Original article

POST REPRODUCTIVE HEALTH

Post Reproductive Health
2022, Vol. 28(3) 149–157

DOI: 10.1177/20533691221116769
journals.sagepub.com/home/min
SAGE

Effects of ultra-low dose hormone therapy on biochemical bone turnover markers in postmenopausal women: A randomized, placebo-controlled, double-blind trial

Lucia Costa-Paiva[1], Maria Celeste O Wender[2], Rogerio B Machado[3], Luciano M Pompei[4], Eliana A Nahas[5], Jorge Nahas-Neto[5], Sonia Y Del Debbio[6], Mariangela Badalotti[7] and Achilles M Cruz[8]

Abstract

Objective: Evaluate the effects of ultra-low-dose hormone therapy (Ultra-LD HT) with 17β-estradiol 0.5 mg and norethisterone acetate 0.1 mg (E2 0.5/NETA 0.1) versus placebo on bone turnover markers (BTM) in postmenopausal women.

Study Design: A multicenter, double-blind, randomized, placebo-controlled study was performed with 107 participants who received one tablet daily of E2 0.5/NETA 0.1 or placebo for 24-weeks. Bone formation markers-N-terminal propeptide of type I procollagen (PINP) and Bone-specific alkaline phosphatase (BSAP), and bone resorption markers-C-telopeptide of type I collagen (CTX-I) and N-telopeptide crosslinked of type I collagen (NTX) were assessed before and at 12 and 24-weeks of treatment.

Results: Women treated with E2 0.5/NETA 0.1 had a significant reduction in the PINP marker from baseline (58.49 ± 21.12 μg/L) to week 12 (48.31 ± 20.99 μg/L) and week 24 (39.16 ± 16.50 μg/L). Placebo group, the PINP marker did not differ significantly. The analysis of the BSAP indicated a significant increase in the placebo group (13.8 ± 5.09 μg/L and 16.29 ± 4.3 μg/L, at baseline and week 24, respectively), whereas in the treatment group the values did not change. The analysis of the NTX marker showed a significant reduction only in the treatment group (43.21 ± 15.26 nM/mM and 33.89 ± 14.9 nM/mM, at baseline and week 24, respectively). CTX-I had a significant decrease in the treatment group from baseline (0.3 ± 0.16 ng/L) to week 12 (0.21 ± 0.14 ng/L) and week 24 (0.21 ± 0.12 ng/L).

Conclusion: Women receiving E2 0.5/NETA 0.1 experienced reductions in bone resorption and formation markers, an expected effect during the anti-resorptive therapy, suggesting a protective bone effect with the Ultra-LD HT.

Keywords

bone mineral density, bone turnover marker, ultra-low-dose hormone therapy, estrogen, menopause

[1]Department of Obstetrics and Gynecology, Faculty of Medical Sciences, State University of Campinas-UNICAMP Campinas, São Paulo, Brazil
[2]Department of Obstetrics and Gynecology, Federal University of Rio Grande do Sul–UFRGS, Rio Grande do Sul, Brazil
[3]Department of Gynecology and Obstetrics, Jundiai School of Medicine, Jundiai, Brazil
[4]Department of Gynecology and Obstetrics, ABC School of Medicine, Santo Andre, Brazil
[5]Department of Gynecology and Obstetrics, Botucatu School of Medicine UNESP-Sao Paulo State University, Botucatu, Brazil
[6]Paulista Center for Clinical Research-CEPIC, Sao Paulo, Brazil
[7]Department of Gynecology and Obstetrics, PUC-RS, Porto Alegre, Brazil
[8]Department of Clinical Research, Libbs Farmacêutica Ltda, Sao Paulo, Brazil

Corresponding author:
Lucia Costa-Paiva, Department of Obstetrics and Gynecology-CAISM/Unicamp, Alexander Fleming 101, Campinas City, Sao Paulo State, Brazil.
Email: paivaepaiva@uol.com.br

Introduction

The decrease in estrogen levels secondary to ovarian insufficiency in postmenopause is associated with vasomotor symptoms, genitourinary symptoms, and loss of bone mass. Estrogen hormone therapy (HT) is the gold standard treatment for symptom relief and prevention of osteoporosis.[1] Although improving climacteric symptomatology, HT may be associated with undesirable effects and an increased risk of breast cancer and thromboembolic phenomena. For this reason, it has been recommended to prescribe the lowest effective dose to provide benefits and minimize risks.[2,3]

Ultra-low dose hormone therapy (Ultra-LD HT) with daily administration of 0.5 mg 17β-estradiol (E2) and 0.1 mg norethisterone acetate (NETA) is available in some countries is effective in alleviating vasomotor symptoms with a good tolerability profile.[4–7] However, data are limited about which is the lowest dose of HT needed to maintain the benefits of bone loss prevention.

