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Effects of black cohosh (*Cimicifuga racemosa*) on bone turnover, vaginal mucosa, and various blood parameters in postmenopausal women: a double-blind, placebo-controlled, and conjugated estrogens–controlled study

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ABSTRACT

Objectives: In this study, the effects of the *Cimicifuga racemosa* (CR) preparation CR BNO 1055 on markers of bone metabolism, hormones, sex hormone-binding globulin (SHBG), lipid metabolism, vaginal maturity, and routine laboratory parameters were compared with those of conjugated estrogens (CE) and placebo.

Design: Sixty-two postmenopausal women were included in this double-blind study. Treatment duration with CR (daily dose corresponds to 40 mg of herbal drug), CE (0.6 mg/day), or placebo was 12 weeks. Markers of bone turnover (bone-specific alkaline phosphatase, CrossLaps), estradiol, follicle-stimulating hormone, luteinizing hormone, SHBG, triglycerides, total cholesterol, high-density cholesterol, low-density cholesterol, and routine clinical chemistry parameters were determined from blood samples. Vaginal "maturity index" was determined from vaginal smears.

Results: The analyses of bone turnover markers indicated beneficial effects for CR and CE on bone metabolism. CR stimulated osteoblast activity, whereas CE inhibited osteoclast activity. Whereas CE showed strong estrogenic effects on vaginal mucosa, CR showed weak estrogen-like activity. No significant effects were seen on coagulation markers and liver enzymes in the blood. CR was well tolerated.

Conclusion: These results suggest that CR has beneficial bone remodeling and weak estrogen-like effects in the vaginal mucosa.

Key Words: Phytoestrogens – Black cohosh – *Cimicifuga racemosa* – Bone turnover – Vaginal mucosa – CR BNO 1055.

Because of recent findings that the proven beneficial effects of hormone therapy (HT) on climacteric symptoms, bone turnover, and vaginal milieu[1-6] may be outweighed by serious risks such as an increased risk of breast cancer and of cardiovascular diseases such as myocardial infarction or stroke,[7-11] HT compliance has decreased. Therefore, physicians and patients are looking for alternatives to estrogens to treat menopause symptoms and maintain bone strength. A selective estrogen receptor modulator that is devoid of undesired estrogenic effects in the mammary gland and in the uterus, prevents climacteric symptoms, and has beneficial effects on bone would be an alternative to HT.[12,13]

Herbal preparations are a promising alternative to HT because there is strong evidence that some herbal extracts reduce climacteric symptoms and improve

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bone metabolism in postmenopausal women. One of these herbal alternatives is extract of the rhizome of black cohosh (*Cimicifuga cemosa* [CR]). Several clinical studies with CR extracts showed beneficial effects on climacteric symptoms. Unfortunately, the majority of the studies was either open or not placebo-controlled.[14-16] In a double-blind, placebo-controlled study, significantly reduced climacteric symptoms were reported with CR treatment when compared with placebo.[17] In this early study, however, double the daily recommended dose (ie, corresponding to 80 mg instead of 40 mg of the herbal drug) was administered. In a recent study, the therapeutic effects of the CR preparation CR BNO 1055 on climacteric symptoms were compared with those of conjugated estrogens and placebo.[18] The effects of CR BNO 1055 and the conjugated estrogens on major climacteric symptoms were comparable and both were significantly superior to placebo in reducing climacteric symptoms. However, in contrast to the conjugated estrogens, placebo and CR BNO 1055 did not increase the thickness of the endometrium.

The preclinical research conducted during the last 10 years has led to an increased knowledge about the effects of CR. In vitro binding studies with recombinant estrogen receptors (ERα and ERβ) demonstrated that the extract does not bind to either of these two estrogen receptors. Experiments with rodents demonstrated that CR extracts do not have uterotropic effects.[19,20] These results were the basis of the conclusion that CR extracts do not contain estrogenic compounds. However, the CR extract BNO 1055 displaced radiolabeled 17β-estradiol (E_2) from cytosolic preparations from porcine uteri and from human endometrium.[21] Therefore, it was suggested that yet-unknown substances of the CR extract BNO 1055 are able to bind to a yet-unknown estrogen-binding protein.

In rats, CR BNO 1055 had the desired effect in the hypothalamus of suppressing pulsatile luteinizing hormone (LH) release and therefore, most likely, hot flushes.[22] Recently, it was shown that CR extracts contain substances that bind to serotonin receptors, and this was suggested to be the mechanism by which hot flushes are relieved.[23] However, it was shown that effects of E_2 and CR extract BNO 1055 on gene expression of two estrogen-regulated genes in the bone were comparable. Effects of the CR extract BNO 1055 and of E_2 were observed in the bones of ovariectomized rats, which were substituted over a period of 12 weeks with both drugs.[19] Both preparations reduced gene expression of *IGF1* and *TRAP*.[19,22] This finding indicates that the ovariectomy-induced increased activity of osteoblasts and osteoclasts was reduced to a normal equilibrium present in intact animals. In the animals treated with E_2 and CR BNO 1055, the more than 50% loss of bone mineral density (BMD) of the metaphysis of the tibia was almost totally prevented by E_2, whereas the rats treated with the CR extract BNO 1055 showed a partial prevention that was significant when compared with the untreated control group.[19,24] Beneficial effects on markers of bone turnover and on femoral BMD have also been demonstrated with another extract of black cohosh.[25]

In the vagina, E_2 causes epithelial proliferation that allows for bacterial production of lactate and subsequent acidification, which protects the uterus against ascending bacterial infections. Similar, though very mild, effects have been seen in women treated with black cohosh extracts. Another ovariectomized rat study demonstrated a mild vaginotropic effect of CR BNO 1055.[22] Estrogens act in various cells to increase blood-clotting parameters, which might lead to increased thromboembolic events. No information concerning such effects of black cohosh is available.

