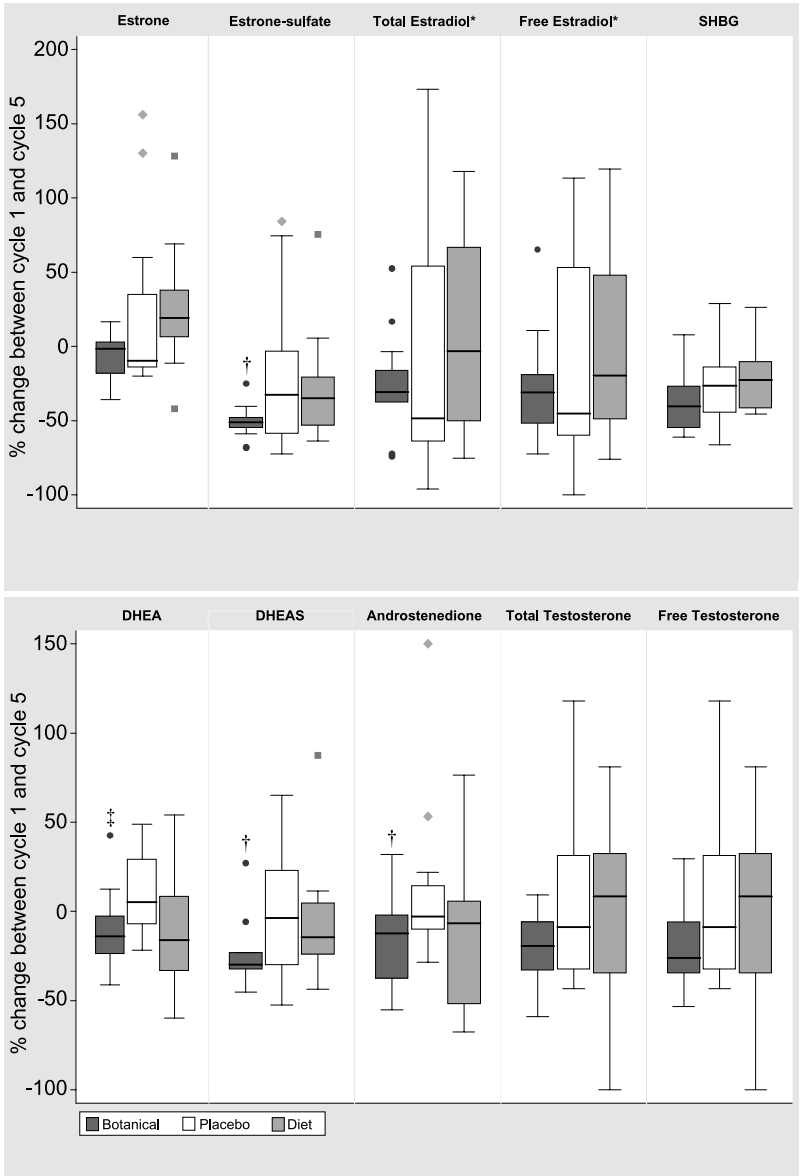

Figure 2. Box plots of percentage changes in serum concentrations of estrogens, SHBG, and androgens during the early follicular phase from cycles 1 to 5. Box plots indicate median, interquartile range, upper and lower adjacent values, and outside values. *, outside values not shown for one placebo group participant, values = 398.41% for change in total estradiol and 374.24% for change in free estradiol. †, $P < 0.10$ when compared with placebo; ‡, $P < 0.05$ when compared with placebo.

whom were unwilling to be randomized to the dietary change arm. Posting and broadcasting community announcements yielded 67.5% ($n = 27$) of our participants and was a successful recruitment strategy.

Few intervention studies have investigated sex steroid hormone-related biomarkers in premenopausal women due to the fact that it can be labor intensive for both the investigator and participant to track menstrual cycles and time biomarker data collection accordingly. In our study, participants were able to track their menstrual cycles and provide fasting morning blood and urine samples during specified phases of their menstrual cycles. Adherence to the study protocol was good, and no major side effects were reported.

A large degree of variability in the biomarker outcomes (as shown by wide 95% confidence intervals) was observed in the placebo group, and this was more evident for the estrogens than androgens. This is likely due to the fact that estrogen concentrations within and between individuals vary considerably over the menstrual cycle, whereas the androgens typically do not have as much variability within an individual (29). Thus, despite our efforts to control for day of cycle, measuring estrogens was a methodologic challenge. It is unclear why the baseline biomarker variability was larger among women in the placebo group compared with the intervention groups; it may be due to chance or small sample size. The observed variability within individuals in the placebo group is within the expected range of the measures. This large variation has the effect of increasing sample size requirements for intervention studies examining changes in estrogen concentrations.

The botanical supplement contained a combination of six botanicals: *C. longa, C. scolymus, R. officinalis, S. chinensis, S. marinum*, and *T. officinalis*. Animal and *in vitro* studies suggest possible mechanisms of action of these agents on steroid hormones and related enzyme systems. *S. marianum* (milk thistle) has been shown to inhibit cholesterol (a precursor to sex steroid hormones) biosynthesis in rats (30), decrease testosterone metabolism to 6-β-hydroxytestosterone *in vitro* (31), decrease CYP3A4 activity *in vitro* (but not *in vivo*; ref. 32), and elevate glutathione *S*-transferases in rats (32). In mice, *R. officinalis* (rosemary) increased 2- and 6-hydroxylation of estradiol and estrone, decreased 16α-hydroxylation of estradiol, and increased glucuronidation of estradiol and estrone (33). Also in mice, *C. longa* (turmeric) has been shown to decrease CYP1A and CYP3A activity and increase glutathione *S*-transferase activity, but to have no effect on CYP19, COMT, or UDP-glucuronosyltransferase activity (34). In rats, *T. officinalis* (dandelion) tea decreased CYP1A2 and CYP2E activity and greatly increased UDP-glucuronosyltransferase

activity (35). *In vitro* studies report conflicting results on the effect of *S. chinensis* (schisandra) on CYP3A4 activity (36, 37). *C. scolymus* (artichoke) has been shown to inhibit cholesterol biosynthesis *in vitro* (38), but there have been conflicting results *in vitro* and in rats about choleretic effects (39). Prior studies also have shown that supplementation with soy isoflavones (40), flaxseed (41), and indole-3-carbinol (18) may decrease concentrations of procarcinogenic sex steroid hormones and their metabolites in premenopausal women.