Oral doses of 1 and 2 mg 17β-estradiol[8] or 0.625 mg conjugated equine estrogens (CEE)[9] are known to be highly effective to reduce the risk of fractures. At doses below 1 mg E2, some studies have shown more modest results in increasing bone density and decreasing bone markers[10,11] and there is no strong body of evidence evaluating the fractures risk. The Ultra-LD HT demonstrated an increase in bone density in the spine and hip in women treated for 24 months with estradiol 0.5 mg and norethisterone 0.25 mg.[12]

Bone mineral density (BMD) assessment using dual-energy X-ray absorptiometry (DXA) scan is the current gold standard test for the diagnosis of osteoporosis. However, considering that BMD does not change in the short term, the evaluation of molecular markers of bone metabolism are novel tools that rapidly detect the dynamics of bone remodeling concerning bone formation and resorption during therapies for osteoporosis. Levels of bone markers decrease rapidly with antiresorptive therapies, and the levels reached after 3–6 months of therapy are more strongly associated with fracture outcome than changes in BMD.[13]

We aimed to evaluate the effects of an oral ultra-low dose HT containing 17β-estradiol 0.5 mg + norethisterone acetate 0.1 mg (E2 0.5/NETA 0.1) compared to placebo in symptomatic postmenopausal women. BT markers, N-terminal propeptide of type I procollagen (PINP), C-terminal telopeptide of type I collagen (CTX-I), N-terminal telopeptide of type I collagen (NTX-I), and bone-specific alkaline phosphatase (BSAP) were evaluated as secondary endpoint from baseline to 12 and 24 weeks of treatment.

Methods

Sample size

The sample size calculation was based on the primary outcome of the study, mean variation in the frequency of vasomotor symptoms between the baseline period and after 24 weeks of treatment with the combination E_2 0.5/NETA 0.1, compared to placebo according to CHOICE Study.[5] The formula for two independent samples was used, based on a single-tailed T-student test with errors $\alpha = 2.5\%$ (unilateral) and $\beta = 20\%$. A standard deviation of 40% for a distinction between groups was assumed based on the observed results by Notelovitz and Mattox (2000).[14] The number of participants was calculated for each treatment arm to demonstrate the superiority of the E2 0.5/NETA 0.1 group compared to placebo with 80% power and a 5% bilateral significance level. Considering a loss rate of up to 50%, we chose to include 120 patients in the study, 60 in each treatment group.

For the secondary analysis of bone markers, the variation in sample power comparing treatment and (week 24 versus baseline) was considered. The calculated powers obtained were as follows: PINP 99%, NTX 78%, BSAP 51%, and CTX-I 72% from the t-test using a corrected alpha of 1.25%.

Participants

A Phase III, comparative, double-blind, multicenter, randomized, placebo-controlled study was conducted. The protocol was approved by the Ethics Committee of each participating center and informed consent was obtained from each participant before starting data collection. CONSORT guidelines were followed, and the study was cataloged in the International Standard Randomized Controlled Trial Number (ISRCTN registry 76005731).

Healthy postmenopausal women were recruited and enrolled at seven Brazilian Climacteric Centers. Inclusion criteria were women with an intact uterus, age between 45 and 60 years, amenorrhea for at least 12 months, with follicle-stimulating hormone (FSH) $\geq$ 30 mIU/mL and estradiol $\leq$30 pg/mL, BMI $\geq$19 and $\leq$35.0 kg/m^2, presenting at least 7 episodes/day of moderate or severe vasomotor symptom or at least 50 episodes/week of moderate or severe vasomotor symptom in the pre-selection evaluation. The results of the study related to the effects on vasomotor symptoms will be reported in another publication. Exclusion criteria: surgical menopause, use of hormonal oral therapy in the last 8 weeks or transdermal HT in the last 4 weeks, abnormal oncologic colpocitology, cervical intraepithelial neoplasia (CIN), or cervical cancer; history or suspected of breast or endometrial cancer, history of arterial

or venous thromboembolism, diabetes mellitus, hypertension (systolic blood pressure >150 mmHg or diastolic blood pressure>90 mmHg), endometrial thickness, heavy smoking or use of drugs with effects on bone metabolism like glucocorticoids, GnRH analogs, anticonvulsants, anticoagulants, immunosuppressive drugs, levothyroxine, calcitonin, and bisphosphonates in the last 12 months.

Intervention

Participants were randomized (1:1) to one of the two treatment groups as follows: E2 0.5 mg/NETA 0.1 mg given orally once daily in blister packs of 28 tablets or placebo with identical characteristics. All women received calcium supplementation (500 mg)/Vitamin D (200 IU) orally once daily.

Bone turnover markers

The level of biochemical BTMs was assessed according to the recommendations of the International Osteoporosis Foundation[15] before treatment and after 12 and 24-weeks. Blood and urine samples were collected in fasting conditions between 7 a.m. and 9:30 a.m. Bone formation markers measured were bone-specific alkaline phosphatase (BSAP) and amino-terminal propeptide of type 1 procollagen (PINP). Bone resorption markers were cross-link C-terminal telopeptide (CTX-I) and terminal N-telopeptides (NTX). Serum was analyzed for BSAP (Advia automated chemiluminescence immunoassay), CTX-I (Electrochemical luminometer, Modular), and PINP (Electrochemical luminescence, E511 Roche). Urine was analyzed for measured NTX (Electrochemical luminometer, Vitro Johnson) three times and compared between the two groups.