Due to the desired effects and the lack of estrogenic activity in the uterus and endometrium, respectively, it was proposed that the CR extract BNO 1055 may have organ-selective beneficial activity[19,22] of which the mechanisms of action are not yet clear. These effects are obviously not mediated by the two known estrogen receptors, but possibly involve the activation of enhancers and/or repressors of estrogen-receptor activity.

Concern has been expressed about a putative hepatotoxicity of black cohosh preparations because a case of acute liver failure has been associated with the use of this herbal preparation.[26] Hepatotoxicity was extensively discussed at a recent National Center for Complimentary and Alternative Medicine / National Institutes of Health workshop (http://nccam.nih.gov/news/pastmeetings/blackcohosh_mtngsumm.htm), and it was proposed that appropriate safety parameters should be used in clinical trials. Therefore, parameters that might indicate hepatotoxicity were investigated in the serum of patients to examine the effects of the ethanolic CR extract BNO 1055 on climacteric symptoms. In view of the similarly scarce information concerning antiosteoporotic and vaginotropic effects of black cohosh and the lack of knowledge about effects on lipids or blood clotting factors, additional data from these patients will also be presented.

METHODS

The primary endpoints of this double-blind, placebo-controlled study have been published previously.[18] In the present follow-up article, secondary objectives were to compare the effects of CR BNO 1055 extract on markers of bone turnover, menopause hormones, sex hormone binding-globulin (SHBG), lipids, parameters of vaginal cytology, and routine laboratory parameters with those of conjugated estrogens and placebo.

Study design

A description of the methods used for this randomized, double-blind comparison of the CR preparation CR BNO 1055 with conjugated estrogens and placebo can be found in a previous publication focusing on climacteric symptoms.[18] Therefore, the methods described here are abridged and mainly focus on those parameters reported in the present article.

The study was conducted between 1998 and 2000 in 13 study centers in the Czech Republic and was in accordance with the ICH-GCP guidelines. The sample size calculation of 30 postmenopausal women per treatment group was done on the basis of an estimation of clinical relevant differences concerning the primary endpoint, reduction of the climacteric symptoms, which was ascertained by the first version of the menopause rating scale.[27]

The balance of the three treatments was guaranteed by using a randomly permuted block design.[28] The treatment duration was 12 weeks. Data and blood samples were collected at week –2 (start of run-in period), at week 0 (baseline), and after 4, 8, and 12 weeks of treatment.

Before any study-related measurements were made, all patients gave written informed consent after they had received sufficient verbal and written information concerning the purpose, type, risks, benefits, and duration of the study, and about treatment alternatives.

Inclusion and exclusion criteria

Women who fulfilled the following inclusion criteria were eligible for enrollment: postmenopausal, aged 40 to 60 years, last menstrual bleeding at least 6 months ago, body mass index ≤ 30 kg/m^2, postmenopausal hormone values ($E_2 \leq 40$ pg/mL, follicle-stimulating hormone [FSH] ≥ 25 mIU/mL) at the start of the run-in period (week –2) and at baseline (week 0), at least three hot flushes per day within the run-in period (as documented in the diary), sum scores of menopause rating scale (MRS, items 1-6) ≥ 1.7 at week –2 and at baseline, and sum scores of MRS (item 1: hot flushes) ≥ 0.3 at week –2 and at baseline. The exclusion criteria are listed in Table 1.

Trial medications

The study medication was provided as hard gelatin capsules with magnesium stearate and lactose as bulk excipients. All capsules were identical in size, shape,

TABLE 1. *Exclusion criteria*

- Signs of ovulatory or anovulatory cycles during the run-in period (as evidenced by vaginal bleeding, E_2 levels > 40 pg/mL, FSH levels < 25 mIU/mL)
- Body mass index > 30 kg/m^2 (This was originally not an exclusion criterion, but after recruitment, these women were excluded because of their increased risk of hypertrophic endometria or less risk of developing osteoporosis due to estrogen production in the fat tissue, which made them a distinct subgroup of patients who would have been unevenly distributed among the three groups.)
- Hysterectomy
- Nonresponder to pretreatment with estrogens
- Any contraindication for estrogen or progestin therapy
- Unresolved genital bleeding
- Suspicion/existence of estrogen-dependent mammary and/or endometrial carcinoma
- Endometrial thickness > 5 mm
- Endometriosis
- Existing or past thromboembolism
- Phlebitis
- Acute or chronic hepatic lesion
- Metabolic disorders of bile pigments
- Diabetes mellitus
- Sickle cell anemia
- Clinically relevant hypertriglyceridemia or hypercholesterolemia
- Past myocardial infarction
- Genital neoplasms
- Severe varicosis
- Known hypersensitivity to the investigational drugs or their ingredients
- Concomitant treatment with estrogenic substances
- Concomitant treatment with psychotropics, antidepressants, hypnotics, or sedatives
- Poor general condition or alcohol and/or drug abuse

weight, appearance, and taste. The daily dose consisted of two capsules. The first group received a preparation of dried aqueous/ethanolic (58% v/v) extract BNO 1055 of the rhizome of *Cimicifuga racemosa* (black cohosh; Klimadynon/Menofem; Bionorica AG, Neumarkt, Germany). Each capsule (batch no. 805005) contained 1.66 to 2.86 mg of native extract corresponding to 20 mg of herbal drug.

The second group received a daily dose of two capsules with 0.3 mg CE per capsule. Each capsule (batch no. 71011210) contained Oestrofeminal (Heinrich Mack Nachf., Illertissen, Germany, consisting of estrone 75% to 85%, equilin 6% to 15%, equilenin up to 4%, estradiol-17α, 17α-dihydro-equilin, 17α-dihydro-equilenin 2% to 8%). The third group received matching placebo capsules (batch no. 805001).

