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ORIGINAL STUDY

Effects of E2/P4 oral capsules on bone turnover in women with vasomotor symptoms

Michael R. McClung, MD,[1,2] Risa Kagan, MD, NCMP,[3] Shelli Graham, PhD,[4] Brian Bernick, MD,[4] Sebastian Mirkin, MD,[4] and Ginger Constantine, MD[5]

Abstract

Objective: To evaluate bone turnover markers (BTM) in the REPLENISH trial (NCT01942668).

Methods: REPLENISH evaluated oral estradiol/progesterone (E2/P4) for the treatment of moderate to severe vasomotor symptoms (VMS) in postmenopausal women with a uterus. Eligible women for this analysis had ≥50 moderate to severe VMS/wk, were <5 years since last menstrual period, and had BTM measurements at baseline, and months 6 and 12. Percent changes for three BTM (bone-specific alkaline phosphatase [BSAP], C-terminal telopeptide of type I collagen [CTX-1], and N-terminal propeptide of type I procollagen [P1NP]) assessed by immunoassay methods were evaluated from baseline to months 6 and 12 for the 1 mg E2/100 mg P4, 0.5 mg E2/100 mg P4, and placebo groups.

Results: A total of 157 women (40-61 y, 69% White) were analyzed. Mean baseline values ranged from 14.0 to 14.3 U/L for BSAP, 0.34 to 0.39 ng/mL for CTX-1, and 76.9 to 79.3 ng/mL for PINP. Mean differences in percent change from baseline for both E2/P4 doses versus placebo significantly decreased at months 6 and 12 and ranged from −8% to −16% for BSAP (all, $P < 0.05$), −30% to −41% for CTX-1 (all, $P \leq 0.001$), and −14% to −29% for PINP (all, $P < 0.01$).

Conclusions: REPLENISH data provide support for a potential skeletal benefit of E2/P4 when it is used for the treatment of moderate to severe VMS. Further studies are warranted.

Key Words: Bone markers – BSAP – CTX-1 – Estradiol – PINP – Progesterone – Vasomotor symptoms.

Video Summary: http://links.lww.com/MENO/A894.

Bones are constantly being remodeled in a tightly coupled balance between resorption and formation processes, which are necessary for the maintenance and overall health of bone.[1] When this balance becomes uncoupled, such that resorption exceeds formation, the net bone loss can result in the disordered skeletal architecture of osteoporosis. As key modulators of bone metabolism, estrogens play an important role in the growth and maturation of bone and in the regulation of bone turnover in adults. When levels of circulating estrogens decline during menopause, bone resorption increases, leading to reduced bone mineral density (BMD) and increased risk of osteoporotic fractures.[2] In menopausal women with vasomotor symptoms (VMS), hormone therapy can effectively treat their symptoms and can also reduce bone turnover and prevent bone loss.[3,4]

The REPLENISH trial (NCT01942668) was a phase 3 study that evaluated four doses of 17β-estradiol and progesterone (E2/P4) combination capsules (TX-001HR) for the treatment of

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From the [1]Oregon Osteoporosis Center, Portland, OR; [2]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, Australia; [3]University of California, San Francisco and Sutter East Bay Medical Foundation, Berkeley, CA; [4]TherapeuticsMD, Boca Raton, FL; and [5]EndoRheum Consultants, LLC, Malvern, PA.
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These data were published in an abstract submitted to the Endocrine Society 2020 (June 2020; online meeting) but was not presented.
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Address correspondence to: Michael R. McClung, MD, Oregon Osteoporosis Center, 2881 NW Cumberland Road, Portland, OR 97210.
E-mail: mmcclung.ooc@gmail.com

menopausal, moderate to severe VMS in women with a uterus.[5] Statistically significant improvements in the frequency and severity of moderate to severe VMS were observed with the two highest E2/P4 (1/100 and 0.5/100 [mg/mg]) doses, meeting the study's coprimary endpoints.[5] The 1 mg E2/100 mg P4 dose was approved by the Food and Drug Administration for the treatment of moderate to severe VMS in women with a uterus.[6] The 0.5/100 E2/P4 dose is currently under review at the Food and Drug Administration for the same indication.