Laboratory tests

Laboratory evaluations were conducted to determine serum levels of follicle-stimulating hormone (FSH) and estradiol using chemiluminescence. Evaluation of metabolic safety was performed concerning complete blood count. The evaluation of thyroid-stimulating hormone (TSH) and glycated hemoglobin was performed only at the beginning of treatment. Evaluations were performed in the morning, before, and after 12- and 24-weeks of treatment. Analyses were performed using the Sysmex XE–2100D, Variant II Turbo–Biorad, Modular E170, Centaur, and Advia 2400 equipment.

Safety was assessed by analyzing the data obtained in clinical records, physical examination, blood pressure measurement, weight, gynecological examination and laboratory tests, mammography, a cervical pap smear, transvaginal ultrasound, and the analysis of the adverse events occurring during the study.

Adherence

The patients were instructed to bring the package(s) of the drug used, at each visit. The calculation of adhesion treatment was carried out by the researcher at each visit counting the number of missing and returned tablets and dividing this value by the "ideal number of tablets" being considered an adhesion of 80% or greater.

Statistical analyses

The homogeneity of the groups was evaluated by Student's t-test (quantitative variables) and Chi-square test (categorical variables). Analysis was performed by intention-to-treat (ITTe) efficacy and all randomized participants who had taken at least one dose of the study medication and who performed at least one post-baseline assessment were included for the ITTe population.

The BTMs were analyzed by treatment group at baseline, week 12 and week 24, and linear longitudinal models using treatment as a fixed effect, visit as repeated, center as random, baseline value as covariate, and interaction between visit and treatment, to compare the difference between the Ultra-LD HT group and the placebo group. If the interaction was significant at 5%, groups were analyzed separately regarding the changes between baseline using contrast analysis.

For all analyses, the level of significance was $p < .05$. For these statistical analyzes was used Statistical Analyzes System software (SAS).

Results

A total of 192 women were screened in the study, of which 73 were considered as screening failure. One hundred and nineteen women were randomized, being 59 in the E2 0.5/NETA 0.1 and 60 in the placebo group. Efficacy analysis (ITTe analysis population) consisted of all patients randomized, who met the inclusion/exclusion criteria of the protocol, who used the medication study and who performed at least one post-baseline assessment of the primary efficacy variable (52 participants in the ultra-low HT and 55 in the placebo groups) (Figure 1).

The clinical baseline characteristics of the studied population according to the treatment group are shown in Table 1. The mean ± SD age of participants was 53.8 ± 3.9 years. Most of the women were white with no differences in demographic or clinical characteristics between the groups.

Women treated with E2 0.5/NETA 0.1 had a significant ($p < .001$) reduction in the PINP marker from baseline