After the run-in-period of 2 weeks, patients started with the study medication at baseline (day 0). They were randomized and the first pack of study medication was supplied. Appropriately packed and labeled individual containers with 70 capsules were distributed at baseline (for weeks 1-4), at week 4 (for weeks 5-8), and at week 8 (for weeks 9-12).

The study medications were produced according to the standards of Good Manufacturing Practice. Certificates of analysis before and during the study and descriptions of the investigational products were filed with the study documentation.

Investigated parameters

At week 0 (baseline) and after 4, 8, and 12 weeks, blood samples were collected in the morning before any food intake for the determination of markers of bone turnover (bone formation: bone specific alkaline phosphatase [BAP]; bone degradation: CrossLaps = C-terminal telopeptides of collagen type I), estradiol-17β (immunoreactive levels), LH, FSH, and SHBG, parameters of lipid metabolism (total cholesterol, high-density lipoprotein [HDL] cholesterol, and low-density lipoprotein [LDL] cholesterol, triglycerides). Because E_2 and FSH were important inclusion criteria, they were additionally examined 2 weeks before baseline at week –2. The hormones and CrossLaps were measured with the ELECSYS System (Roche-Diagnostics, Mannheim, Germany), whereas the levels of BAP were analyzed by an enzymatic assay (Hitachi/Roche-Diagnostics, Mannheim, Germany). Lipids were analyzed by enzymatic color tests. Specifications of the assays are given in Table 2.

At baseline (week 0) and after 12 weeks, the degree of the maturity of the vaginal epithelium as a measure of an estrogenic effect was determined using the "maturity index."[29] For these investigations, vaginal cells were taken with a brush and smeared on a microscope slide, and immediately fixed and stained according to Papanicolaou. The maturity index was determined by counting 500 cells and calculating the percentage of parabasal, intermediary, and superficial cells.

Routine safety laboratory parameters (creatinine, urea, uric acid, total protein, sodium, potassium, calcium, iron, total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), γ-glutamyl transferase (γGT), alkaline phosphatase, serum glucose), and a complete blood workup that consisted of erythrocyte, leukocyte, and thrombocyte, and differential blood count, hemoglobin, and hematocrit were analyzed at baseline and at the end of treatment. At the same time points a blood coagulation status was done in the form of an analysis of the activated thromboplastin time (in seconds) and of the thromboplastin time as international normalized ratio (INR). Blood pressure, heart rate (sitting/standing), and body weight were determined at all visits.

Statistical analyses

Variables were calculated as changes from baseline to endpoint values. For each variable, multiple comparisons of the treatment groups were performed by analysis of covariance (ANCOVA). Baseline values and center effects were included as covariates in the ANCOVA model. Changes in the number of superficial cells of the vaginal cytological examination were tested by Wilcoxon signed rank test. In general, *P* values less than or equal to 0.05 were

TABLE 2. *Specification of variables in the serum*

Assay characteristics/variable	E_2 (pg/mL)	FSH (mIU/mL)	LH (mIU/mL)	SHBG (nmol/L)	BAP (U/L)	CrossLaps (ng/mL)	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
Sensitivity	< 5	< 0.1	0.1	0.35	0.01	0.01	3	3	3	4
Coefficient of variance	< 5 %	< 5 %	< 5 %	< 4 %	< 1%	< 7 %	< 3 %	< 2 %	< 2 %	< 3 %

TABLE 3. *Demographic characteristics*

	CR BNO 1055		Conjugated estrogens		Placebo	
Age (y)						
Mean ± SD	52.25	± 3.19	52.32	± 3.03	54.05	± 4.36
Median (n)	52.50	20	52.00	22	54.00	20
Weight (kg)						
Mean ± SD	67.00	± 8.05	67.86	± 9.3	65.15	± 7.72
Median	67.50		69.00		65.00	
Height (cm)						
Mean ± SD	164.20	± 6.57	167.00	± 5.27	164.05	± 5.6
Median	165.50		167.00		164.00	
E_2 (pg/mL)						
Mean ± SD	19.45	± 10.05	17.04	± 9.23	14.57	± 9.52
Median	20.00		19.07		12.81	
FSH (mIU/mL)						
Mean ± SD	83.92	± 33.47	80.97	± 25.64	70.43	± 19.6
Median	79.90		82.79		69.92	
BAP [U/L]						
Mean ± SD	54.8	± 14.34	52.85	± 21.48	56.06	± 23.31
Median (n)	56.00	20	54.50	20	55.00	18
CrossLaps [ng/mL]						
Mean ± SD	0.25	± 0.20	0.24	± 0.12	0.25	± 1.6
Median (n)	0.22	17	0.24	19	0.23	17

ALP, alkaline phosphatase.

considered statistically significant. Statistical analyses were accomplished with SAS Version 8.2.

RESULTS

Patients

A total of 97 patients were randomized. These women were included in the intention-to-treat (ITT) analyses. From this population, 2 patients dropped out prematurely due to withdrawal of informed consent after baseline and were lost to follow-up. In total, 33 women were excluded from the ITT analyses due to protocol violations: 5 with less than three hot flushes per day during the run-in period, and 16 who were not definitely postmenopausal (signs of ovulatory or anovulatory cycles during the run-in period as evidenced by vaginal bleeding, E_2 levels > 40 pg/mL, and/or FSH levels < 25 mU/mL). Twelve adipose women (body mass index > 30 kg/m^2) were excluded for reasons detailed in Table 1. The remaining 62 women were included in the per-protocol analysis (CR BNO 1055: n = 20; conjugated estrogens: n = 22; placebo: n = 20). Characteristics of those patients remaining in the study at baseline (age, height, weight, hormone concentrations, and concentrations of bone formation and degradation markers) were comparable in all treatment groups (Table 3). The analysis of pill-count data demonstrated a high level of compliance in all three treatment groups.

