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Women's Health

Influence of progestagens on bone health. Bone changes related to ovulatory disturbances and low progesterone levels

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Purpose To enhance trans-disciplinary understanding of bone changes in women, between the boundaries internal medicine, experimental orthopedics/surgery and gynecology.

Major sources of information A two-decade literature archive on factors affecting female bone metabolism, supplemented by a search of the recent experimental publications, and including work from our own working group on bone and females hormones.

Data synthesis in the model context In experiments in human osteoblast cultures from female femur bone, which were sufficiently estrogenized to induce progesterone receptors (PGR), progesterone showed remarkable dose-response curves explaining many clinical observations. In bone research negating progesterone effects, the fact that PGR induction needs a minimum of 4–7 days of estrogen exposure and may need a female genetic endowment is often neglected. There is insufficient information on female animals in many bone models.

Incorporation the new understanding into clinical and/or research relevance While ovulation itself shows parallels with inflammatory processes for a short time, lack of progesterone or its receptor may prolong this state of inflammation. Progestin resistance is a feature of endometriosis, and 19% of women with early stage endometriosis are anovulatory. Bone marrow derived tem cells are known to play a role in endometriosis, but bone loss has only been evaluated regarding estrogen deprivation treatment in this diesease. Based on clinical observations of premenopausal women presenting with both endometriosis and osteoporosis without prolonged estrogen-suppressive treatment, a joint mechanism involving inflammatory mechanisms may play a role.

Conclusion Chronic inflammatory processes may be maintained by anovulation and lack of progesterone and may preferentially affect women with PCOS (for whom this has already been investigated) and also with endometriosis. This may also partly explain the preponderance of women in osteoporotic disease.

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Introduction

Following the life span in a chronological order, the influence of progestogens is best recognized in five clinically relevant situations. Starting with puberty, new biochemical pathways between ovary and bone will be briefly reviewed taking a translational approach between clinical and basic science findings. Aspects of the relative energy deficit-syndrome (RED-S) affects bone more severely in teenagers than in adolescents and when peak bone mass is not achieved, the attenuation of progesterone's effects on osteoblasts may play a role.

The review will then move on to PCO-syndrome in which anovulation is highly prevalent, and also review data on anovulation prevalence in normal cycle length.

Among the mechanisms which determine the fate of pre-osteoblasts to become either osteoblasts or adipocytes, RUNX2 is a major differentiation factor. The expression of RUNX is reported to be mediated by activation of the progesterone receptor. Whether this may be one of the mechanisms responsible for the loss of trabecular bone, which accelerates in a substantial number of women during perimenopause, when ovulation rates and progesterone decline before the cessation of estrogen production, needs to be further studied. Finally, postmenopausal hormone therapy containing either estrogen only or combined estrogen-progestin in cyclic or continuous dosage combinations show added bone benefit for the latter.

The influence of progesterone is slower, and more subtle than the more visible changes associated with estrogen or it's decline, androgens and growth hormone. Yet, over the approximate 3–4 decades of women's reproductive phase, the association of bone-building with ovulation is relevant, not only by preparing a surplus reservoir of trabecular bone from which large amounts of calcium may be mobilized in short time for mineralization the fetal skeleton in the third trimenon of pregnancy.

Puberty and the bone-ovary-connection

Bone metabolism and ovarian function are closely linked: two oocyte-specific factors, bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9 (GDF-9), play crucial roles in determining folliculogenesis, ovulation rate and litter size in sheep and mice [1]. BMP-15 prevents the transition of small antral follicles into preovulatory follicle by inhibiting the production of FSH receptor mRNA in granulosa cells [2]. Therefore, FSH cannot bind to the granulosa cells, this inhibits FSH dependent progesterone production and luteinization, subsequently granulosa cells do not differentiate. BMP-15 defects have been implicated in female sterility, PCOS, primary ovarian insufficiency (POI) and endometriosis [3]. Women with PCOS have been noted to have higher levels of BMP-15, while missense mutations of the protein have been identified in females with POI [4]. Several Growth Differentiation Factors (GDF) and Bone Morphogenic Proteins (BMP) are also involved in bone healing. The onset of puberty and eventually menarche, is governed by many factors, both genetic and envrironmental. An overview of recent genetic factors important for the establishment of menstrual cycle regularity and the coordination of ovulation is shown in Fig. 1. Energy homeostasis plays a major part in the fine-tuning, and one of the new players in the crosstalk between bone and ovary belongs to the hypocretin gene family, encoding for neuropeptides that regulate arousal, wakefulness, and appetite, Orexins.

