

Original Article

Estrogen-progestin therapy causes a greater increase in spinal bone mineral density than estrogen therapy - a systematic review and meta-analysis of controlled trials with direct randomization

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Abstract

Objective: To assess whether progesterone (P4) or osteoblast P4 receptor-acting progestin (P) contributed to estrogen (E) therapy-related increased areal bone mineral density (BMD) in randomized controlled trials (RCT) with direct randomization to estrogen (ET) or estrogen-progestin (EPT) therapy. **Methods:** Systematic literature searches in biomedical databases identified RCT with direct randomization and parallel estrogen doses that measured spinal BMD change/year. *Cyclic* P4/P was included in this random effects meta-analysis only if for \geq half the number of E-days. **Results:** Searches yielded 155 publications; five met inclusion criteria providing eight dose-parallel ET-EPT comparisons in 1058 women. Women averaged mid-50 years, <five years into menopause and took conjugated equine E daily at 0.625 mg with/without 2.5 mg medroxyprogesterone acetate (MPA). The weighted mean EPT minus ET percentage difference in spinal BMD change was +0.68%/year (95% CI 0.38, 0.97%) ($P=0.00001$). This result was highly heterogeneous ($I^2=81\%$) but this may reflect the small number of studies. **Conclusion:** Estrogen with an osteoblast P4R-acting progestin (EPT) in these five published RCT provides Level 1 evidence that MPA caused significantly greater annual percent spinal BMD gains than the same dose of ET. These data have implications for management of vasomotor symptoms and potentially for osteoporosis treatment in menopausal women.

Keywords: Estradiol, Progesterone, Spine Areal Bone Mineral Density, RCT Meta-analysis, Osteoporosis

Introduction

Menopausal ovarian hormone therapy (OHT), is either estrogen alone (ET) or estrogen-progesterone/progestin

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treatment (EPT)¹. OHT has been prescribed for decades to prevent areal bone mineral density (BMD) loss in menopausal women (≥ 1 year since last flow)^{2,3} or to treat osteoporosis⁴. A meta-analysis of ET and EPT randomized controlled trials (RCT) found major increases in BMD and a trend to decreased spine and non-vertebral fractures². This meta-analysis considered ET and EPT as a single therapy, however, which may or may not be justified². Women's Health Initiative RCT data showed fracture prevention in both ET and EPT vs. their respective placebos^{5,6}.

Estrogen is the bone-active component of OHT acting as an anti-catabolic agent and decreasing bone resorption⁷. ET also increases gut calcium absorption and indirectly suppresses excess parathyroid hormone production leading to fragility fracture prevention^{8,9}. But balanced bone resorption (catabolism) and formation (anabolism) appear



