Menstrual Cycle and Bone Health in Adolescents

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The intriguing notion of the menstrual cycle's having an acute impact on bone metabolism is examined as an expression of estrogen changes manifest as fluctuations in calcium-regulating hormones or biomarkers of bone formation/resorption. The effects of estrogen, progesterone, androgens, and follicle-stimulating hormones on bone health are also reviewed here. To date, the balance of evidence suggests that the menstrual cycle may exert a significant effect on bone metabolism. Further research needs to be conducted, however, to define these hormonal relationships.

Key words: **androgens; bone metabolism; estrogen; follicle-stimulating hormone (FSH); progesterone**

Menstrual Cycle

The relationship between sex hormones and bone health in general has been well documented,[1] prompting the intriguing question of whether the large fluctuations of sex hormones during the menstrual cycle may have an acute impact on bone metabolism. Over the past 30 years, about a dozen studies have been published that examine this question. Although the results have been inconclusive, some data suggest that the cyclic changes in sex hormones may significantly affect bone metabolism during the normal ovulatory menstrual cycle.

One of the earliest studies in this area was published by Pitkin and colleagues in 1978.[2] They examined selected calcium-regulating hormones in blood samples obtained at least every other day from seven adult women throughout one ovulatory cycle. They found that parathyroid hormone (PTH) levels rose through the follicular phase, peaked around the luteinizing hormone (LH) surge (30% higher than baseline), and declined through the luteal phase. Ionic calcium showed a reverse pattern, with decreasing levels before ovulation that then increased with and after the LH surge. Because increased estrogen is associated with ovulation and the luteal phase, these authors inferred from their data that bone resorption is inhibited, thereby leading to decreased levels of circulating calcium seen in the very early follicular phase, with the subsequent stimulation of PTH to restore blood calcium.

These interesting findings were noted within the investigative community, and attempts were made to replicate and extend this work, with mixed results. Three studies conducted over the next decade found no changes over the menstrual cycle in 1,25-dihydroxyvitamin D [$1,25(OH)_2D$],[3–5] PTH,[4,5] or total (nonionic) calcium.[4] In contrast, two studies were published over the same time period which, at least in part, lent support to the findings of Pitkin *et al.* First, Gray and colleagues reported that serum concentrations of $1,25(OH)_2D$ doubled on day 15 of an ovulatory menstrual cycle compared to the serum concentration of $1,25(OH)_2D$ on day 1 in seven healthy adult women. Mid-cycle increases in $1,25(OH)_2D$ were not found in five women who were on oral contraceptives who did not experience endogenous variations in sex hormones.[6] Similar findings of mid-cycle increases in $1,25(OH)_2D$ were reported by Tjellesen and colleagues.[7] Also lending support to calcium-regulating hormones' changes across the menstrual cycle, Zitterman *et al.* in a more recent study measured relevant hormone levels at five different points across an ovulatory menstrual cycle in nine adult women. Peak PTH concentrations occurred on day 3 after ovulation and decreased thereafter through the luteal phase.[8]

Over the past 15 years, the trend in measurement of bone metabolism related to the menstrual cycle has turned toward use of serum and urine biochemical markers of bone formation and bone resorption. Although the findings are not universal,[9] the balance of evidence from three studies is in support of variation

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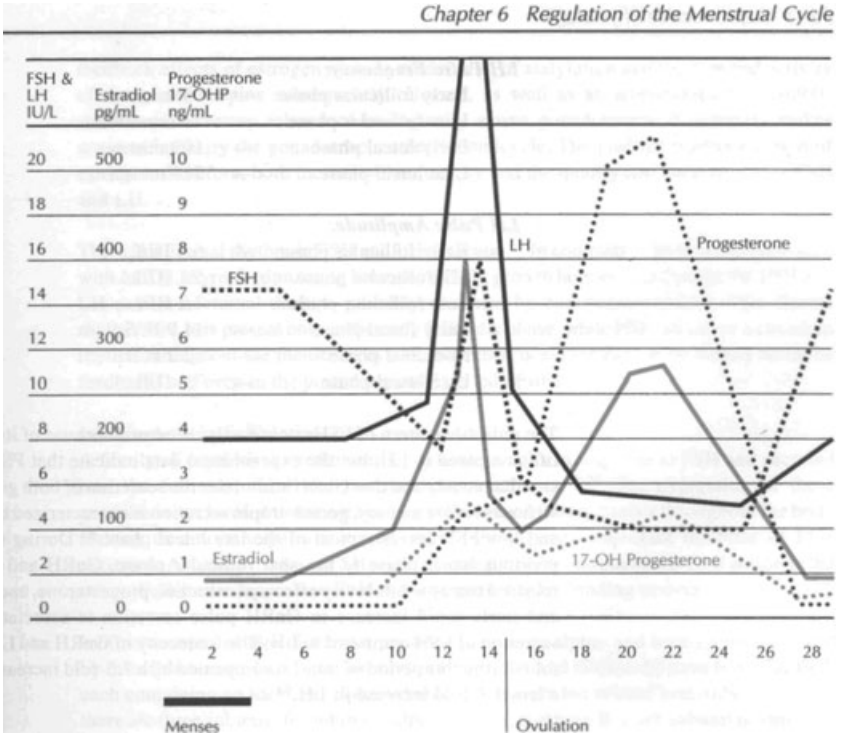