The Orexin A — mediated increase of Progesterone synthesis depends on endogenous BMP activity

Dysfunction of osteoblastic bone formation and matrix mineralization plays a key role in the pathological development of osteoporosis. The orexin peptide orexin-A, a highly excitatory neuropeptide hormone, possesses various biological functions by activating its specific G protein-coupled receptors, orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R) [5]. A recent study by Fujita et al. investigated the effects of orexin A on ovarian steroidogenesis by using rat primary granulosa cells that express both OX1 and OX2 receptors for orexins. Treatment with orexin A enhanced progesterone-, but not estradiol-biosynthesis induced by FSH, whereas it did not affect basal levels of progesterone or estradiol [6]. When endogenous BMP actions were blocked by noggin or BMP-signaling inhibitors, orexin A failed to increase levels of progesterone synthesis induced by FSH treatment, suggesting that endogenous BMP activity in granulosa cells might be involved in the enhancement of progesterone synthesis by orexin A. Thus, orexin A may enhance FSH-induced progesterone production, and the functional interaction between the orexin and BMP systems may affect progesterone synthesis.

Relative energy deficiency, anovulation and risk of fracture

The female athlete triad (FAT) was defined by the American College of Sports Medicine (ACSM) as low energy availability (low EA), functional hypothalamic amenorrhoea and osteoporosis. In low EA, lutein dysfunction first develops, followed by anovulation and, subsequently, oligomenorrhea, leading to amenorrhea. In athletes with one of the factors of FAT, the risk of a stress fracture is 2.4–4.9 times higher and may increase the risk of fracture throughout the lifespan. Low

EA is the starting point of the female athlete triad (FAT), which also includes functional hypothalamic amenorrhoea and osteoporosis. [8]. Among 390 athletes enrolled in a study, 36 developed new stress fractures within 3 months of registration. The risk for stress fractures due to the Triad in teenage athletes was higher than for athletes in their 20 s. In teenage female athletes, secondary amenorrhea, low BMD for the whole body, and a low ratio of actual body weight to ideal body weight increased the risk for stress fractures by 12.9 times, 4.5 times, and 1.1 times, respectively. [9].

Recently, the concept of female athlete triad has been expanded by the International Olympic Committee (IOC) to the Relative Energy Deficiency-Syndrom (RED-S), which includes the FAT but also other body systems and psychological factors [10].

Anovulation

Since anovulation may occur in low estrogen states such as relative energy deficiency or even in orthorexic dietary restraint, as well as with high estrogenic circumstances e.g. from functional perimenopausal ovarian cysts, the association with bone changes has been variable in the literature. Also, the proof of ovulation is methodologically elaborate (unlike the *likelihood* of ovulation, which is currently offered by many cycle-apps), since proof needs either two ultrasound examinations (one to make sure a dominant follicle developed, one proving it's rupture) or a progesterone measurement taken in the second half of the cycle, sufficiently distant from both ovulation and the following menstruation to be discriminatory. In a study by Niethammer et al., 176 cycles in healthy premenopausal women, FSH, 17β-estradiol (E2) and progesterone (P4) as well as bone alkalic phosphatase (BAP), pyridinoline (PYD) and C-terminal crosslinks (CTX) were measured during the follicular and during the luteal phase [11]. The probability and timing of ovulation was self-assessed by a monitoring device. In addition, bone density of the lumbar spine was measured by quantitative computed tomography (QCT) at baseline and at the end of the study. Analysis was restricted to blood samples taken more than three days before the following menstruation. 56.8 % of the analyzed cycles (n = 118) were ovulatory by two criteria (ovulation symbol shown on the monitor display and LP