The dietary intervention included increasing cruciferous vegetables, dark leafy greens, fiber and water, and limiting caffeine and alcohol consumption. Although no prior studies have examined this combination of dietary changes, studies on the effects of the separate aspects of the dietary intervention have shown decreases in procarcinogenic sex steroid hormones and their metabolites in premenopausal and postmenopausal women. For example, a high-fiber diet decreased serum estrone and estradiol in premenopausal women (42), and cruciferous vegetable consumption increased the urinary 2-hydroxyestrone/16α-hydroxyestrone ratio in postmenopausal women (22). Higher alcohol consumption in premenopausal women was shown to increase estrone, estradiol, estriol, and DHEAS (43), and caffeine intake was positively associated with urinary 2-hydroxyestrone and 16α-hydroxyestrone concentrations in an observational study of women ages 42 to 52 years (44). In a prior randomized, controlled trial in 213 healthy premenopausal women, a high-fiber, high-fruit and vegetable, and low-fat diet, which is a similar combination dietary intervention protocol to that used here, resulted in a decrease in serum total estradiol, but no change in free estradiol, SHBG, or progesterone over 12 menstrual cycles (45).

The dietary intervention tested in this study resulted in modest weight loss, but no detectable hormonal changes. There are limited and inconsistent data on the effects of dietary changes on hormonal parameters in premenopausal women, and it is possible that weight loss due to dietary changes may play a more important role than the dietary changes themselves (46). Weight loss in premenopausal women due to a very low-energy diet has been associated with an increase in SHBG and a decrease in testosterone (47). Weight loss in postmenopausal women due to dietary changes has been associated with decreases in circulating estrogens (48), but the data are inconsistent in premenopausal women (49), most likely because the majority of premenopausal estrogens are produced in the ovaries as opposed to adipose tissue. It is unclear if the critical factor in any of the hormonal changes associated with weight loss is a decrease in total adiposity, a decrease in visceral adiposity, or a shift in energy balance, and it is unclear if these changes persist over time (50). Future studies testing the effects of a specific dietary intervention on hormonal outcomes, as opposed to the effects of weight loss per se, should consider keeping study participants weight stable by controlling for caloric intake via the use of an isocaloric diet.

The changes in androgens that we observed in this study are potentially important in light of recent data relating premenopausal androgens with breast cancer risk. A recent study within the European Prospective Investigation into Cancer and Nutrition showed associations between high premenopausal testosterone, androstenedione, and DHEAS concentrations and increased premenopausal and postmenopausal breast cancer risk, after accounting for phase of menstrual cycle (2). However, to our knowledge, no studies have examined the effects of changing premenopausal androgens levels on subsequent breast cancer risk, and there are no data indicating what magnitude of change would be required to decrease breast cancer risk. A randomized, controlled trial in 104 postmenopausal women with high baseline testosterone levels tested the effects of a low-fat, low-carbohydrate diet that was high in low glycemic index foods, monounsaturated and omega-3 fatty acids, and phytoestrogens and showed a decrease in testosterone levels after 5 months (51). However, it is unclear if this type of intervention would have the same effects in premenopausal women and if the effects would persist over time.

This was a pilot study designed primarily to test feasibility and was not sufficiently powered to detect changes in serum and urinary biomarker levels. The dietary intervention component of the intervention was administered *ad libitum* and monitored by self-report; therefore, we do not know if our null findings in the dietary intervention were due to a true lack of effect, low power to detect a difference, or poor adherence to the protocol. However, there was little evidence of poor adherence to the protocol based on self-report data. Each of the botanical agents in the combination supplement potentially affects sex hormone biosynthesis and metabolism via different pathways and/or in opposite directions. Furthermore, by studying a combination botanical supplement, a lack of effect for many of our outcomes may have been due to lack of an additive effect of the botanicals, the botanicals working in opposite pathways, or a low dose. Although we observed changes in early-follicular phase androgens with the botanical supplement, the biological significance and clinical relevance of these changes are unknown. Further, the study population primarily consisted of a group of highly motivated white women with high socioeconomic status and high complementary and alternative medicine use; thus, the generalizability of these findings may be limited.

These analyses are considered exploratory and will need to be confirmed by future, larger studies. Overall, the naturopathic interventions tested had no substantial effects on estrogen measures that could be detected in the context of this pilot study. However, early-follicular phase androgens decreased with the botanical supplement. Given the growing data suggesting a role of androgens in breast cancer etiology, further controlled studies that are appropriately powered for detecting changes are warranted to validate these findings. Such studies also can include other surrogate markers for breast cancer such as mammographic breast density (52). In addition, further studies are needed to elucidate the effects and mechanisms of the botanical agents alone and in combination on androgen and estrogen metabolism.

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A Pilot and Feasibility Study on the Effects of Naturopathic Botanical and Dietary Interventions on Sex Steroid Hormone Metabolism in Premenopausal Women

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