FIGURE 1. Depiction of menstrual cycle with parallel changes in bone biomarkers and calcium-regulating hormones. (Adapted from Speroff *et al*.[67])

in bone metabolism reflecting the changes in sex hormones over the menstrual cycle.[8,10,11] The studies were conducted in three separate international communities, but had similar designs, including the use of healthy women with established ovulatory menstrual cycles; blood and urine samples for selected measures of bone resorption[8,10,11] and formation[8,11] each collected at different points (range 5–12 times) across one menstrual cycle. In all three studies, markers of bone resorption were high in the early follicular phase, when estrogen levels are typically low. The markers of bone resorption were low during and after ovulation into the luteal phase, when estrogen levels are typically high. Similarly, in one study, the marker for bone formation was lower in the follicular phase than in the luteal phase, again reflecting an expected response to changes in circulating concentrations of estrogen.[8] Although these data convey compelling evidence for menstrual cycle–related changes in bone metabolism that would parallel changes in sex hormones, particularly estrogen, other data in these studies are not so convincing. For example, in one of these studies, there was no change in biomarkers of bone formation across the cycle despite demonstrated changes in estrogen.[11] Second, no correlation was found between bone biomarkers and sex hormone levels in two of the three studies.[8,10] However, it was noted in the third study that significant correlations were found between estrogen concentrations and biomarkers of bone resorption obtained 6 to 8 days earlier.[11] This finding may reflect the unsurprising delayed effect between change in estrogen exposure and signs of bone response. This finding also may account for the lack of significant relationships between biomarkers and estrogen concentrations in the other two studies.[8,10]

In summary, the intriguing notion of the menstrual cycle's having an acute impact on bone metabolism has been examined as an expression of estrogen changes seen as changes in calcium-regulating hormones or biomarkers of bone formation/resorption. To date the balance of evidence suggests that there may indeed be a significant effect of the menstrual cycle on bone metabolism. However, further research needs to be conducted to define these hormonal relationships.

Estrogen

Responding to a variety of mechanical, hormonal, and biochemical stimuli, the skeleton is a dynamic

system in which modeling and remodeling occur as an ongoing process in more than 10 million "multicellular units" (20% of the trabecular bone surface). By this activity, the skeleton maintains its integrity and plasticity. As a critical hormone in this process, estrogen is central to the growth and development of bone during childhood as well as in maintenance of bone integrity during adulthood. Several comprehensive reviews have been published describing the mechanisms of action of estrogen on bone across the lifetime.[1,12–15]

During childhood, increases in bone size and shape occur under a process termed bone modeling, which means that the classic lifelong relationship between bone resorption and formation favors bone formation at this stage of development. Estrogen is intimately involved, along with growth hormone and insulin growth factors, in bone modeling. Linear growth occurs through ossification of the growth plates at the terminal ends of long bones, and radial bone growth occurs by periosteal (outer surface) apposition along with endosteal (inner surface) formation. Puberty is terminated by epiphyseal closure, also driven by estrogen, by which time almost all of peak bone mass has been achieved.[12] The remaining bone mass is accrued by a process called "consolidation," in which bone mass in increased without a change in bone length, but rather by continued periosteal apposition and internal thickening of the trabeculae.[12]

In contrast, after puberty is completed and while a woman is still estrogen-sufficient, bone remodeling predominates, during which the relationship between bone resorption and formation is "coupled", that is, the amount of bone formed and resorbed is about equal and bone mass is maintained. However, in an estrogen-deficient state, of which the menopause is the classic example, the relationship between bone resorption and formation is "uncoupled," and increased bone resorption, outstripping bone formation, results in loss of bone mass. The lack of estrogen is associated with cortical thinning and loss of bone from the endosteal surface, with resultant enlargement of the bone marrow cavity and decreased bone mass.[16]

Estrogen's effect on bone can also be viewed from a biomechanical perspective. During childhood and adolescence, bones continually adapt to mechanical challenges due to increasing bone length and muscular action. It is well recognized that estrogen has a major modulating effect on how bone responds to mechanical stimuli, thereby inducing important structural changes in the bone. According to a model proposed by Frost, the main action of estrogen is to lower the mechanostat setpoint on inner bone surfaces.[17] In other words, under the influence of estrogen, relatively small mechanical loads have a large effect on bone; the result is increased bone on the endosteal surface. This model of estrogen action is supported by the observation that estrogen levels in early pubertal girls are negatively associated with marrow cavity size, but positively associated with cortical thickness.[18] In contrast, given an estrogen-deficient state, the bone senses a smaller mechanical load than with the same degree of stimulation in an estrogen-sufficient state; the result is similar to a "disuse" mode, which leads to rapid loss in bone mass as is seen in early menopause.[17]