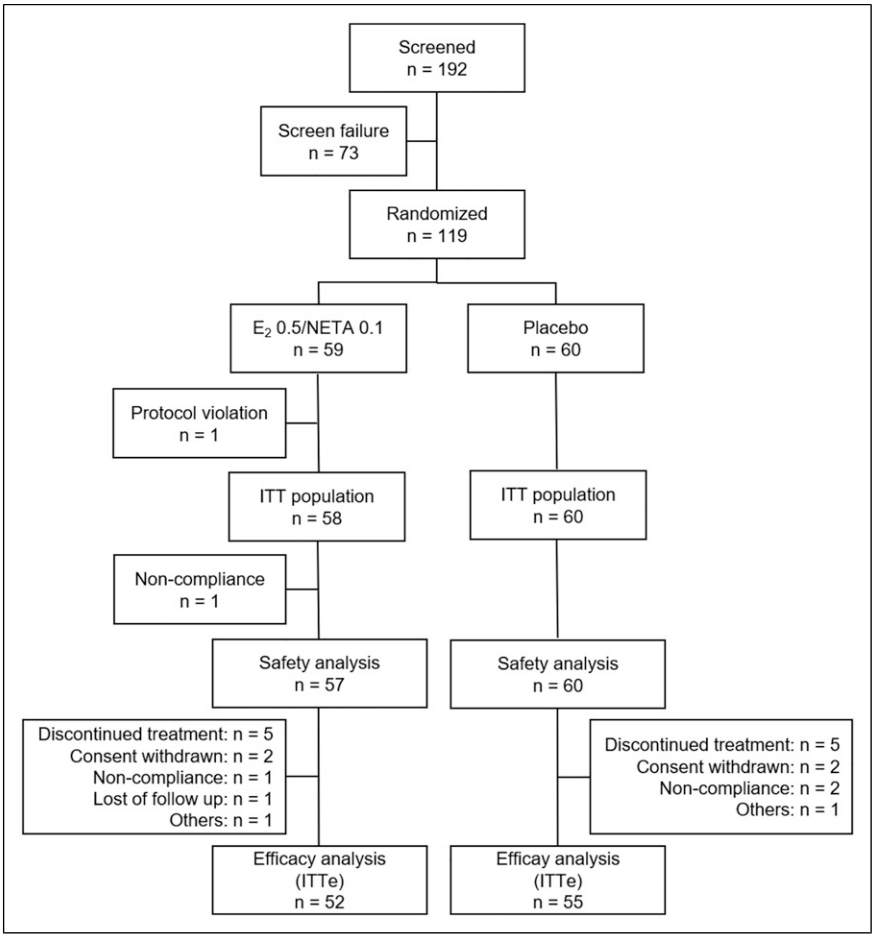

Figure 1. Flowchart of participants through the trial. E2 0.5/NETA 0.1 = 17β-estradiol 0.5 mg + norethisterone acetate 0.1 mg; ITT: Intention to treat analysis; ITTe: Intention to treat efficacy analysis.

(58.49 ± 21.12 µg/L) to week 12 (48.31 ± 20.99 µg/L) 95% CI for change from baseline (-14.8; -5.9) and week 24 (39.16 ± 16.50 µg/L) 95%CI for change from baseline (-23.6; -14.9).

In the placebo group, the PINP marker did not differ significantly (Figure 2).

The analysis of the BSAP indicated a significant ($p <$ .001) increase in the placebo group (13.8 ± 5.09 µg/L and 16.29 ± 4.3 µg/L, at baseline and week 24, respectively), 95%CIs: (0.4; 2.5) – week12 - baseline (1.1; 3.9) – week24 - baseline whereas in the active group the values did not change (Figure 3). The analysis of the NTX marker showed a significant ($p <$ .001) reduction only in the treatment group (43.21 ± 15.26 nM/mM and 33.89 ± 14.9 nM/mM, at baseline and week 24, respectively) 95%CIs: (-9.9; 0.4) – week12 from baseline; (-14.9; -5.1) – week 24 from baseline as shown in Figure 4. Finally, the analysis of CTX-I showed a significant ($p <$ .001) decrease only in the treatment group from baseline (0.3 ± 0.16 ng/L) to week 12 (0.21 ± 0.14 ng/L) and week 24 (0.21 ± 0.12 ng/L) 95%CIs: (-0.14; -0.05) – week 12 from baseline; (-0.14; -0.04) – week 24 from baseline (Figure 5). The results of laboratory evaluations for safety analysis did not show significant changes in blood count, renal or hepatic function, fasting glucose, insulinemia, and lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides). There were no changes in blood pressure, body weight, and clinical and gynecological examination over the 24 weeks (data not shown).

A total of 105 adverse events were reported after randomization, 69 (65.7%) in the treatment group and 36 (34.3%) in the placebo group. Overall, the majority (98.6%) of the adverse events reported in the treatment group were non-severe and mild (85.5%), with transient symptoms and without interference in the patient's daily activities. Two (1.7%) women had serious events, one (1.8%) in the

Table 1. Demographic and clinical baseline characteristics of participants according to the treatment group. Data are presented as mean ± standard deviation (SD) and range or as number (%).

Variable	E2 0.5/NETA 0.1 (n = 58)	Placebo (n = 60)	All subjects (n = 118)
Age (years)			
Mean (± SD)	53.4 (4.0)	54.1 (3.7)	53.8 (3.9)
Range	45–60	45–60	45–60
Race – n (%)			
White	45 (77.6)	45 (75.0)	90 (76.3)
Black	6 (10.3)	9 (15.0)	15 (12.7)
Asian	1 (1.7)	1 (1.7)	2 (1.7)
Other	6 (10.3)	5 (8.3)	11 (9.3)
Bodyweight (kg)			
Mean (± SD)	69.0 (11.4)	68.9 (9.7)	68.9 (10.5)
Range	48.7–98.0	50.0–98.0	48.7–98.0
Body mass index (kg/m^2)			
Mean (± SD)	27.2 (3.8)	27.6 (3.5)	27.4 (3.7)
Range	20.5–35.1	21.4–34.8	20.5–35.1
Systolic blood pressure (mmHg)			
Mean (± SD)	120.1 (10.3)	119.4 (11.4)	119.7 (10.9)
Range	100–150	90–150	90–150
Diastolic blood pressure (mmHg)			
Mean (± SD)	75.8 (7.0)	76.9 (8.1)	76.4 (7.6)
Range	60–90	50–90	50–90
Smokers – n (%)	11 (19.0)	12 (20.0)	23 (19.5)
Estradiol (pg/ml) mean (± SD)	17.3 (6.7)	15.1 (5.1)	16.2 (6.0)

E2: 17β-estradiol; NETA: norethisterone acetate.