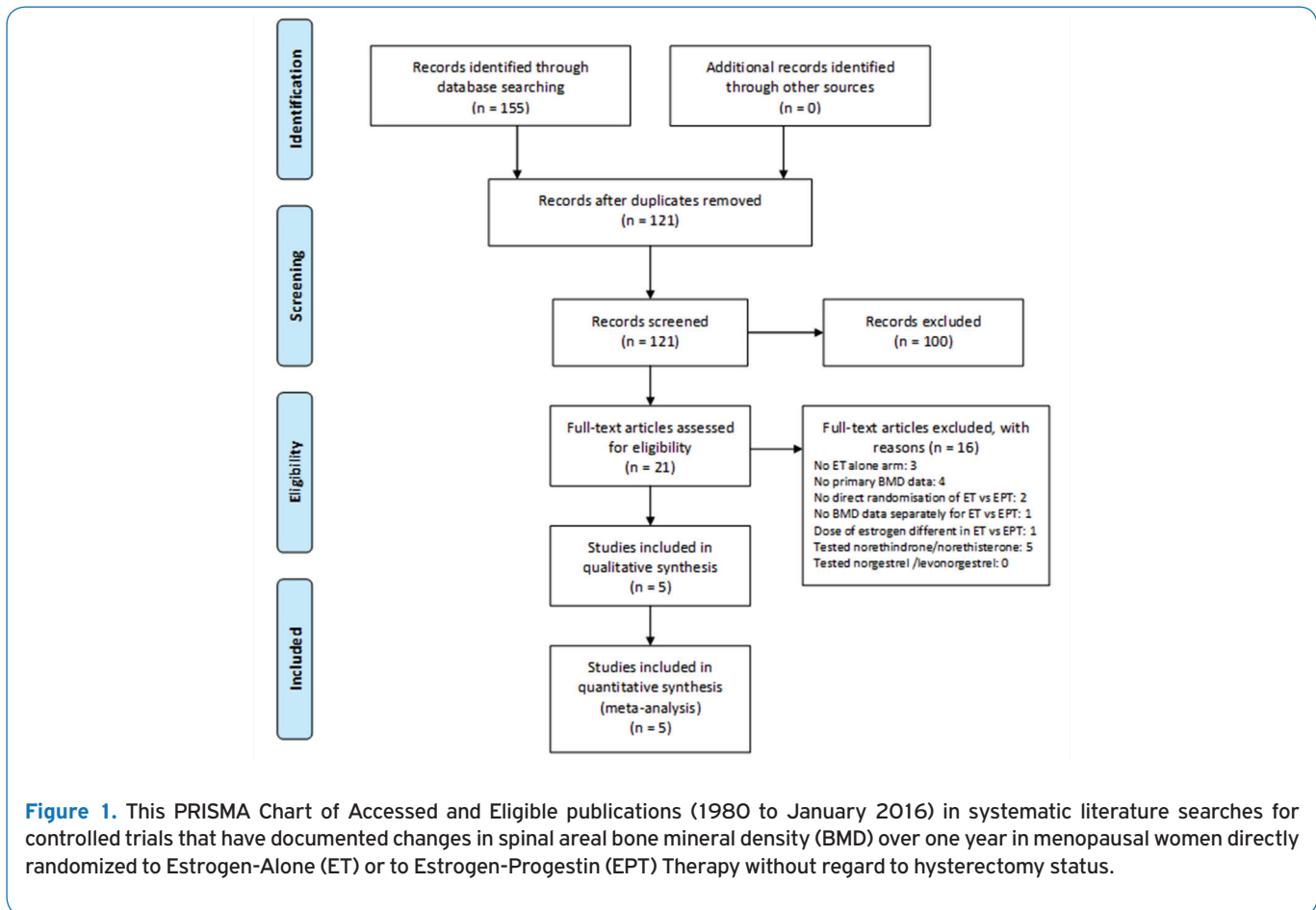


Figure 1. This PRISMA Chart of Accessed and Eligible publications (1980 to January 2016) in systematic literature searches for controlled trials that have documented changes in spinal areal bone mineral density (BMD) over one year in menopausal women directly randomized to Estrogen-Alone (ET) or to Estrogen-Progestin (EPT) Therapy without regard to hysterectomy status.

optimal for preservation of adult BMD and strength¹⁰. In osteoporosis treatment - since resorption and formation are coupled at the bone mineralization unit (BMU) level¹¹ - it is theoretically important to facilitate both. Because of coupling, however, anti-catabolic therapies (such as estrogen, bisphosphonates, calcitonin, selective estrogen receptor modulators and denosumab) also inhibit bone anabolism¹². An additional bone remodeling complexity is that resorption and formation have differing *in vivo* time courses; resorption in human bone takes about three weeks while new bone formation in the same BMU takes at least three months¹³.

Given estrogen's proliferative and thus potentially carcinogenic endometrial effects¹⁴, the addition of a progesterone/progestin to estrogen (EPT) is recommended to prevent endometrial hyperplasia/carcinoma in women who have not undergone hysterectomy¹⁵. Since estradiol and progesterone appear to act synergistically on bone within the normal-length, ovulatory menstrual cycle^{16,17}, EPT as dual-mechanism therapy may also be advantageous for bone physiology. Endogenous estradiol decreases bone resorption and progesterone appears to increase bone formation^{16,18}. Most synthetic, not androgen-derived progestins, e.g. medroxyprogesterone acetate (MPA) act

through osteoblast nuclear progesterone receptors (P4R) and the Wnt/b catenin system to increase osteoblast numbers and bone matrix formation¹⁹.

It is currently controversial whether ET and EPT differ in BMD effects. One of the pioneers of OHT for osteoporosis, asserted that: "progestogens have neither a direct effect on osteoporosis nor an additive effect when used as a component of hormone replacement therapy"²⁰. However, other clinician-scientists suggest progestin-specific additions to BMD gains^{21,22}. New data now show some non-estrogen anti-catabolic agents have additive BMD effects when paired with anabolic therapy²³.