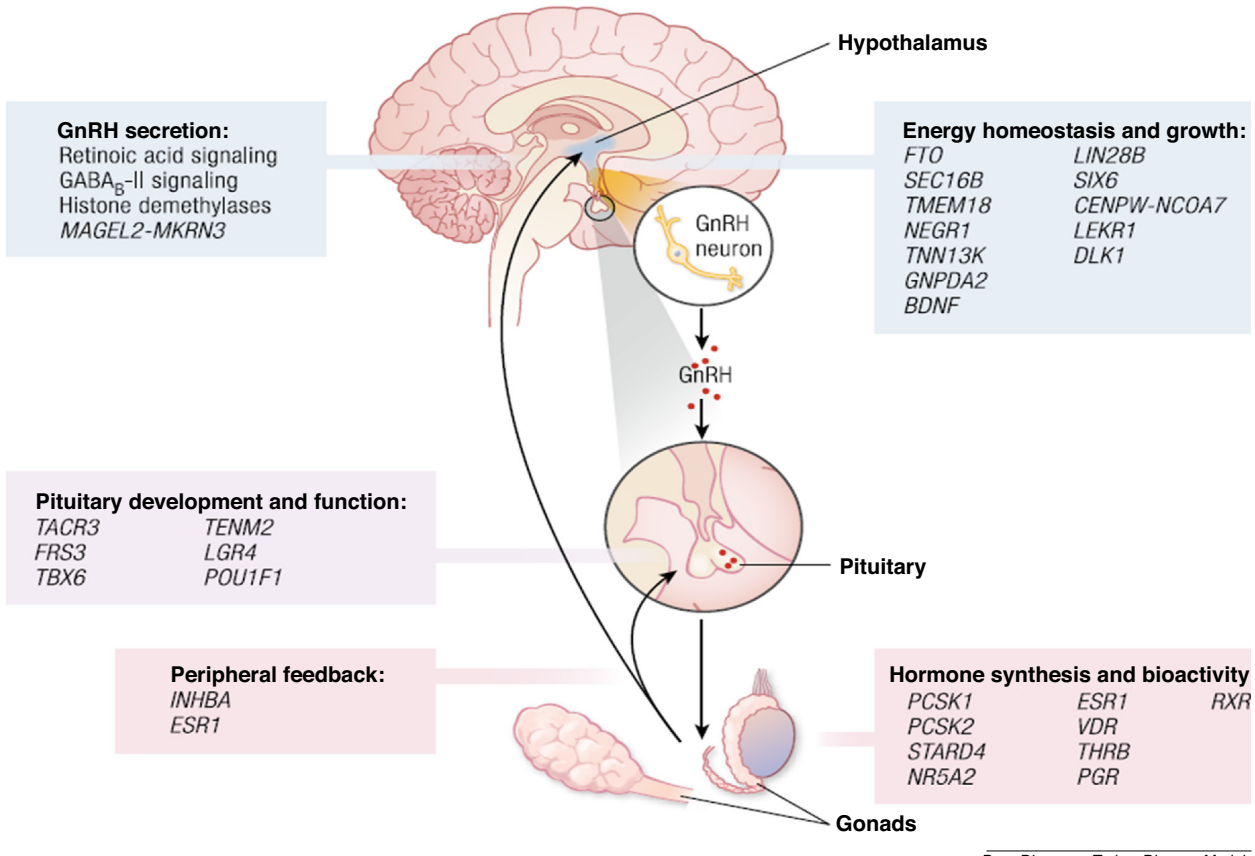

Fig. 1. This figure shows the complexity of neuroendocrine and other variables now known to influence the maturation of the reproductive neuroendocrine system leading to menarche [5].
Source: Ref. [7].