Hormones and SHBG

Immunoreactive levels of E_2 were significantly higher after 4, 8, and 12 weeks in the group treated with conjugated estrogens compared with the placebo group. No such effect was seen with CR BNO 1055 (Fig. 1). The difference between immunoreactive E_2 levels in the placebo and the CR BNO 1055 group was not significant. The effects of the three treatments on serum FSH levels are shown in Figure 2. The conjugated estrogens suppressed serum FSH significantly at all three time points whereas no significant effect was seen on serum LH levels (data not shown). The black cohosh extract had no significant effect on both hormones. Due to limited amounts of serum, the measurement of SHBG concentrations was possible only in the serum of 16 women treated with CR BNO 1055, 20 treated with conjugated estrogens, and 15 treated with placebo at the end of the treatment period. A slight decrease in SHBG levels was observed with placebo and CR BNO 1055 treatment, whereas treatment with conjugated estrogens resulted in a significant increase in SHBG levels (Fig. 3).

Markers of bone turnover

Serum analyses of markers of the bone turnover in the postmenopausal women indicated that CR BNO 1055 and conjugated estrogens had beneficial effects.

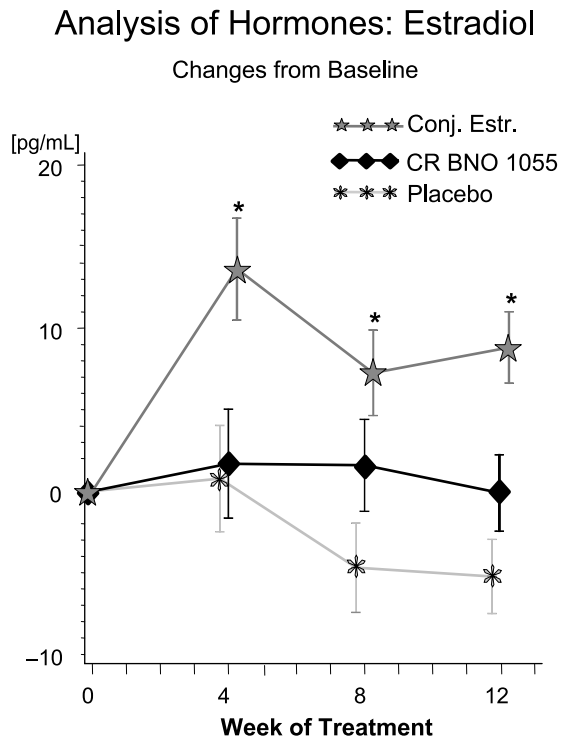

FIG. 1. Treatment with conjugated estrogens increased E_2 levels at all measurement points when compared with placebo and CR BNO 1055. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ vs placebo.

A reliable marker for bone degradation the C-terminal breakdown product of bone-specific collagen-1α1, referred to here as CrossLaps, increased slightly but not significantly with placebo. With CR BNO 1055, CrossLaps did not increase, whereas treatment with the conjugated estrogens produced a significant decrease in CrossLaps after 4 and 12 weeks (Fig. 4).

The concentrations of bone-specific alkaline phosphatase (bALP), which is a metabolic marker for bone formation, were significantly increased after 4 weeks of treatment with conjugated estrogens in comparison with placebo, but this effect was not seen after 8 and 12 weeks of treatment. A significant increase above placebo values of BAP was demonstrated with CR BNO 1055 treatment after 12 weeks (Fig. 5).

Vaginal cytology

Whereas treatment with conjugated estrogens significantly ($P < 0.0001$) increased the number of superficial cells when compared with placebo, a slight increase of the number of superficial cells was observed with CR BNO 1055 treatment, and this effect approached significance ($P = 0.0542$). In the course of the study, a further decrease in the number of superficial cells was observed with placebo (Fig. 6) in comparison with the baseline values.

Lipid metabolism

Significant effects on serum cholesterol (total cholesterol, HDL, and LDL cholesterol) were not observed with CR BNO 1055 or conjugated estrogens treatment (data not shown).

Serum triglyceride levels were significantly higher in women treated with conjugated estrogens or CR BNO 1055 treatments than in placebo-treated women (Fig. 7).

Hematologic and clinical chemistry parameters and coagulation status

The analyses of hematologic and clinical chemistry parameters are detailed in Table 4. There was no indication of significant systematic treatment effects. In particular, none of the three treatments had an influence on the investigated parameters of blood coagulation such as activated thromboplastin time (in seconds) and thromboplastin time as the INR. Hepatic enzymes (ie, SGOT, SGPT, γ-GT) remained unaffected, with a tendency toward decreased levels, with CR BNO 1055 treatment.

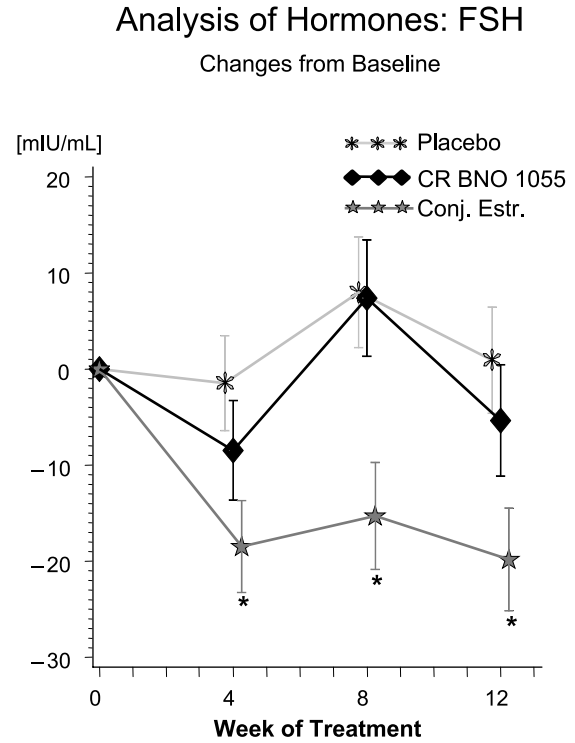

FIG. 2. Treatment with conjugated estrogens decreased serum FSH levels at all time points when compared with placebo. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ vs placebo.