Our research question was: Is there a difference in the annual change in spinal BMD within RCT in which menopausal women were **directly randomized** to the same dose of estrogen alone (ET) or with progesterone or an osteoblast P4R-acting progestin (EPT)? We tested the hypothesis that progesterone/progestin would add significantly to estrogen-related spinal BMD gains.

Materials and methods

All included, published articles are assumed to have followed International Medical Association Declaration of

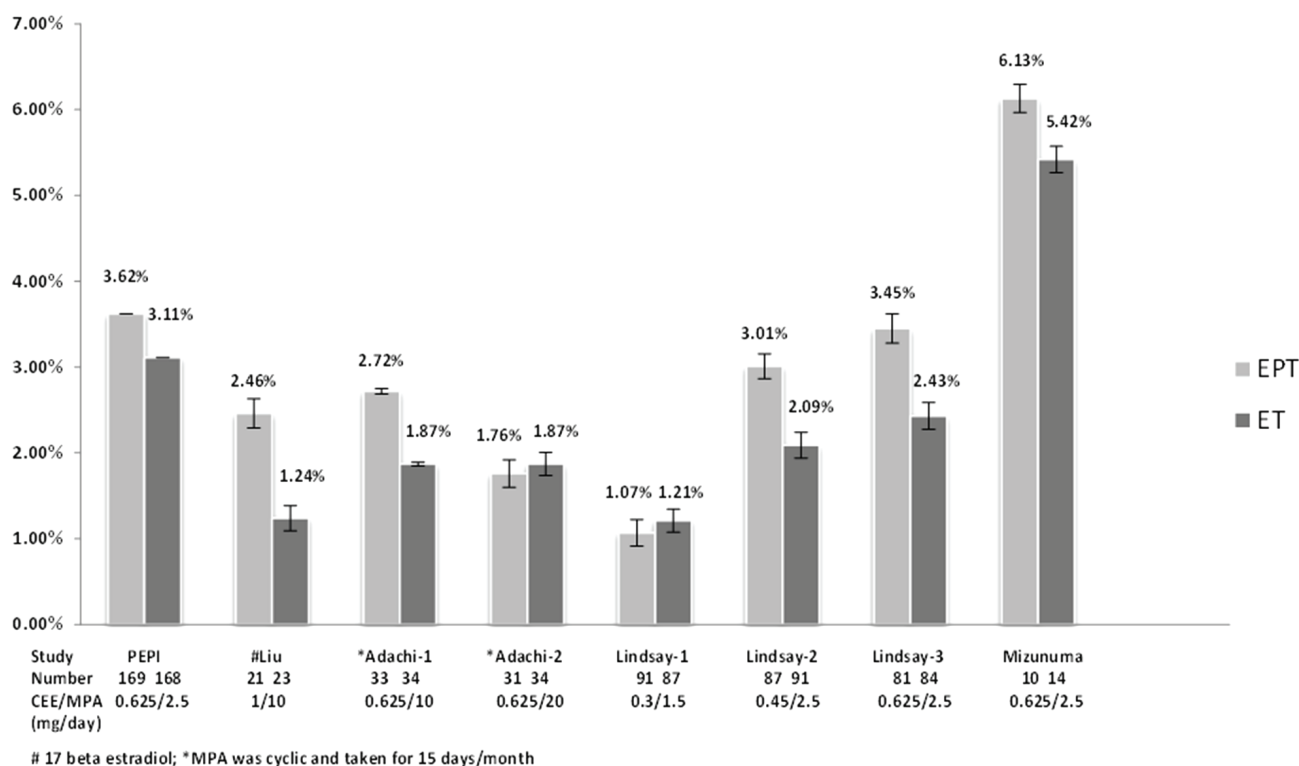


Figure 2. Bar graph of Percentage Annual Change in Spinal area Bone Mineral Density (BMD) from Randomized Controlled Trials of Estrogen-Alone Therapy (ET) versus Estrogen-Progestin Therapy (EPT). The study is identified underneath each bar by the abbreviated name of the study (e.g. PEPI) or the last name of the first author. Below that is the number of women in each arm. Finally, the medication and dose-comparisons are shown as a fraction with the dose of CEE or E₂ on top and the dose of MPA on the bottom. Above each bar graph the mean percent change in spine BMD is shown to the nearest tenth of a percentage with SD.