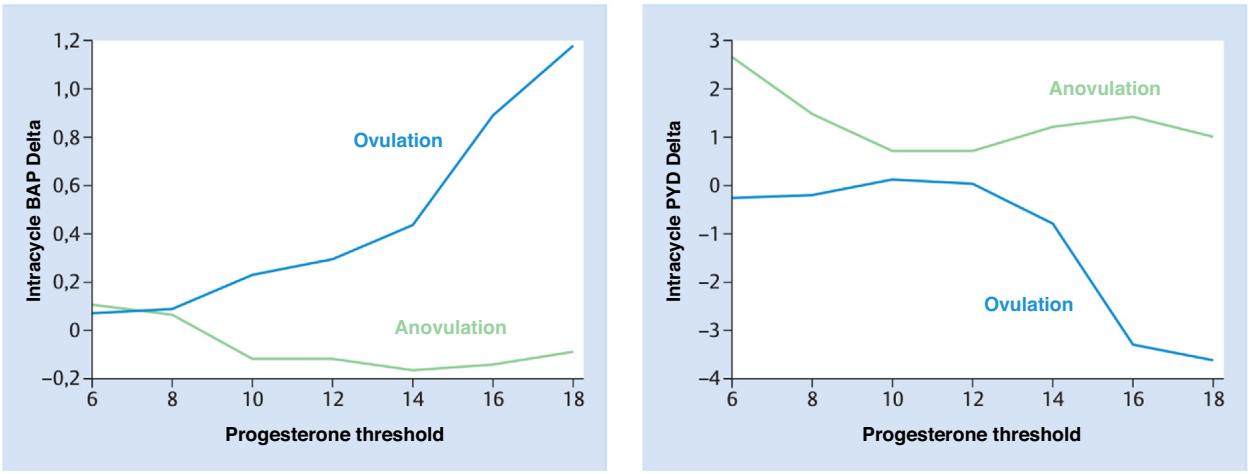

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Fig. 2. Within-woman analysis across ovulatory cycle phases of the changes in markers of bone resorption, pyridinoline (PYD), and of formation, bone-specific alkaline phosphatase (BAP).
A (left) shows the intra-cycle changes of the bone formation marker bone alkaline phosphatase (BAP) with rising progesterone threshold. The x-axis displays the rising progesterone threshold, the y-axis shows the difference (Delta) in serum concentration of BAP between FP and LP ([LP] – [FP]). Negative values indicate the luteal phase values are lower than follicular phase values. The upper (blue) line depicts the results for ovulatory cycles, while the lower (green) line shows the delta values for anovulatory cycles. In ovulatory cycles, the bone formation marker BAP was higher during the luteal phase than during the follicular phase in the participating women. This difference seems to grow with higher progesterone thresholds applied for the discrimination between ovulatory and anovulatory cycles. [15]
B (right) shows the intra-cycle changes of the bone resorption marker pyridinoline (PYD) with rising progesterone threshold. The x-axis displays the rising progesterone threshold, the y-axis shows the difference (Delta) in serum concentration of pyridinoline (PYD) between FP and LP ([LP] – [FP]). Negative values indicate the luteal phase values are lower than follicular phase values. The lower (blue) line depicts the results for ovulatory cycles, while the upper (green) line shows the delta values for anovulatory cycles. Bone resorption declined in the luteal phase of ovulatory cycles, while remaining higher in anovulatory cycles. The difference seems to grow with higher progesterone thresholds applied for discrimination between ovulatory and anovularory cycles [15]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Source: [11].

progesterone > 6 ng/mL), 33.1 % were possibly ovulatory by one criterion (ovulation symbol shown on the monitor display or LP progesterone > 6 ng/mL), and 10.2 % were anovulatory by both criteria). Ovulation in the previous cycle and in the same cycle did not significantly influence the mean absolute concentrations of the bone markers. However, bone formation (BAP) was higher (n.s.) in the luteal phase of ovulatory cycles than in anovulatory cycles and the relative changes within one cycle were significantly different for bone resorption (CTX) during ovulatory vs. anovulatory cycles ($p < 0.01$). In 68 pairs of cycles following each other directly, both ovulation in the previous cycle and ovulation in the present cycle influenced CTX, but not the differences of other bone markers. The authors concluded that ovulatory cycles reduce bone resorption in their luteal phase and that of the following cycle [11]. Which threshold of progesterone is needed for bone effects is not yet known (Fig. 2).