Bone remodeling can be evaluated with bone turnover marker (BTM) assays, which measure collagen breakdown products and other molecules released from osteoclasts and osteoblasts during the process of bone resorption and formation.[7,8] High bone turnover in postmenopausal women, as assessed by these BTMs, is associated with bone loss, while interventions that reduce BTMs lead to preservation or improvement of BMD. In this post hoc study of the REPLENISH trial, the objective was to evaluate BTM changes in postmenopausal women treated with the 1/100 and 0.5/100 E2/P4 doses compared with those treated with placebo.

METHODS

Study design

The REPLENISH trial (NCT01942668) was a 12-month, phase 3, multicenter, randomized, placebo-controlled, double-blind study that evaluated TX-001HR for the treatment of moderate to severe VMS in postmenopausal women with a uterus.[5] Description of the study design has been previously published.[5] Briefly, healthy postmenopausal women between the ages of 40 and 65 years, with an intact uterus, body mass index $\leq$34 kg/m^2, and seeking treatment for VMS associated with menopause were eligible to enroll in the study. Postmenopausal status was defined as spontaneous amenorrhea for at least 12 months or a screening serum follicle-stimulating hormone level greater than 40 mIU/mL for at least 6 months, or bilateral oophorectomy at least 6 weeks prior to screening. Major exclusion criteria included recent use of medications that could alter the activity of estrogen or progesterone, having a contraindication or allergy to estrogen and/or progesterone, and heavy smoking ($\geq$15 cigarettes/d). Women could not be enrolled in the study if they had a history of thromboembolic disorder, coronary artery or cerebrovascular disease, chronic liver or kidney disorder, diabetes, or other endocrinological diseases. Concomitant medications that could affect bone turnover such as estrogens, progestogens, and SERMs were not allowed; however, women could have been using these products prior to the study, which may impact bone turnover. Use of bone-specific agents, such as alendronate and risedronate, was not an exclusion criterion. All participants gave written informed consent.

Women with moderate to severe hot flushes ($\geq$7/d or $\geq$50/wk) at baseline were included in a VMS substudy and were randomized 1:1:1:1:1 to daily E2/P4 (mg/mg) of 1/100, 0.5/100, 0.5/50, or 0.25/50, or placebo for 12 months. The remaining participants were randomized 1:1:1:1 to the four active doses. The safety population included all women who took at least one dose of the study drug for analysis of drug safety. The trial was conducted at 117 US sites in accordance with Good Clinical Practice; the study protocol was approved by a central or local institutional review board at each study site.

The REPLENISH primary efficacy and safety endpoints have been previously described.[5,9] Primary efficacy endpoints were changes in the frequency and severity of moderate to severe VMS from baseline to weeks 4 and 12 in the VMS substudy.[5] The primary safety endpoint was the incidence of endometrial hyperplasia in women who completed the 12-month treatment.[9]

Bone turnover marker assessments

Serum BTMs were assessed from fasting blood samples obtained at baseline, month 6 and month 12. The analysis was restricted to women included in the VMS substudy with $<$5 years since their last menstrual period and BTM measurements available for all timepoints. Women with $<$5 years since their last menstrual period were chosen as they would likely have the greatest change in their BTMs. The markers of bone formation, bone-specific alkaline phosphatase (BSAP, MicroVue, Quidel, San Diego, CA) and N-terminal propeptide of type I procollagen ([P1NP], Abbexa, Houston, TX), and the marker of bone resorption, C-terminal telopeptide of type I collagen (CTX-1, Serum CrossLaps, US Immunodiagnostic Systems, Gaithersburg, MD) were assessed by enzyme-linked immunoassay methods in batch assays by Syneos Health (Morrisville, NC). Samples were blinded to Syneos Health employees. The coefficient of interassay variability was $\leq$7.6% for BSAP, $<$12% for P1NP, and $\leq$10.9% for CTX-1. The BTMs were analyzed in September 2019 from blood samples collected between 2013 and 2016; samples were frozen to -80°C during storage. Long-term stabilities at -20°C/-80°C were validated to 458 days for BSAP in matrix and to 444 days for CTX-1 in matrix. No long-term stabilities were demonstrated for the P1NP assays.