Helsinki principles in their conduct of research with human participants.

Literature search strategy

Systematic searches (**Supplemental Material**) were conducted of RCT in menopausal women using the electronic databases, Ovid MEDLINE (1949 to 2013), Ovid Embase (1974-2013), Google Scholar and Web of Science and updated to 2016. Abstracts from American Society for Bone and Mineral Research meetings were screened, and publication reference lists assessed for additional publications. Medical subject headings and text words were supplemented with synonyms for BMD, RCT, menopausal women and OHT with estrogen/estradiol, progestin/progesterone or both.

Inclusion and exclusion criteria

Articles were eligible if they were RCT in menopausal women directly (ignoring hysterectomy status) comparing estradiol/estrogen plus P4 or an osteoblast P4R-acting progestin (EPT) with estradiol/estrogen alone (ET) and measured spinal BMD change by dual energy X-ray

absorptiometry (DXA) over at least one year with the same estrogen agent/dose in both arms. Eligible RCT used intent-to-treat analysis. The progesterone/progestin dose needed to be for \geq half the ET duration (i.e. for ≥ 14 days/28 day ET). For comparability among studies with/without multi-year data, we included only the first year's data. The progestins, norethisterone and norethisterone acetate were excluded since they are metabolized into estrogen, and likely act through, an osteoblast estrogen as well as a testosterone receptor²⁴. Norgestrel and levonorgestrel were excluded because they act through osteoblast androgen receptors. All trial protocols (except for the one in those with hysterectomy²³) had ethics review-board approved endometrial safety precautions in place.

When publications did not include complete intent-to-treat data, we contacted the authors. Publications in non-English languages were excluded as were studies in premenopausal women or in menopausal women with bone-affecting illnesses or who were taking other bone-active therapies. Non-human studies, review articles, commentaries, letters without original data and cross-sectional studies were excluded.

Table 1. Baseline demographics of participants, dosage schedules and bone mineral density (BMD) values are provided for this Meta-analysis of Spinal BMD Changes per Year on Estrogen Therapy (ET) compared with Estrogen-Progestin Therapy (EPT) in trials that directly randomized women to one or the other group.

		Study Group							
		+PEPI	Liu	Adachi MPA: 10	Adachi MPA: 20	Lindsay CEE: 0.3	Lindsay CEE: 0.45	Lindsay CEE: 0.625	Mizunuma
Number of Participants	ET*	168	23	34	34	87	91	84	14
	EPT^	169	21	33	31	91	87	81	10
Age (years)	ET	56.2 (3.9)	52.0 (3.8)	53.5 (7.4)	53.5 (7.4)	52.2 (3.9)	51.9 (3.6)	52.1 (3.1)	55.1 (1.2)
	EPT	56.4 (3.9)	52.9 (3.9)	55.0 (6.8)	53.6 (7.7)	51.4 (3.5)	51.6 (3.9)	51.5 (4.2)	53.7 (1.1)
Ethnicity (% Caucasian)	ET	91	-	-	-	90	89	92	0 (100% Asian)
	EPT	89	-	-	-	88	98	90	0 (100% Asian)
Years Into Menopause (≥1 y with no flow)	ET	-	<5	12.8 (8.8)	12.8 (8.8)	2.3 (1.0)	2.5 (1.0)	2.3 (0.9)	5.1 -
	EPT	-	<5	12.8 (11.1)	12.7 (9.4)	2.3 (1.0)	2.5 (0.9)	2.2 (0.9)	3.0 -
Dosage (mg unless noted)	ET	0.625 CEE	1 mg 17β E ₂	0.625 CEE	0.625 CEE	0.3 CEE	0.45 CEE	0.625 CEE	0.625 CEE
	EPT	0.625 CEE 2.5 MPA daily	1 μg 17β E ₂ 10 MPA daily	0.625 CEE 10 MPA: 15 d/mo.	0.625 CEE 20 MPA: 15 d/mo.	0.3 CEE 1.5 MPA daily	0.45 CEE 2.5 MPA daily	0.625 CEE 2.5 MPA daily	0.625 CEE 2.5 MPA daily
Spine BMD at Baseline (g/cm ²)	ET	0.966 (0.146)	1.140 (0.101)	1.040 (0.135)	1.040 (0.135)	1.140 (0.150)	1.135 (0.155)	1.174 (0.153)	0.842 (0.101)
	EPT	0.972 (0.171)	1.132 (0.146)	1.047 (0.160)	1.067 (0.154)	1.139 (0.145)	1.152 (0.171)	1.144 (0.164)	0.830 (0.107)
Total Hip BMD at Baseline (g/cm ²)	ET	0.860 (0.119)	-	-	-	0.942 (0.121)	0.954 (0.136)	0.979 (0.136)	-
	EPT	0.854 (0.13)	-	-	-	0.944 (0.122)	0.956 (0.147)	0.965 (0.144)	-
Femoral Neck BMD at baseline (g/cm ²)	ET	-	0.866 (0.020)	0.771 (0.121)	0.771 (0.121)	-	-	-	0.679 (0.099)
	EPT	-	0.873 (0.027)	0.769 (0.118)	0.786 (0.114)	-	-	-	0.661 (0.084)