Progesterone effects in primary human osteoblasts

The influence of concentration and cyclicity of progesterone (P) on the proliferation and differentiation of human osteoblasts (HOBs) was studied by Schmidmayr et al. [12]. Cells were cultured for 14 and 28 days respectively in medium containing either estradiol at a concentration of 10^{-10} M or no estradiol. From day eight on, progesterone (P) was added in concentrations between 10^{-6} M and 10^{-10} M. The proliferation of HOBs was measured by asssaying hexosaminidase; differentiation was determined by measuring alkaline phosphatase (ALP).

In this study, seven days of exposure to physiologic levels of progesterone (6.4×10^{-7}–10^{-9} M) led to a significant increase in ALP concentrations of up to 70% ($p = 0.004$–0.019), while supraphysiologic progesterone concentration (6.4×10^{-6} M) showed a significant (50%) reduction of ALP ($p = 0.028$). After 21 days of P exposure, ALP increased up to 2.7-fold ($p = 0.000$–0.004) in the physiologic progesterone range, while supraphysiologic concentrations showed a decrease of ALP by 80% ($p = 0.03$). This effect was independent of pre- or co-treatment with estradiol. The effect in HOBs reached its peak at concentrations of 10^{-9} M progesterone, corresponding to known serum levels in the luteal phase of ovulatory cycles. These results support the concept of an osteoanabolic function of progesterone. By contrast, supraphysiologic progesterone concentrations suppressed the differentiation marker ALP in vitro. This could explain the clinical observation of decreased bone density after long-term

use of premenopausal depot progestins, and also - in part - the uncommon entity of osteoporosis in pregnancy [12].

Polycystic ovary syndrome (PCOS)

PCOS accounts for approximately 90% of anovulation infertility, affecting 5–10% of woman of reproductive age. In women with PCOS, GDF9 mRNA is decreased in all stages of follicular development compared to women without PCOS [3]. In particular, levels of GDF9 increase as the follicle develops from primordial stages to more mature stages [4]. Women with PCOS have considerably lower expression of GDF9 in primordial, primary and secondary stages of folliculogenesis [3]. GDF9 expression is not only reduced in women with PCOS but also delayed [4].

Studies on bone metabolism in PCOS have revealed a possible influence of chronic inflammation [13]. Park et al. showed that apart from its involvement in ovulation, the progesterone receptor plays an additional essential role: it attenuates ovulatory inflammation and Prostaglandin E 2 (PGE2) synthesis in granulosa cells by inhibiting the nuclear factor κB (NF-κB), a transcription factor, also involved in bone osteoclast activation. When the expression of PGR is ablated in granulosa cells, a hyperinflammatory condition may be manifested by excessive PGE2 synthesis, immune cell infiltration, oxidative damage, and neoplastic transformation of ovarian cells [14],

While ovulation itself shows parallels with inflammatory processes for a short time [15], lack of progesterone or its receptor may prolong this state of inflammation [13,14].

Prevalence of anovulation in normal length cycles

In an epidemiological study in Norway, ovulation was assessed in 3168 women aged 41.7 yrs (mean; interquartile range, [IQR] 36.8–45.5), with a normal cycle length of 28 days (d) (IQR 28 to 28) and body mass index (BMI) 26.3 kg/m 2 (95% CI 26.1–26.4). Parity was 95.6%, 30% smoked, 61.3% exercised regularly and 18% were obese. 1545 women with a serum progesterone level on cycle days 14 to -3 were presumed to be in the luteal phase. Of these, 63.3% of women were assumed to have had an ovulatory cycle (n = 978) and 37% (n = 567) were anovulatory [16] using a threshold of ≥9.54 nmol/L progesterone as proof of ovulation.

Ovulation involves the neurotransmitter control of amplitude and frequency of the LH pulse generator in the hypothalamus, changes which are seen in mild eating disorders such as restrained eating and orthorexia, and athletes as discussed above.

