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

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Abstract

**Objective:** Evaluate the effects of ultra-low-dose hormone therapy (Ultra-LD HT) with 17\(\beta\)-estradiol 0.5 mg and norethisterone acetate 0.1 mg (E2 0.5/N ETA 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/N ETA 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/N ETA 0.1 had a significant reduction in the PINP marker from baseline (58.49 ± 21.12 \(\mu\)g/L) to week 12 (48.31 ± 20.99 \(\mu\)g/L) and week 24 (39.16 ± 16.50 \(\mu\)g/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 \(\mu\)g/L and 16.29 ± 4.3 \(\mu\)g/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/N ETA 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

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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. 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-estradiol8 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 markers10,11 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 anti-resorptive 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 E2 0.5/NETA 0.1, compared to placebo according to CHOICE Study. The formula for two independent samples was used, based on a single-tailed T-student test with errors α = 2.5% (unilateral) and β = 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) ≥ 30 mIU/mL and estradiol ≤30 pg/mL, BMI ≥19 and ≤35.0 kg/m², 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 colposcopy, 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 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 luminescent, 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
(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

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**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.
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

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**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/m²)        |                          |                 |                        |
| 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.*
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 years10,21–23 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

Figures 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.

Figures 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.
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 17\(\beta\)-estradiol 0.5 mg with norethisterone acetate 0.1 mg on BMT. 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 17\(\beta\)-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.

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**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**

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**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).

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