+These are intent-to-treat data from the Postmenopausal Estrogen Progestin Investigation (PEPI); *ET=estrogen alone therapy; EPT= estrogen with progesterone or osteoblast progesterone receptor-acting progestin; BMD=areal bone mineral density; CEE=conjugated equine estrogen; MPA=medroxyprogesterone acetate; mo. = month.

Data collection

All publications were screened for eligibility by two reviewers (JCP and AG) based on inclusion/exclusion criteria. Articles were initially selected based on abstracts but final decisions required full texts. Disagreements between reviewers were resolved by consensus.

Statistical analysis

The primary outcome was change in areal BMD in the lumbar spine by dual energy X-ray absorptiometry (DXA) during the first year of OHT on estrogen alone (ET) or estrogen with progesterone/progestin (EPT) treatment. Primary data were reported as percentage change so this was analysed. BMD total hip (TH) and femoral neck (FN) data were available in a few studies but were insufficient for quantitative synthesis.

Meta-analysis of spine BMD percentage change per year was performed using the Cochrane analysis tool Revman (<http://tech.cochrane.org/revman>).

Results

The initial literature search strategy in 2013 retrieved 41 publications of which four were duplicates²⁵. A subsequent search in 2016 retrieved 155 publications; 121 remained after removal of duplicates. Following full-text review, five publications were eligible providing eight progesterone/progestin dose comparisons as shown in the PRISMA diagram (Figure 1). As per our protocol, studies were excluded for not having direct randomization to ET and EPT (e.g. for having separate placebo arms for EPT and ET as in the Women's

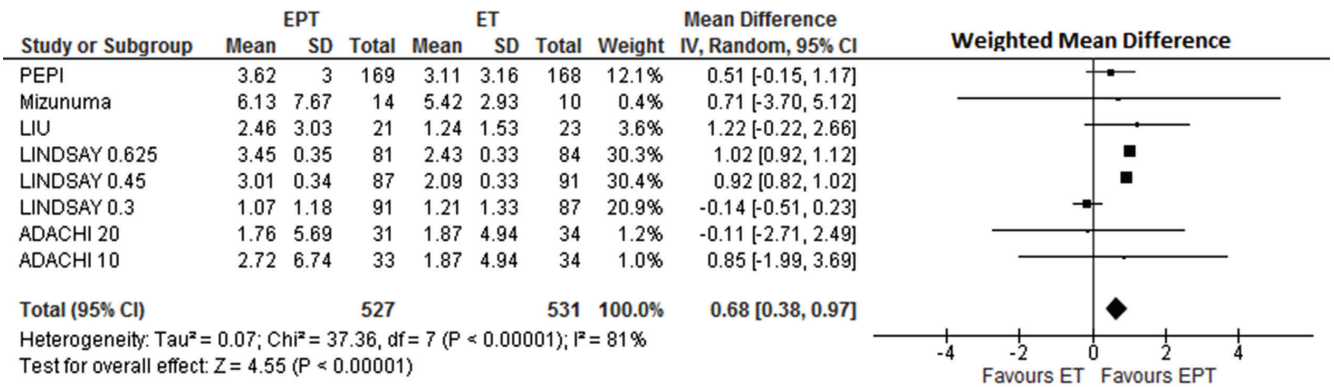


Figure 3. Forest plot comparing the Weighted Mean Difference in Percentage Annual Change in Spinal areal Bone Mineral Density (BMD) by Dual Energy X-ray Absorptiometry on Estrogen-Alone Therapy (ET) versus Estrogen-Progestin Therapy (EPT). This random effects meta-analysis model shows heterogeneity of the studies by I².