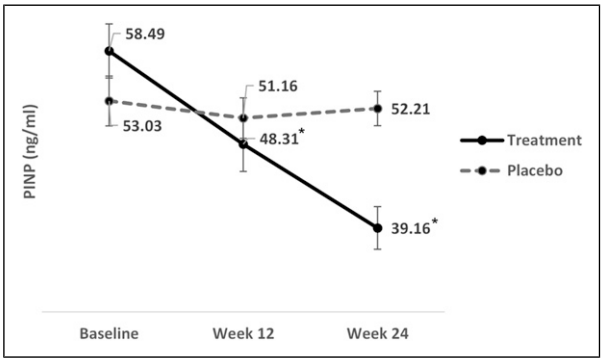

Figure 2. PINP marker on the baseline, week 12 and week 24 in placebo and treatment groups. The values are mean ± SD. *p < .001 compared to baseline of the same group. PINP: N-terminal propeptide of type I procollagen; SD: standard deviation.

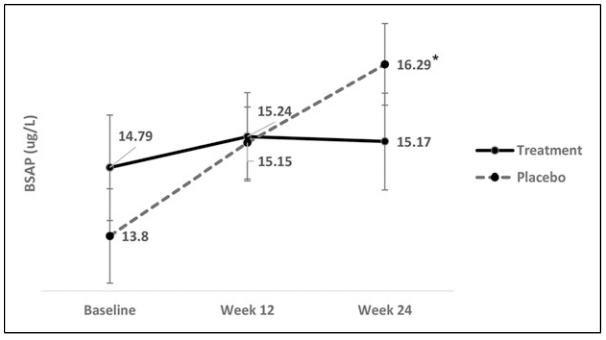

Figure 3. BSAP marker on the baseline, week 12 and week 24 in placebo and treatment groups. The values are mean ± SD. *p < .001 compared to baseline of the same group. BSAP: Bone-specific alkaline phosphatase; SD: standard deviation.

treatment group (cholecystectomy), and one (1.7%) in the placebo group (gallbladder stones).

The most common adverse reactions (≥1%) were vaginal bleeding, increased blood pressure, headache, dysmenorrhea, breast pain, pelvic pain, hypercholesterolemia, nausea, and dyslipidemia. Vaginal bleeding was the most frequently reported adverse event. The event was reported by 14 (24.6%) women in the treatment group and in 4 (6.7%) in the placebo group and was related to the study medication according to the investigator's blind assessment. It is noteworthy that no patient interrupted treatment due to an adverse event. No cases of venous or arterial thromboembolism were reported in the study.

Discussion

This study showed that the ultra-LD HT with E2 0.5/NETA 0.1 reduced the bone turnover by a reduction in the levels of

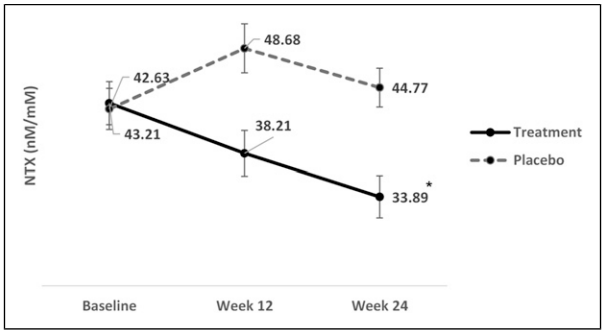

Figure 4. NTX marker on the baseline, week 12 and week 24 in placebo and treatment groups. The values are mean ± SD. *p < .001 compared to baseline of the same group. NTX: N-telopeptide crosslinked of type I collagen; SD: standard deviation.

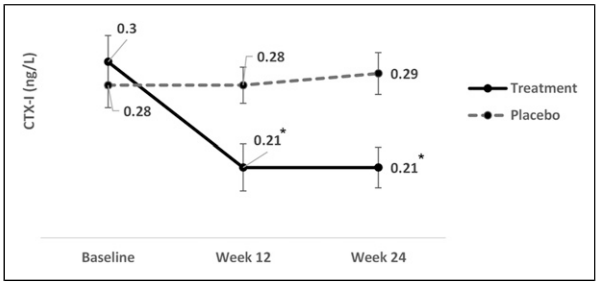

Figure 5. CTX-I marker on the baseline, week 12 and week 24 in placebo and treatment groups. The values are mean ± SD. *p < .001 compared to baseline of the same group. CTX-I: C-telopeptide of type I collagen; SD: standard deviation.

bone resorption markers (NTX and CTX-I) and bone formation marker (PINP). These findings suggest that this dose of 17β-estradiol may have beneficial effects in preventing bone loss in postmenopausal women. Studies with different low or ultra-low dose therapies also have shown that these doses of estrogen can decrease resorption and prevent bone loss.[11,12,16–20]

Ultra-LD HT formulations vary in composition and route of administration. Some of these are transdermal 17β-estradiol from 0.014 to 0.25 mg/d and 17β-estradiol by oral route from 0.5 to 0.25 mg associated with NETA (0.1–0.250 mg/d) or dihydrogesterone. A systematic review of the effects of HT with different doses of estrogen showed that bone response seems to be directly related to the dose, as the reduction in resorption at lower doses is slightly less than at high doses.[10,17] The authors reported an increase in BMD of the lumbar spine with conventional doses (1–2 mg estradiol) ranging from 2.5% to 8.5% in 1–4 years[10,21–23] compared to 2%–6.2% at lower doses (<1 mg/d).[11,12,18,19] Gambacciani et al. (2008)[12] found an increase in the spine and hip BMD in women treated for 24 months with the same regimen of ultra-LD estradiol used in the present study. The only difference is that the dose of norethisterone acetate was slightly higher (0.25 mg) than the dose used in the present study (0.1 mg/d).