Statistical analysis

Percent changes from baseline to months 6 and 12 for each BTM marker were calculated and compared between the 1/100 and 0.5/100 E2/P4 doses and placebo. *P* values were calculated for between-group comparisons using the mixed model for repeated measures with treatment, visit, and treatment-by-visit interaction as factors; baseline as covariate; and subject as repeated measure unit. Statistical analysis was performed with SAS v 9.2 or higher (SAS Institute, Cary, NC).

RESULTS

Demographics and disposition

Of the 1,260 women in the REPLENISH trial treated with 1/100 E2/P4, 0.5/100 E2/P4, or placebo, a total of 157 women (with $<$5 y since their last menstrual period and BTM measurements at all timepoints) were included in this post hoc analysis (56 women each from the 1/100 and 0.5/100 E2/P4 groups and 45 women from the placebo group).

TABLE 1. *Demographic and baseline characteristics*

	1 mg E2/100 mg P4 ($n = 56$)	0.5 mg E2/100 mg P4 ($n = 56$)	Placebo ($n = 45$)
Age, y	52.2 ± 3.8	52.3 ± 3.1	52.6 ± 4.1
Range	40-61	45-60	45-60
Race, *n* (%)			
White	39 (70)	36 (64)	33 (73)
Black or African American	17 (30)	19 (34)	12 (27)
Other[a]	0	1 (2)	0
Years since menopause, y	2.0 ± 1.3	2.4 ± 1.4	2.4 ± 1.1
Range	0.5-4.9	0.5-4.9	0.6-4.8
BMI, kg/m^2	26.3 ± 3.5	27.9 ± 4.4	27.0 ± 4.1
Range	19-33	18-35	19-34
BMI tertile, *n* (%)			
<25 kg/m^2	22 (39)	17 (30)	16 (36)
25 to <30 kg/m^2	24 (43)	16 (29)	19 (42)
≥30 kg/m^2	10 (18)	23 (41)	10 (22)
Alcohol history, *n* (%)			
Current	30 (54)	41 (73)	28 (62)
Former	7 (13)	3 (5)	3 (7)
Never	19 (34)	12 (21)	14 (31)
Smoking history, *n* (%)			
Current	11 (20)	17 (30)	8 (18)
Former	16 (29)	12 (21)	11 (24)
Never	29 (52)	27 (48)	26 (58)
Estradiol, pg/mL	6.8 ± 6.8	8.7 ± 9.9	6.5 ± 4.8
Median	4.6	5.4	4.6
Estrone, pg/mL	24.2 ± 17.8	25.9 ± 11.3	25.7 ± 9.2
Median	19.9	22.4	23.9
BSAP, U/L	14.3 ± 4.0	14.3 ± 4.5	14.0 ± 4.6
Median	13.7	12.8	12.7
P1NP, ng/mL	79.3 ± 21.8	77.9 ± 24.9	76.9 ± 23.7
Median	75.0	68.4	73.6
CTX-1, ng/mL	0.39 ± 0.15	0.38 ± 0.18	0.34 ± 0.14
Median	0.35	0.34	0.33

Data expressed as mean ± SD, unless otherwise stated.
BMI, body mass index; BSAP, bone-specific alkaline phosphatase; CTX-1, C-terminal telopeptide of type I collagen; E2, estradiol; P1NP, N-terminal propeptide of type I procollagen; P4, progesterone.
[a]Other could include American Indian/Alaska Native, Native Hawaiian/Pacific Islander, and Unknown.

The majority of women were White (69%) with a mean age of 52 years and BMI of 26 to 28 kg/m^2 (Table 1). Baseline estradiol levels were slightly numerically higher for women in the 0.5/100 E2/P4 group (8.7 pg/mL) versus the 1/100 E2/P4 (6.8 pg/mL) and placebo (6.5 pg/mL) groups. Mean baseline value ranges were 14.0 to 14.3 U/L for BSAP, 76.9 to 79.3 ng/mL for PINP, and 0.34 to 0.39 ng/mL for CTX-1. None of the women reported using medications to treat osteopenia or osteoporosis.