Health Initiative^{5,6}). Others were excluded for having no ET arm, for having differing doses of estrogenic hormones in the two arms or because they studied androgenic progestins metabolized to estrogens (norethindrone, norethisterone, or their acetate metabolites)(n=5)²⁴. No norgestrel or levonorgestrel studies were found. We excluded the cyclic MPA and cyclic progesterone arms in the Postmenopausal Estrogen-Progestin Investigation (PEPI) trial since these agents were given for only 12 days/month with continuous ET. We excluded trials with: data only for bone biomarkers; non-menopausal women; diseased populations; or cross-sectional designs. Unpublished intent-to-treat analysis data from PEPI²⁶ were obtained from Drs. Elizabeth Barrett-Connor and Gail Laughlin.

Five eligible RCT provided eight estrogen-dose parallel comparisons and randomized menopausal women directly to ET or EPT; they were published in 1996-2005²⁶⁻³⁰. The primary outcome was DXA-based BMD change in the lumbar spine reported as percentage change per year [%/y] over the first year. The estrogen interventions were primarily conjugated equine estrogen (CEE) in doses of 0.625, 0.45, or 0.3 mg/d; one trial administered 1 mg oral 17 β -estradiol/d (E₂)²⁹. The progesterone/progestin in all eligible studies was MPA in doses of 1.25 to 20 mg with 2.5 mg/d being the most common dose (Figure 2, Table). Most trials prescribed both estrogen and progestin daily; MPA was prescribed cyclically for 15 days/month in one²⁷.

As shown in the Table, participants were in early in menopause (1-11 years since final flow) except those in one²⁷ who averaged 12.8 years. The PEPI trial did not provide this information²⁶. The average age of participants was in the early to mid-50s; women's ethnicity was primarily Caucasian except for being apparently 100% Asian in one³⁰. Baseline mean BMD (SD) values in the lumbar spine (lumbar levels L2-L4), total hip, femoral neck and trochanter are shown by study arm. Trial sizes ranged from >300²⁶ to fewer than 50

women^{29,30}. Trial durations ranged from one to three years with an average duration of two years.

The percentage spinal BMD change per year on each dose-comparison is shown graphically (Figure 2). These data indicate that there was a numerically greater spinal BMD gain on EPT versus ET in all of the comparisons except on 0.3 mg/d CEE or on CEE 0.625 with 20 mg cyclic MPA^{27,28}. Percentage changes in the total hip were available in the PEPI²⁶ and Lindsay²⁸ trials but no final BMD data were provided; femoral neck change data were reported in the Liu²⁹, Adachi²⁷ and Mizunuma³⁰ trials. Although these hip BMD measurements indicated increases in all active arms, none were reported to be statistically different between ET versus EPT.

A meta-analysis was performed using a random effects model analyzing the weighted mean differences in percent spinal BMD changes between EPT and ET with results shown as a Forest plot (Figure 3). Within-study EPT arms compared with the ET ones showed a highly significantly greater weighted mean difference in spinal BMD percent change/year (y) documented as +0.68%/y (95% confidence interval 0.38, 0.97%; P=0.0001) on EPT. There also was, however, a highly significant degree of heterogeneity with I²=81%. This may simply reflect the small number of available studies³¹. Or it may be related to differences in mean years into menopause (from 2.3 to 12.8 years), and in race (most Caucasian and one Asian), the differing estrogen and progestin doses as well as varying sample sizes.

Discussion

This meta-analysis of RCTs that directly assigned menopausal women to estrogen (ET) or to estrogen-progestin treatment (EPT) irrespective of hysterectomy documented that combined estrogen and a progestin therapy (medroxyprogesterone, acting through the P4 osteoblast

receptor) caused two-thirds of a percentage greater spinal BMD gain per year than estrogen alone. This is the first time, to our knowledge, that a meta-analysis has been performed of RCT assessing BMD changes in studies that directly randomized menopausal women to ET versus EPT. These data support additive effects of MPA to those of estrogen on gain in spinal BMD. These publications, however, did not provide histomorphometric or bone biomarker data to assess the mechanism for the MPA-added BMD gain. However, all except one (0.3 mg/d) of the estrogen doses are known to suppress bone resorption²⁸. RCT biomarker data further show that MPA does not decrease the very high resorption following premenopausal ovariectomy³². Since MPA acts through the osteoblast P4R¹⁹, it likely stimulated bone formation³³.