Progesterone receptor activity and RUNX2 — the osteoblast differentiation factor

The LH surge induces specific transcription factors that regulate the expression of a myriad of genes in periovulatory follicles to bring about ovulation and luteinization.

Runt-related transcription factor 2 (RUNX2), also known as core-binding factor subunit alpha-1 (CBF-alpha-1) is a protein that in humans is encoded by the *RUNX2* gene. RUNX2 is a key transcription factor associated with osteoblast differentiation.

Jo and Curry found that LH or hCG-induced Runx1 mRNA expression in rats was inhibited by a progesterone receptor antagonist. The expression was mediated by the activation of the progesterone receptor and epidermal growth factor receptor. Finally, knockdown of Runx1 mRNA by small interfering RNA decreased progesterone secretion and also reduced levels of mRNA for Cyp11a1, Hapln1, Mt1a, and Rgc32 [17].

Core Binding Factors (CBFs) are a small group of heterodimeric transcription factor complexes composed of DNA binding proteins, RUNXs, and a non-DNA binding protein, CBFB. The LH surge increases the expression of Runx1 and Runx2 in ovulatory follicles, while Cbfb is constitutively expressed. To investigate the physiological significance of CBFs, a conditional mutant mouse model was generated, in which granulosa cell expression of Runx2 and Cbfb was deleted. Mutant mice failed to develop corpora lutea, as evident by the lack of luteal marker gene expression, marked reduction of vascularization, and excessive apoptotic staining in unruptured poorly luteinized follicles, consistent with dramatic reduction of progesterone by 24 h after hCG administration [18].

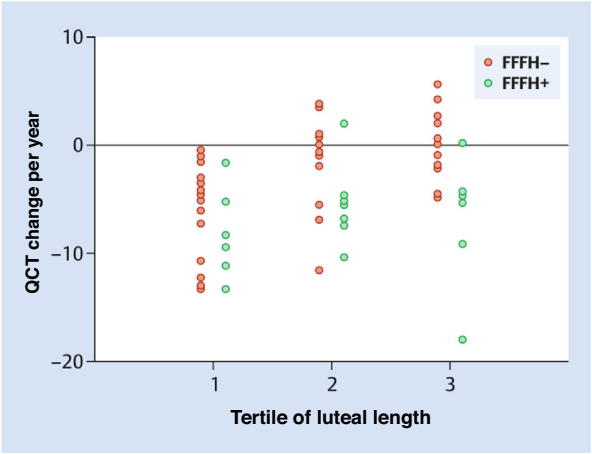

Fig. 3. This figure shows the 66-woman premenopausal Vancouver cohort divided across the X-axis into tertiles of the mean annual luteal phase length and showing on the Y axis the rate of QCT spinal trabecular bone change. Within the 3 pairs of dot columns, women in the respective right columns (green dots) had a family history of fragility fracture (FFFH+), while women in the respective left columns reported no family history of fragility fracture (FFFH-). Thus, the rate of trabecular volumetric bone loss was influenced by both genetics and by luteal phase length. Reprinted with permission from (23). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Source: [19].