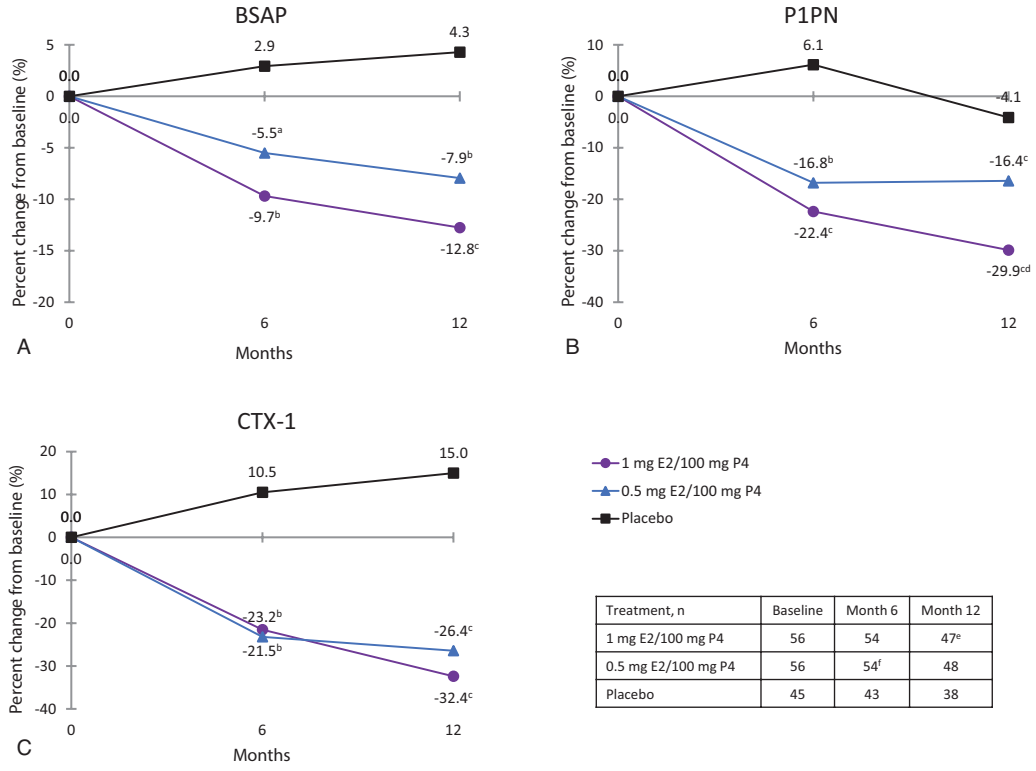
Bone turnover markers

A total of 443 BSAP samples were analyzed in 15 different analytical runs, 443 P1NP samples in 18 different analytical runs, and 443 CTX-1 samples in 14 separate analytical runs. For all three BTMs, mean differences in percent changes from baseline with both E2/P4 doses versus placebo significantly decreased at months 6 and 12 (Fig. 1). No significant differences were observed between the two E2/P4 doses at months 6 and 12, except for the P1PN marker at month 12 ($P < 0.016$). With E2/P4, mean differences from placebo from baseline to months 6 and 12 ranged from −8% to −16% for BSAP, from −14% to −29% for P1NP, and −30% to −41% for CTX-1 (Table 2). Reductions for BSAP and P1NP appeared to have a dose-dependent pattern, with the 1/100 E2/P4 dose reducing BTMs the most.

DISCUSSION

Hormone therapy is the most effective treatment for moderate to severe postmenopausal vasomotor symptoms, but it has also been shown to prevent bone loss and fracture.[10-12] In the REPLENISH trial, markers of both bone formation and resorption were significantly reduced from baseline for up to 12 months with E2/P4 (1/100 and 0.5/100) compared with placebo.

Markers of bone turnover are valuable for assessing the dynamic nature of bone remodeling in response to antiremodeling therapies.[13] Reductions in bone turnover with estrogens, bisphosphonates, and denosumab have been associated with preservation or increases in BMD in young postmenopausal women with low bone mass.[14-18] Short-term changes in BTMs are predictive of longer-term increases in BMD.[19] The responses of BTM to antiremodeling therapies in women with postmenopausal osteoporosis have also been shown to be predictors of vertebral but not nonvertebral fracture outcomes.[20,21] In the Foundation for the National Institutes of Health Bone Quality meta-regression of 14 randomized, placebo-controlled fracture endpoint trials with BTM data, strong relationships were observed between treatment-related BSAP or PINP changes and vertebral fracture risk reduction.[20] Treatment-associated reductions in BSAP levels compared with placebo of 12% and 30% correlated with reductions in