Higher doses of MPA than 10 mg/d, however, may stimulate the osteoblast *glucocorticoid* receptor (e.g. 20 mg/d cyclic MPA in²⁷) thus *inhibiting* bone formation³⁴. MPA may also bind to the osteoblast androgen receptor (AR)³⁵; androgens have bone-formation stimulating effects³⁶. However, MPA appears to bind more strongly to the P4R than to the AR³⁵. Most recently, additional mechanisms have been discovered, by which progesterone and its metabolites influence the effect of estrogen-receptor binding to the nuclear DNA, increasing our understanding of intracellular crosstalk of estrogen and progesterone receptors³⁷.

These results of estrogen-progestin menopausal therapy parallel observational prospective studies of spine BMD changes in premenopausal women with regular cycles but ovulatory disturbances (estradiol dominant) versus normally ovulatory menstrual cycles (with balanced estradiol and progesterone)¹⁷. In that analysis, significantly more negative spinal BMD changes (-0.86%/y, $p=0.040$) occurred in those with usually regular cycles but more frequent ovulatory disturbances and thus absent or lower progesterone levels¹⁷. This RCT meta-analysis result is also analogous to results of other therapies in which anti-catabolic agents are combined with anabolic ones²³.

An RCT in otherwise healthy, normal-weight premenopausal women with hypothalamic amenorrhea/oligomenorrhea and regular cycles but ovulatory disturbances showed significantly greater spinal BMD gain on cyclic MPA therapy versus placebo (+2.2% vs -2.0%/y; ANOVA $P=0.0001$)³⁸. Thus there already is Level 1 evidence that MPA increases spinal BMD in premenopausal women with abnormal cycles or ovulation.

Although anti-catabolic therapies such as ET, denosumab and various bisphosphonates significantly increase BMD and effectively decrease fractures³⁹⁻⁴¹, it has been hypothesized that the catabolism/anabolism imbalance or the high mineralization they may cause could increase the risk for "adynamic" or brittle bones⁴². The ideal osteoporosis therapy may be both anti-catabolic and anabolic^{23,43}.

Fracture prevention is the ultimate goal of osteoporosis therapy, thus the question: is the +0.68%/y greater spinal BMD gain on EPT versus ET clinically important in preventing fragility fractures? A meta-analysis of anti-catabolic therapy-related BMD gains showed that a 1% spinal BMD

increase correlated with an 8% decrease in non-vertebral fractures⁴⁴. Although measurements of BMD and bone strength intrinsically differ, these data suggest there may be a greater decrease in fracture risk on EPT than on ET. Women's Health Initiative hormonal RCT data suggest similar BMD and fracture prevention results on ET and EPT each compared versus its own placebo, but they did not directly compare ET with EPT^{5,6}. Long-term WHI follow-up data, however, showed significant hip fracture prevention persisted for a longer duration in those previously on EPT compared with those who were on ET in the past⁴¹.

Anabolic treatment may have a stronger fracture-prevention effect than anti-catabolic therapy; e.g., an RCT recently showed the BMD change/spine fracture prevention effect of parathyroid hormone (anabolic) was greater than that of alendronate (anti-catabolic)⁴⁵. Furthermore, a meta-analysis of RCT of incident fracture on OHT suggested greater decreased absolute fracture risk on EPT¹. Thus the additional BMD gain from osteoanabolic MPA or progesterone co-therapy with an anti-catabolic therapy is likely important for fracture prevention. RCT data are needed and remain to be performed.

It is a strength of this study that we performed comprehensive literature searches and a quantitative comparison of two formerly common hormonal osteoporosis therapies. Although no longer recommended for osteoporosis treatment or prevention¹, EPT or ET continue to be important for treatment of intense menopausal hot flashes/night sweats (vasomotor symptoms, VMS). Another strength is that we limited progestins, given their wide heterogeneity³⁵, to those acting through the osteoblast P4R.