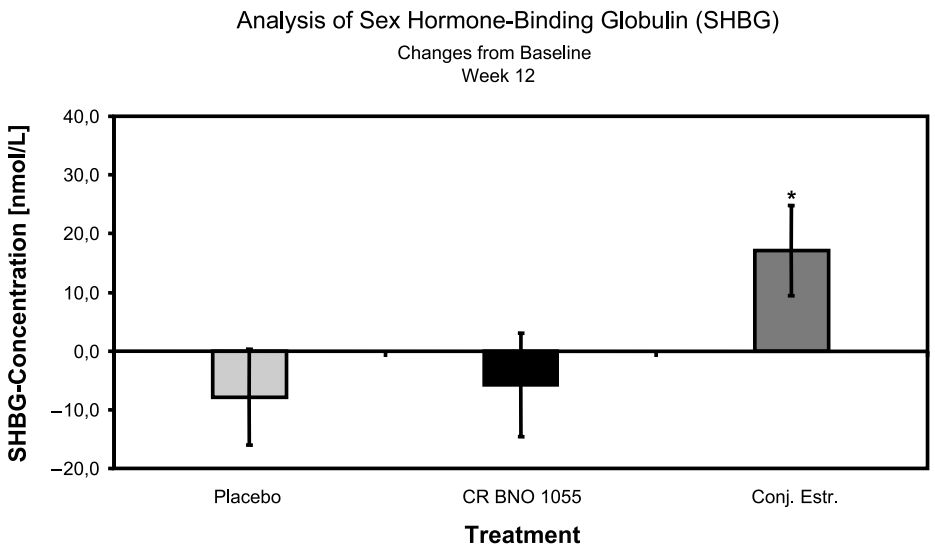

FIG. 3. Serum levels of SHBG were significantly higher in the women treated with conjugated estrogens than in those treated with CR BNO 1055 or placebo. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ vs placebo and CR BNO 1055.

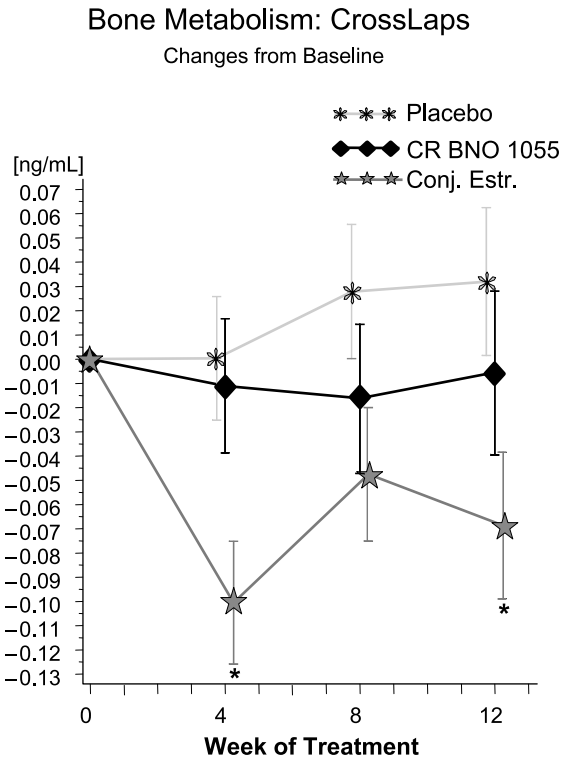

FIG. 4. Serum levels of CrossLaps were significantly lower in the conjugated estrogens group. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ versus placebo.

Blood pressure and heart rate

There were no relevant treatment-related effects on heart rate or on blood pressure in the sitting or standing position. Vertigo was reported as an adverse event in one woman in the placebo group; and vertigo, headache, and hypertension were reported by one woman in the CR BNO 1055 group. In both cases changes in blood pressure and heart rate were not observed.

Body weight

After treatment week 12, there were no significant differences in mean body weight.

DISCUSSION

For this presentation, secondary parameters of a study designed to investigate the therapeutic effects of the black cohosh preparation CR BNO 1055 on climacteric symptoms were evaluated. The favorable outcome on this primary parameter was comparable to the effects of conjugated estrogens and superior to the effects of placebo, a finding that was published previously.[18] Given that this was a small study with a relatively short follow-up period, it was surprising to observe clear treatment effects. In a previous clinical study[30] with another black cohosh extract and in animal experiments with the CR extract BNO 1055,[22] a significant decrease in LH concentration was shown. Hence, hypothalamic mechanisms, which may include serotoninergic effects of CR extracts, may be responsible for the reduction of climacteric symptoms.[23]

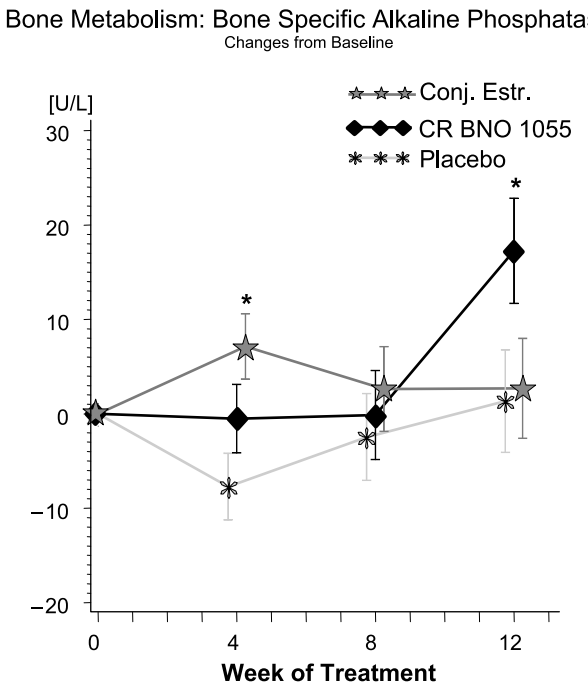

FIG. 5. Serum levels of the BAP remained unchanged with placebo, whereas in comparison with placebo and the conjugated estrogens a significant increase was observed after 12 weeks of CR BNO 1055 treatment. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ vs placebo.