We evaluated BTMs that are optimal manner to monitor adherence and treatment effects on bone remodeling.[24,25] The markers provide an idea of bone turnover faster than the change in BMD since changes in treatment-induced BMD can take up to 3 years. Thus, bone remodeling markers provide a rapid response to treatment and are predictive of fracture risk reduction than BMD measures.[25–28]

In the treatment with antiresorptive agents such as HT and bisphosphonates, a reduction in resorption markers is expected, followed by a reduction in formation markers.[26,28] In the present study, the HT treatment led to a reduction in bone turnover with less resorption evidenced by the reduction of the NTX marker (-21.5% in the ultra-low dose group vs +5.0% in the placebo) and CTX-I marker (-30.0% in the ultra-low dose group vs +3.5% in the placebo), which were statistically significant at the final visit when comparing to the baseline.

There was no significant change in the BSAP marker in the treatment group. In the placebo group, this bone formation marker increased, however, only in the ITTe population, which may mean mere chance. About the bone formation marker PINP, the results of the present study showed that there was less bone formation, which was clear from the reduction in the levels of this marker (-33.0% in the ultra-low dose group vs -1.5% in placebo), which was expected, since when there is a reduction in bone resorption, consequently there is also reduction in formation due to the interaction between osteoclast and osteoblast. Reductions in PINP have also been observed in large trials with antiresorptive treatment.[29]

Few studies have evaluated the effects of Ultra-LD on bone remodeling markers. Rubinacci et al. (2003)[18] evaluated the effects of the ultra-LD transdermal therapy with E2 0.025/NETA 0.125 mg versus placebo for 24 months. The authors observed, in addition to the increase in BMD, a decrease in the values of bone remodeling markers. In the study by Ettinger et al. (2004)[19] the transdermal therapy with E2 0.014 mg or placebo was associated with calcium and vitamin D supplementation and the levels of osteocalcin and specific BSAP were lower in the estradiol group compared to the placebo group (p < .001). Another study[16] evaluated the same transdermal formulation associated with calcium 1.300 mg and vitamin D 1.000 IU and observed a 15% reduction in osteocalcin and a 26% reduction in BSAP in the hormonal versus the placebo group.

In the study by Prestwood et al. (2003),[11] the group treated with E2 0.25 mg orally versus placebo treatment had an immediate effect on bone resorption with a 28% reduction in NTX after 3 months and a 43% reduction after 12 months, followed by a reduction in BSAP after 12 months of treatment.

Increased levels of bone resorption markers are associated with a two-fold increase in the risk of non-vertebral fractures in women over 65 years[30,31] and under 65 years[13,30] when compared to normal levels. A meta-analysis carried out by the Joint Working Group on Standardization of Biochemical Markers of Bone Turnover showed that a greater reduction in the levels of bone remodeling markers was associated with a greater reduction in the fracture risk. There was a significant association between PINP and the risk of fracture, where the fracture risk gradient for each increase in PINP was 1.23, in addition to showing a significant association between CTX-I and fracture risk gradient, GR = 1.18. These markers can predict the risk of fracture regardless of bone density.[26]

Therefore, changes in the levels of NTX and PINP markers in the present study indicate a reduction in bone resorption with this ultra-low dose regimen. This is the first study that evaluated the effects of the association of ultra-LD oral of 17β-estradiol 0.5 mg with norethisterone acetate 0.1 mg on BTM. Importantly, new approaches in menopausal HT showed that there are no data on incidence and risk of fracture in postmenopausal women using ultra-LD HT.[32]

The safety and tolerability results show that HT with 17β-E2 0.5 mg/NETA 0.1 mg is safe and well-tolerated. The profile of adverse events reported was compatible with the safety of the formulation used in a continuous combined regimen. Vaginal bleeding was more frequent in the treatment group compared to the placebo group. This finding is under the literature data, where the incidence of vaginal bleeding is higher in the groups submitted to HT compared to the placebo.[33] It was demonstrated the high safety and tolerability with the HT of ultra-LD administered in the continuous combined regime, concluding that the safety profile is favorable for the studied drug.[34]

The limitation of this study was the follow-up time was relatively short and the women who participated were mostly young postmenopausal who may not exhibit the same bone effects of this dose that the older postmenopausal women and a adjust for randomization since use of recent hormone replacement therapy before the study begins, that is, a 4 or 8-week washout could have some residual interference in bone remodeling. However, we emphasize that baseline measurements of baseline formation and resorption markers did not show differences between groups, which minimizes the risk of this bias. A strength of the study is the fact that it is a randomized placebo-controlled trial that provides more evidence about the effects of this association on bone remodeling. In addition, it should be noted that the evaluation of these markers has not yet been considered for this ultra-LD HT.