The limitations of this study are that, despite the millions of menopausal women who have used OHT, only about 1000 women were studied by direct randomization to ET or EPT. It is a limitation that BMD change on ET has primarily been studied with conjugated equine estrogen and only one study in this meta-analysis used oral micronized estradiol. It is also a limitation that EPT in comparison with ET has only been studied with MPA as the progesterone/progestin and not with oral micronized progesterone or the other progestin (e.g. dydrogesterone) neither of which has increased breast cancer risk on estrogen-co-therapy⁵². These data are also limited by their high degree of heterogeneity that is likely biased by the small number of included studies³¹. However, our meta-analysis used a random-effects model that does not assume homogeneity of effects. Therefore these results are likely robust to heterogeneity.

Menopausal OHT, with estradiol and progesterone or other non-MPA progestins, continues to be indicated and commonly used for the treatment of symptomatic menopausal women with VMS⁴⁶ although it is no longer considered appropriate for the prevention or treatment of osteoporosis^{5,6,47}. Related to this, it is important that a previous 1-year RCT directly comparing CEE versus MPA for VMS effects showed equivalent and effective control of hot flashes/night sweats on both single hormone therapies⁴⁸. Also an RCT of oral micronized progesterone (alone) versus placebo showed significant VMS efficacy⁴⁹.

Current gynecology guidelines recommend that estrogen-treated menopausal women without a uterus should not be prescribed progesterone/ progestin⁴⁶. VMS treatment, however, is significantly more effective on EPT than ET in a meta-analysis of RCT⁵⁰. A progesterone vs placebo RCT for VMS⁴⁹ also showed no significant short-term adverse effects on weight, blood pressure, lipids, coagulation or inflammation⁵¹. Progesterone with estrogen does not increase breast cancer risk^{52,53}, although MPA with estrogen does⁵. This breast cancer safety of estrogen/progesterone versus progestins was recently confirmed by systematic review and meta-analysis⁵⁴. Also, estradiol/estrogen or EPT, ideally of bio-identical estradiol and progesterone, will continue to be prescribed for menopausal women who are younger than age 40 or have primary ovarian insufficiency⁵⁵.

We currently have many anti-catabolic therapies that increase BMD and prevent fractures. These agents, however, may also have negative immune effects⁵⁶. Although combined anti-resorptive/anabolic therapies (i.e. denosumab or bisphosphonate plus parathyroid hormone) are promising²³, combined therapy increases treatment risks and limitations (e.g. the 2-year limited duration of parathyroid hormone therapy) and most involve a degree of patient burden (e.g. intravenous or subcutaneous injections or the fasting required for oral therapy). The spine BMD effects of these anti-catabolic therapies combined with progesterone deserve examination in randomized controlled trials.

In summary, this meta-analysis in more than 1000 menopausal women randomized directly to either estrogen alone (ET) or estrogen-progestin (EPT) documented that EPT had significantly and importantly greater spinal BMD gains than ET. The relevance of this observation is that, for spinal BMD change, progesterone (or osteoblast progesterone receptor acting progestins) have additive, positive effects with estradiol that are greater than the change on estradiol alone.

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Author roles

JcP conceived of the idea, with AG reviewed publications for inclusion, contacted other authors for intent-to-treat data and wrote the original and revised drafts of the manuscript; VRS-K advised on roles of EPT and ET in today's therapy and reviewed/revised drafts of manuscript; DG created the original search strategy and assisted in the PRISMA Figure; JDA provided advice about potential publications and reviewed/revised drafts of manuscript; SK commented on mechanisms and benefits and drawbacks of osteoporosis therapies; AG performed the follow-up literature search, with JcP decided on publication inclusion, performed the meta-analysis and reviewed/revised drafts of the manuscript. All authors have approved the submitted manuscript.

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Supplemental Material

Initial Literature search strategy:

1	proges*.mp.
2	random:.mp.
3	or/1-2
4	(Norethindrone or Norethisterone).mp.
5	3 not 4
6	low dos*.mp.
7	5 not 6
8	exp *Estrogens/
9	7 and 8
10	exp *Bone Density/
11	9 and 10 (151)
12	(bone adj5 chang*).mp.
13	9 and 12
14	11 or 13
15	limit 14 to english language
16	adachi *.au.
17	Medroxyprogesterone.mp.
18	16 and 17
19	liu *.au.
20	17 and 19
21	8 and 20 (35)
22	(Progesterone or Medroxyprogesterone).mp.
23	estradiol.mp.
24	22 and 23
25	8 and 24
26	13 or 14 or 18 or 20 or 21
27	limit 26 to English language
28	limit 27 to humans