Such an LH-reducing effect of CR BNO 1055 was not seen in the present study, but conjugated estrogens also failed to suppress pituitary LH release over the entire investigation period. The estrogens did, however, suppress serum FSH levels at all three investigated time points, an effect not seen in the CR BNO 1055-treated women. The gonadotropin-releasing hormone pulse generator is known to be intimately involved in generating hot flushes[31]and to selectively modulate either pituitary LH or FSH secretion.[32] This may be the mechanism by which estrogens reduce the number of hot flushes, whereas serotoninergic[23] or dopaminergic[21] components in black cohosh may address other, not-yet-identified mechanisms.

The increase of immunoreactive E_2 levels with conjugated estrogens treatment in the course of their metabolism was expected because the antiserum used to measure these levels cross-reacted to some degree with some of the equine conjugated estrogens or their metabolites. In contrast, with CR BNO 1055 treatment, the absence of an increase in immunoreactive E_2 demonstrated that CR BNO 1055 does not contain immunoreactive estrogen-like material. It was shown in vitro that the ingredients of this black cohosh extract do not bind to recombinant human ERα and ERβ proteins.[21] However, the moderate decrease in FSH at the beginning of the therapy, which was analogous to the conjugated estrogens, suggests that CR BNO 1055 might act in an estrogen-like manner—obviously not

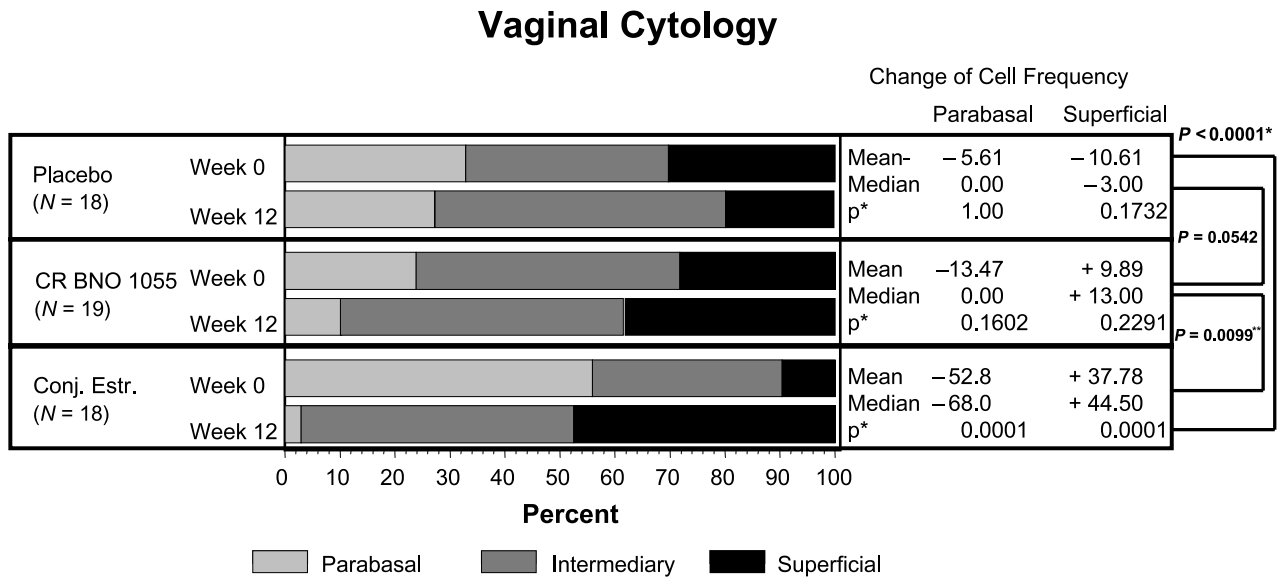

FIG. 6. Treatment with conjugated estrogens significantly increased the number of superficial cells when compared with placebo and CR BNO 1055. After 12 weeks of treatment with CR BNO 1055, the number of superficial cells was slightly increased. When compared with placebo, this effect approached significance. In contrast to both verum preparations, the number of superficial cells further decreased after 12 weeks of treatment with placebo. Shown are the *P* values (Wilcoxon signed rank test) of the differences versus placebo (*) and CR BNO 1055 (**).

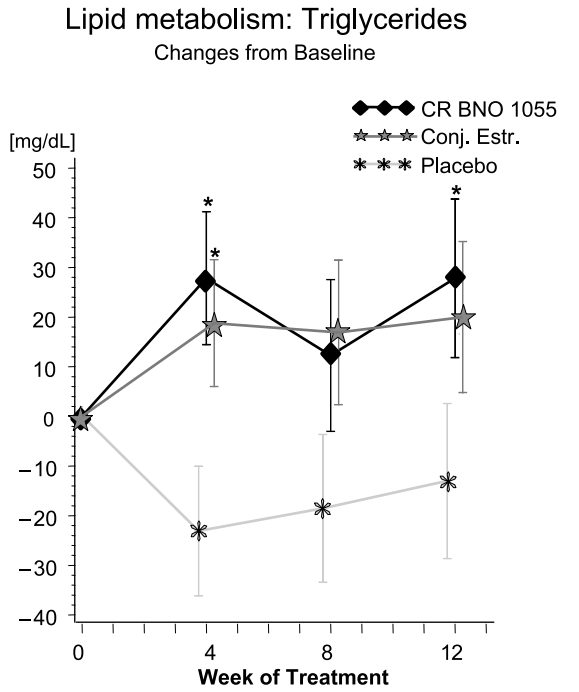

FIG. 7. With the conjugated estrogens and CR BNO 1055 treatments, a significant increase in serum triglyceride levels was observed within 4 weeks. Means adjusted for baseline and center differences and standard errors of the mean are given. *$P < 0.05$ vs placebo.