Conclusion

Our results suggest benefits in the prevention of osteoporosis in young postmenopausal women with ultra-LD HT. For women with less severe symptoms, ultra-LD HT may be sufficient to control symptoms. The introduction of ultra-LD HT may add possibilities to individualize treatment for symptomatic postmenopausal women or for those in whom treatment extension is recommended. Prospective studies with larger sample sizes to assess the effects of ultra-LD HT on reducing the risk of fractures in young and elderly postmenopausal women are necessary to better understand the various bone effects of this formulation.

Acknowledgements

The authors would like to thank all participants in this research and Libbs Farmacêutica LTDA.

Author contributions

LCP was involved in concept and design of this study, data collection, performed analyses, interpreted findings, and wrote the manuscript. MCOW; RBM; LMP; EAPN; JNN; SYODD; MB were involved in data collection, data analysis and revision the manuscript. AMC was involved in concept and design of this study, data collection, performed analyses and wrote/edited the manuscript. All authors discussed the results and to final manuscript and approved the final version.

Declaration of conflicting interests

All author(s) received consultancy fees or research grants as a study investigator from Libbs Farmacêutica Ltda (Brazil). AMC is a medical advisor at Libbs Farmacêutica Ltda and reports funding while conducting the study.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Libbs Farmacêutica Ltda (Brazil) provided funding and material support for this research (Number LB1105).

Ethical approval

The study protocol was approved by the Ethics Committee of each of the participating centers and registered in the International Standard Randomized Controlled Trial Number (ISRCTN registry 76005731).

Guarantor

AMC.

ORCID iD

Lucia Costa-Paiva https://orcid.org/0000-0002-9088-6700

References

1. Maclennan AH, Broadbent JL, Lester S, et al. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. *Cochrane Database Syst Rev* 2004; 2009: CD002978. DOI: 10.1002/14651858.CD002978.pub2.
2. Marjoribanks J, Farquhar C, Roberts H, et al. Long-term hormone therapy for perimenopausal and postmenopausal women. *Cochrane Database Syst Rev* 2017; 1: CD004143. DOI: 10.1002/14651858.CD004143.pub5.
3. The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* 2018; 25: 1362–1387. DOI: 10.1097/gme.0000000000001241.
4. Mattsson LA, Ipsen HE, on behalf of the Study Group, et al. Ultra-low-dose estradiol and norethisterone acetate: bleeding patterns and other outcomes over 52 weeks of therapy. *Climacteric* 2015; 18: 419–425. DOI: 10.3109/13697137.2014.999661.
5. Panay N, Ylikorkala O, Archer DF, et al. Ultra-low-dose estradiol and norethisterone acetate: effective menopausal symptom relief. *Climacteric* 2007; 10: 120–131. DOI: 10.1080/13697130701298107.
6. Sturdee DW, Archer DF, on behalf of the CHOICE Study Inves, et al. Ultra-low-dose continuous combined estradiol and norethisterone acetate: improved bleeding profile in postmenopausal women. *Climacteric* 2008; 11: 63–73. DOI: 10.1080/13697130701852390.
7. Stevenson JC, Durand G, Kahler E, et al. Oral ultra-low dose continuous combined hormone replacement therapy with 0.5 mg 17beta-oestradiol and 2.5 mg dydrogesterone for the treatment of vasomotor symptoms: results from a double-blind, controlled study. *Maturitas* 2010; 67: 227–232. DOI: 10.1016/j.maturitas.2010.07.002.
8. Wells G, Tugwell P, Shea B, et al. The osteoporosis methodology group, The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. *Endocr Rev* 2002; 23: 529–539. DOI: 10.1210/er.2001-5002.
9. Cauley JA, Robbins J, Chen Z, et al. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. *Jama* 2003; 290: 1729–1738. DOI: 10.1001/jama.290.13.1729.
10. Lindsay R, Gallagher JC, Kleerekoper M, et al. Effect of lower doses of conjugated equine estrogens with and without medroxyprogesterone acetate on bone in early postmenopausal women. *Jama* 2002; 287: 2668–2676. DOI: 10.1001/jama.287.20.2668.
11. Prestwood KM, Kenny AM, Kleppinger A, et al. Ultralow-dose micronized 17beta-estradiol and bone density and bone metabolism in older women: a randomized controlled trial. *Jama* 2003; 290: 1042–1048. DOI: 10.1001/jama.290.8.1042.
12. Gambacciani M, Cappagli B, Ciaponi M, et al. Ultra low-dose hormone replacement therapy and bone protection in postmenopausal women. *Maturitas* 2008; 59: 2–6. DOI: 10.1016/j.maturitas.2007.10.007.
13. Garnero P, Sornay-Rendu E, Claustrat B, et al. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *J Bone Miner Res* 2000; 15: 1526–1536. DOI: 10.1359/jbmr.2000.15.8.1526.
14. Notelovitz M and Mattox JH. Suppression of vasomotor and vulvovaginal symptoms with continuous oral 17beta-estradiol. *Menopause* 2000; 7: 310–317. DOI: 10.1097/00042192-200007050-00005.
15. Delmas PD, Eastell R, Garnero P, et al. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. *Osteoporos Int* 2000; 11(Suppl 6): S2–S17. DOI: 10.1007/s001980070002.
16. Huang AJ, Ettinger B, Vittinghoff E, et al. Endogenous estrogen levels and the effects of ultra-low-dose transdermal estradiol therapy on bone turnover and BMD in postmenopausal women. *J Bone Miner Res* 2007; 22: 1791–1797. DOI: 10.1359/jbmr.070707.
17. Peeyananjarassri K and Baber R. Effects of low-dose hormone therapy on menopausal symptoms, bone mineral density, endometrium, and the cardiovascular system: a review of randomized clinical trials. *Climacteric* 2005; 8: 13–23. DOI: 10.1080/13697130400012288.
18. Rubinacci A, Peruzzi E, Modena AB, et al. Effect of low-dose transdermal E2/NETA on the reduction of postmenopausal bone loss in women. *Menopause* 2003; 10: 241–249. DOI: 10.1097/00042192-200310030-00012.
19. Ettinger B, Ensrud KE, Wallace R, et al. Effects of ultralow-dose transdermal estradiol on bone mineral density: a randomized clinical trial. *Obstet Gynecol* 2004; 104: 443–451. DOI: 10.1097/01.aog.0000137833.43248.79.
20. Kalder M, Kyvernitakis I, Hars O, et al. Comparison of combined low-dose hormone therapy vs. tibolone in the prevention of bone loss. *Climacteric* 2016; 19: 471–477. DOI: 10.1080/13697137.2016.1198313.
21. Lees B and Stevenson JC. The prevention of osteoporosis using sequential low-dose hormone replacement therapy with estradiol-17 beta and dydrogesterone. *Osteoporos Int* 2001; 12: 251–258. DOI: 10.1007/s001980170113.
22. Heikkinen J, Vaheri R, Kainulainen P, et al. Long-term continuous combined hormone replacement therapy in the prevention of postmenopausal bone loss: a comparison of high- and low-dose estrogen-progestin regimens. *Osteoporos Int* 2000; 11: 929–937. DOI: 10.1007/s001980070031.2001/02/24.
23. Delmas PD, Confavreux E, Garnero P, et al. A combination of low doses of 17 beta-estradiol and norethisterone acetate prevents bone loss and normalizes bone turnover in