Treatment, n	Baseline	Month 6	Month 12
1 mg E2/100 mg P4	56	54	47[e]
0.5 mg E2/100 mg P4	56	54[f]	48
Placebo	45	43	38

FIG. 1. Percent changes from baseline in (**A**) BSAP, (**B**) P1NP, and (**C**) CTX-1. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ versus placebo; [d]$P < 0.05$ versus 0.5 mg E2/100 mg P4; [e]$n = 46$ for P1NP and CTX-1; [f]$n = 53$ for CTX-1. BSAP, bone-specific alkaline phosphatase; CTX-1, C-terminal telopeptide of type I collagen; E2, estradiol; P1NP, N-terminal propeptide of type I procollagen; P4, progesterone.

TABLE 2. *Changes from baseline in bone turnover markers with E2/P4 versus placebo*

	1 mg E2/100 mg P4 ($n = 56$)		0.5 mg E2/100 mg P4 ($n = 56$)		Placebo ($n = 45$)	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
BSAP, U/L						
Baseline	56	14.34 ± 4.5	56	14.29 ± 4.5	45	14.05 ± 4.6
Month 6	54	12.76 ± 3.7	54	13.26 ± 4.3	43	14.16 ± 4.8
Month 12	47	12.62 ± 3.9	48	12.62 ± 3.4	38	14.67 ± 5.0
LSM % change from placebo						
Month 6	54	−12.5[a]	54	−8.1[b]		
Month 12	47	−16.1[a]	48	−8.3[c]		
P1NP, ng/mL						
Baseline	56	79.32 ± 21.8	56	77.88 ± 24.9	45	76.89 ± 23.7
Month 6	54	59.35 ± 17.6	54	63.94 ± 23.3	43	79.85 ± 25.0
Month 12	46	53.66 ± 18.1	48	60.57 ± 19.2	38	75.40 ± 26.8
LSM % change from placebo						
Month 6	54	−28.6[a]	54	−23.0[a]		
Month 12	46	−28.2[a]	48	−14.2[c]		
CTX-1, ng/mL						
Baseline	55	0.390 ± 0.15	56	0.384 ± 0.18	45	0.344 ± 0.14
Month 6	53	0.287 ± 0.22	53	0.279 ± 0.14	43	0.358 ± 0.14
Month 12	46	0.248 ± 0.10	48	0.262 ± 0.12	38	0.374 ± 0.14
LSM % change from placebo						
Month 6	52	−29.5[c]	53	−30.1[a]		
Month 12	46	−41.1[a]	48	−38.1[a]		

BSAP, bone-specific alkaline phosphatase; CTX-1, C-terminal telopeptide of type I collagen; E2, estradiol; LSM, least square mean; P1NP, N-terminal propeptide of type I procollagen; P4, progesterone.
[a]$P < 0.001$ versus placebo. [b]$P < 0.05$. [c]$P < 0.01$.

risk of vertebral fracture of 30% and 65%, respectively, while a 22% reduction in P1NP compared with placebo was associated with a 30% reduction in vertebral fracture risk. These percent changes in BTMs are similar to those observed in our study, suggesting that the reductions in BSAP and P1NP with 1/100 or 0.5/100 E2/P4 may translate into improved bone strength and reduced fracture risk.

Limitations of these analyses include the fact that BTM assessments were post hoc analyses of the REPLENISH trial and not one of the primary endpoints. Furthermore, we did not have any measurements of BMD nor morphometric fracture data from patients, which would have been useful to determine the effects of E2/P4 on BMD and fracture risk. Finally, BTMs in blood samples may have been degraded during long-term storage. Nevertheless, the current analysis suggests that significant reductions in bone turnover occur with E2/P4, implying a skeletal benefit when E2/P4 is used to treat moderate to severe VMS. Further studies will be needed to better our understanding of the effects of E2/P4 on bone.

CONCLUSION

In the REPLENISH trial, treatment of postmenopausal women with a uterus for moderate to severe VMS with 1/100 E2/P4 or 0.5/100 E2/P4 was associated with reduced markers of bone turnover. These results suggest that postmenopausal women with a uterus who are taking E2/P4 for their moderate to severe vasomotor symptoms may have an additional skeletal benefit while using E2/P4. Further studies are warranted to confirm these results.

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