via ERα or ERβ, but possibly via E_2-binding proteins, of which the presence was demonstrated in the endometrium of pigs and women.[21]

As expected, serum levels of the transport binding protein of sex steroids, SHBG, were significantly higher with conjugated estrogens treatment when compared with placebo, and this effect was not seen in the CR BNO 1055-treated women. In addition, serum concentrations of hepatic enzymes such as SGOT, SGPT, and γ-GT showed a clear tendency toward reduction in CR BNO 1055-treated women, an effect not seen in the women treated with conjugated estrogens. Taken together, these data indicate that neither an increase in liver metabolism nor damaging hepatocellular processes took place in the CR BNO 1055-treated women.

The effects of CR BNO 1055 on markers of bone metabolism indicate bone anabolic effects. The osteoblasts and osteoclasts, cells responsible for bone formation and degradation, are receptive to estrogens.[33-35] In women in the reproductive phase or under estrogen substitution, there exists a balance of activity of both cell types. After resorptive osteoclast activities, osteoblasts react directly with bone formation. The underlying mechanisms of bone metabolism and of the development of osteoporosis after estrogen withdrawal have been extensively studied, and it has been shown that in ovariectomized animals and in postmenopausal women, the activity of both cell types (osteoblasts and osteoclasts) is increased but resorptive osteoclast activity prevails, resulting in reduced bone mineral density and osteoporosis.[36] Therefore, markers of osteoblast and osteoclast activity are increased in postmenopausal women and in ovariectomized rats. Estrogens are able to compensate this effect and to thereby reduce the serum surrogate parameters. Such beneficial effects of black cohosh extracts on blood and urinary bone markers and on bone mineral density have been demonstrated previously in ovariectomized rats[19,25] and confirmed in the present study in postmenopausal women. The black cohosh preparation BNO 1055 has clear beneficial effects on surrogate parameters of bone metabolism. As a parameter of bone formation (ie, osteoblast activity), BAP was measured in the serum. Compared with the placebo and conjugated estrogens groups, a significant increase in BAP concentrations was observed in the CR BNO 1055 group, indicating an increase of osteoblast activity that began within the first 4 weeks of treatment and became statistically significant at 12 weeks. This is, according to our knowledge, the first demonstration of a plant-derived principle with primarily bone-forming properties. The extent of the bone degradation mediated by osteoclasts has been determined by measuring serum concentrations of C-terminal metabolic products of the bone-specific collagen-1 α 1 (the so-called CrossLaps). With placebo, a slight increase in CrossLaps was observed, whereas treatment with conjugated estrogens lowered the concentrations of CrossLaps significantly in comparison with placebo, indicating the well-known antiresorptive effects of estrogens in the bone. CrossLaps with CR BNO 1055 treatment were at an intermediate concentration (ie, between those of the placebo and the estrogen-treated levels), and this finding may also suggest slightly decreased activity of the osteoclasts with possibly favorable effects on bone mineral density. The short treatment period of 12 weeks did not allow for studies of bone mineral density, but it is known from rat experiments that the observed effects on serum surrogate parameters of bone turnover also reflect BMD.[19,25] Studies using longer treatment periods and higher numbers of patients that include measurement of BMD are necessary to verify these assumptions.

In postmenopausal women who are not taking estrogenic treatment, the vaginal "maturity index" is

TABLE 4. *Changes in hematologic and clinical chemistry parameters over the course of the study (differences week 12 – week 0)*

	Placebo	CR BNO 1055	Conjugated estrogens
Hematology			
Thrombocytes (Gpt/L)	29.50 ± 118.16	–3.60 ± 41.32	3.27 ± 44.48
Leukocytes (Gpt/L)	0.13 ± 2.10	0.16 ± 1.29	–0.44 ± 1.39
Erythrocytes (Tpt/L)	–0.11 ± 0.31	–0.12 ± 0.35	–0.08 ± 0.31
Hemoglobin (g/L)	–5.40 ± 12.21	–3.92 ± 6.26	–3.00 ± 6.70
Hematocrit (%)	–1.20 ± 3.05	–1.18 ± 2.71	–0.85 ± 2.29
Basophils (%)	–0.07 ± 0.37	0.04 ± 0.61	0.03 ± 0.50
Eosinophils (%)	–0.48 ± 1.84	–0.36 ± 1.74	0.42 ± 1.86
Bands (%)	0.07 ± 0.75	0.03 ± 2.04	–0.58 ± 1.73
Segmented (%)	0.47 ± 13.45	0.48 ± 10.69	3.00 ± 6.54
Lymphocytes (%)	1.27 ± 12.07	0.14 ± 11.08	–1.84 ± 8.37
Monocytes (%)	–0.11 ± 3.38	0.00 ± 2.28	–0.21 ± 2.53
Clinical chemistry			
Glucose (mmol/L)	0.32 ± 1.21	0.15 ± 0.94	0.07 ± 0.70
Urea (mmol/L)	–0.14 ± 1.12	–0.19 ± 1.43	0.12 ± 1.07
Creatinine (μmol/L)	1.46 ± 11.23	0.89 ± 11.88	0.88 ± 10.59
Uric acid (μmol/L)	–41.71 ± 63.94	4.00 ± 38.12	9.32 ± 67.98
Bilirubin tot. (μmol/dL)	–0.11 ± 0.51	0.10 ± 0.36	–0.15 ± 0.39
Protein tot. (g/L)	0.71 ± 4.71	–2.38 ± 4.38	–0.15 ± 3.88
Iron (μmol/L)	0.34 ± 5.75	0.37 ± 6.05	–4.37 ± 9.66
SGOT (nkat/L)	–52.00 ± 131.29	–135.00 ± 259.73	0.91 ± 115.18
SGPT (nkat/L)	–83.00 ± 174.06	–169.50 ± 240.67	–23.18 ± 301.05
γ-GT (nkat/L)	–58.42 ± 309.47	–65.50 ± 170.52	34.29 ± 290.44
Alk. phosphatase (nkat/L)	53.16 ± 444.12	19.50 ± 225.12	30.00 ± 188.30
Sodium (mmol/L)	1.34 ± 4.39	1.64 ± 3.46	0.16 ± 3.17
Potassium (mmol/L)	–0.03 ± 0.33	0.25 ± 0.50	–0.05 ± 0.44
Calcium (mmol/L)	–0.01 ± 0.17	0.02 ± 0.17	–0.03 ± 0.18
Thromboplastin time (s)	–0.29 ± 3.24	–0.58 ± 5.25	–1.53 ± 7.61
Thromboplastin time as INR	–0.01 ± 0.15	–0.02 ± 0.08	–0.4 ± 0.20