postmenopausal women. *Osteoporos Int* 2000; 11: 177–187. DOI: 10.1007/pl00004180.

24. Szulc P. Bone turnover: Biology and assessment tools. *Best Pract Res Clin Endocrinol Metab* 2018; 32: 725–738. DOI: 10.1016/j.beem.2018.05.003.
25. Jain S and Camacho P. Use of bone turnover markers in the management of osteoporosis. *Curr Opin Endocrinol Diabetes Obes* 2018; 25: 366–372. DOI: 10.1097/med.0000000000000446.
26. Johansson H, Oden A, Kanis JA, et al. A meta-analysis of reference markers of bone turnover for prediction of fracture. *Calcif Tissue Int* 2014; 94: 560–567. DOI: 10.1007/s00223-014-9842-y.
27. Vasikaran S, Eastell R, Bruyere O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int* 2011; 22: 391–420. DOI: 10.1007/s00198-010-1501-1.
28. Szulc P, Naylor K, Hoyle NR, et al. Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. *Osteoporos Int* 2017; 28: 2541–2556. DOI: 10.1007/s00198-017-4082-4.
29. Reginster JY, Sarkar S, Zegels B, et al. Reduction in PINP, a marker of bone metabolism, with raloxifene treatment and its relationship with vertebral fracture risk. *Bone* 2004; 34: 344–351. DOI: 10.1016/j.bone.2003.10.004.
30. Garnero P. Biomarkers for osteoporosis management: utility in diagnosis, fracture risk prediction and therapy monitoring. *Mol Diagn Ther* 2008; 12: 157–170. DOI: 10.1007/bf03256280.
31. Massera D, Xu S, Walker MD, et al. Biochemical markers of bone turnover and risk of incident hip fracture in older women: the Cardiovascular Health Study. *Osteoporos Int* 2019; 30: 1755–1765. DOI: 10.1007/s00198-019-05043-1.
32. Stute P, Becker HG, Bitzer J, et al. Ultra-low dose - new approaches in menopausal hormone therapy. *Climacteric* 2015; 18: 182–186. DOI: 10.3109/13697137.2014.975198.
33. Samsioe G and Hruska J. Optimal tolerability of ultra-low-dose continuous combined 17beta-estradiol and norethisterone acetate: laboratory and safety results. *Climacteric* 2010; 13: 34–44. DOI: 10.3109/13697130903480706.
34. Levin VA, Jiang X and Kagan R. Estrogen therapy for osteoporosis in the modern era. *Osteoporos Int* 2018; 29: 1049–1055. DOI: 10.1007/s00198-018-4414-z.