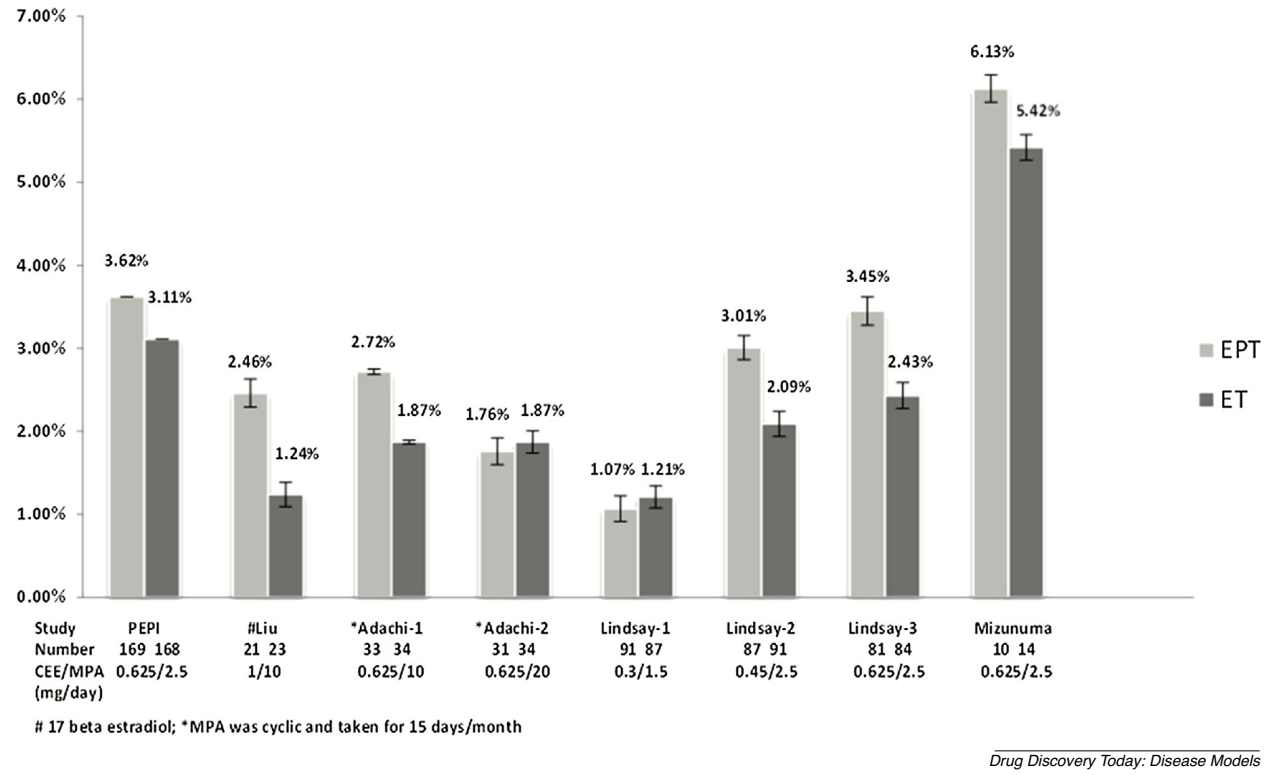

Fig. 4. This bar graph shows eight within-study cohort comparisons from five randomized controlled trial publications of menopausal women randomized to estrogen/estradiol alone (ET) versus estradiol-progesterone/progestin (EPT). Randomization occurred with consideration of hysterectomy status. Annual percentage spinal BMD change (±SD) is shown on the X-axis. Below each bar the number of women in each arm is noted. In addition, below each bar are medication doses: MPA = medroxyprogesterone, CEE = conjugated equine estrogen, E2 = estradiol, P4 = progesterone. Reprinted with permission from (24).
Source: Ref. [20].

Luteal phase length and osteoporosis

A study of 2-year trabecular bone change by quantitative computer-tomography (QCT) measurements in premenopausal patients from a pooled Canadian-German cohort found more bone loss per year in women with shorter luteal length than women with normal luteal length (indicating a greater area under the curve for progesterone), and also found women with a first-degree relative with osteoporosis (mostly the mother) to have more premenopausal bone loss than those without such a family history (see Fig. 3).

Progestin adds bone effect in postmenopausal hormone therapy

In a recent meta-analysis of 8 trials with direct randomization of women to receive either estrogen alone oder combined estrogen-progestin hormone therapy, an additional gain of bone density was noted in 6 of the 8 trials, as shown in Fig. 4.

Conclusion

The interaction between ovulation, progesterone and bone metabolism is complex. Accumulating physiological and clinical evidence however point towards a role for ovulation and progesterone in enhancing bone formation and limiting bone resorption.

Conflicts of interest

During the last five years, my team, my hospital or I have received travel expenses, research compensation for clinical studies, or honoraria from the following organisations: Amgen; Bavarian Government; Bayer; GKM Therapieforschung; Kade/Besins; LGL (Bavarian health agency); Medicover; Novartis; Novo Nordisk; Ricordati; Roche; Vifor.

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