Values are means and standard deviations.

generally shifted toward parabasal cells whereas more superficial cells are found with estrogenic compounds.[29] Because the patients in this study did not take other estrogenic substances, it is highly probable that a possible shift of the maturity index toward intermediary and superficial cells was due to the trial drug. Conjugated estrogens stimulated the vaginal mucosa, which resulted in an increased number of superficial cells. This is a well-known estrogenic effect that decreases pH values, which prevent ascending bacterial infections. Furthermore, estrogens improve lubrication upon sexual arousal. Even though the effect was less obvious than with conjugated estrogens, an increase in the number of superficial cells was observed with CR BNO 1055 administration, which was close to statistical significance when compared with placebo. This effect correlated with a significant decrease in the feeling of a dry vagina, which was published previously.[18] Slightly increased numbers of vaginal superficial cells were also observed in several previous (but not all) clinical studies with another black cohosh preparation.[14,17] This effect may only be seen in postmenopausal women without any estrogenic pretreatment and with a history of menopause lasting longer than 6 months in whom a shift from superficial to parabasal and intermediary cells had occurred. This could explain why in some studies using a black cohosh preparation, in which perimenopausal women with fluctuating endogenous estradiol levels were also included, this desired effect at the vaginal epithelium was not observed.[16]

In ovariectomized rats, estrogens have profound cholesterol-reducing effects that include a reduction in both HDL and LDL.[37] Similar effects were observed on cholesterol and LDL in women with several HT preparations.[38] This effect, however, was not seen in the present study. Neither the conjugated estrogens nor CR BNO 1055 influenced cholesterol, HDL, or LDL. With conjugated estrogens, an increase in serum triglyceride levels in individual cases is known and was observed in the present clinical study under both treatments. The increase in serum triglyceride levels with CR BNO 1055 and conjugated estrogens was certainly not due to a nutritional supply before blood collection and incorporation into chylomicrons, because blood was collected before food intake. Hence, increased triglycerides most likely represent increased hepatic very-low-density lipoproteins, which may increase atherosclerotic risk. However,

estrogens have lipolytic or antilipotrophic effects,[39] and CR extracts may have similar properties.[19]

Concerning hematologic and clinical chemical parameters, coagulation status, blood pressure, heart rate and body weight, no systematic treatment effects of conjugated estrogens or CR BNO 1055 were observed. It should be emphasized that both verum treatments did not alter hemostasis factors. Recently, a case of acute liver failure was reported after treatment with a black cohosh preparation. Therefore, several liver parameters were determined in the serum of our study participants. If anything, a slight reduction in SGOT, SGPT, and γ-GT was noted, which would not indicate hepatotoxicity of the CR BNO 1055 extract. This preparation CR BNO 1055 was well tolerated, and significant adverse effects other than those experienced with placebo were not reported.

Some of the observed effects of the CR preparation BNO 1055 are similar to those of the conjugated estrogens, whereas others differ in quality and/or quantity. Previously, we showed that treatment resulted in a clear improvement of many climacteric symptoms[18] and was comparable to the effects seen in women treated with conjugated estrogens. Both preparations were superior to placebo.[18] Our in vitro tests have shown that CR BNO 1055 has binding activity to cytosolic estrogen binding (receptor) sites of human, rat, or porcine origin, but not to recombinant human estrogen receptor α or β proteins (ERα or ERβ).[21] The mode of action of the CR preparation remains enigmatic, but may involve organ-specific ER enhancer or repressor genes. This may point to a totally new way in which substances elicit organ-specific estrogen-like, hence positive, selective ER modulator effects.

Whether a black cohosh preparation of a given producer has properties similar to an extract of another producer is not known at present. There is evidence that aqueous/ethanolic extracts differ from isopropanolic extracts because different bands exist after thin-layer chromatography of CR extracted with ethanol or isopropanol.[40] Because the active compounds in black cohosh are not known, it is also unknown whether they differ from preparation to preparation. In each case, the user should only use preparations for which efficacy has been shown in clinical studies.

CONCLUSIONS

In conclusion, the beneficial effects on climacteric symptoms of CR BNO 1055 and of conjugated estrogens were reported previously.[18] In the present study, it was shown for the first time that the black cohosh preparation CR BNO 1055 may have antiosteoporotic effects as indicated by serum surrogate parameters of bone turnover. Additionally, the herbal preparation had the desired estrogen-like effects on the vaginal mucosa. The lack of effects on serum liver enzymes and on hemostasis factors may indicate that the CR extract BNO 1055 has no effect on the liver.

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