TABLE 1. Selected sex hormones and their general relationship with bone biomarkers

	Bone action	
Sex hormones	Formation	Resorption
Estrogen	±	↓↓
Progesterone	↑	±
Testosterone	↑	±
DHEA-S	↑?	±?
FSH	–	↑

Despite rapid bone loss seen in estrogen deficiency, there is some compensatory structural adaptation that preserves some of the bone strength as measured by its ability to withstand physical stress. As described above, the loss on the endosteal bone surface caused by estrogen deficiency is associated with stimulation at the outer surface of the bone, termed periosteal expansion. Periosteal expansion helps offset the bone loss on the endosteal surface; therefore, partial mechanical strength is maintained, although in a larger diameter, less dense bone. The contribution of bone mass to bone strength varies as the square of its distance from the neutral axis of the bone (i.e., the center of mass of the cross-section). Therefore, bone strength, expressed as section modulus, can be partially offset with a small increase in outer bone diameter despite substantial losses in bone mass.[19,20]

In addition to estrogen's effect on biomechanical aspects of bone health, there is a complex interaction between estrogen and systemic hormones, locally produced cytokines, and other mediators,[14] either directly at the cellular level or indirectly through effects on calcium-regulating hormones. Since the discovery of estrogen receptor on bone cells 20 years ago,[21,22] investigation has focused on the direct effects of estrogen through its interaction with the osteoblast (bone-forming cell) and the osteoclast (bone-resorbing cell). There appear to be two receptors, alpha and beta, whose patterns of expression are overlapping and are incompletely characterized.[15] Scientific evidence to

date suggests that the primary direct effect by estrogen on bone metabolism is through a combination of genomic and nongenomic action on osteoblasts and osteoclasts that begin with interaction with the estrogen receptor in the osteoblast and ultimately result in production of selected cytokines that affect bone metabolism.[15] Specifically, the initiating step is when estrogen attaches to the receptor in the cytoplasm and the complex undergoes conformational change and diffuses to the nucleus, where it combines with "estrogen receptor elements." This entity joins with the appropriate site on the nuclear DNA and undergoes transcription for the ultimate production of specified proteins. These proteins, such as certain cytokines and other mediators, are released into the local milieu.[1,12,13] Which of these cytokines are crucial for mediating the action of estrogen has not been resolved; a comprehensive list is provided by Rickard *et al.*[15] There are also "nongenomic" mechanisms by which estrogen can have a direct impact upon a bone cell by attaching to the plasma membrane and initiating a biochemical cascade by which certain cellular action relevant to bone metabolism occurs; however, this action is less well-characterized than the genomic response.[15]

Another major effect of estrogen on bone is indirect, through its impact on calcium-regulating hormones. For example, estrogen stimulates production of $1,25(OH)_2D$ from hydroxyvitamin D, which in turn enhances gastrointestinal absorption of calcium and restricts excretion of calcium from the kidney; with increased total body retention of calcium, production of PTH is suppressed.[23] Furthermore, estrogen increases the number of vitamin D receptors, which in turn enhances the impact of vitamin D on the bone cell, especially the osteoblast.[24] Within the osteoblast, $1,25(OH)_2$ appears to stimulate secretion of osteocalcin by osteoblasts, which is a marker of bone formation.[24]

In summary, estrogen is essential to the growth, maturation, and maintenance of the skeleton in both males and females. It exerts a profound effect that is manifested through changes in bone mass as well as biomechanical forces and through direct effects via interaction with estrogen receptors as well as indirect effects on calcium-regulating hormones. Although our understanding is not perfect, estrogen is the most thoroughly characterized of the sex hormones as they relate to bone metabolism.

Progesterone

When compared to estrogen, the role of progesterone in bone metabolism is less clear.[1,13] This lack of clarity is in part because less research has been devoted to studying the effect of progesterone on bone and in part due to its intimate relationship with estrogen, For example, animal studies have shown a synergistic positive activity on bone when estrogen and progesterone are added to bone cell culture together.[25,26] In addition, studies have demonstrated that estrogen upregulates progesterone receptors in rat osteoprogenitor cells in response to progesterone exposure.[27] From the balance of evidence to date, it appears that both estrogen and progesterone contribute to skeletal development in the growing adolescent and to maintenance of bone mass in the adult premenopausal woman.[28]

The main contribution of progesterone in the bone's modeling and remodeling processes is to stimulate bone formation. For example, the administration of progesterone to growing[29] and aged[30] rats that had undergone ovariectomy was associated with enhanced bone formation. In addition, multiple studies have demonstrated similar findings, specifically through enhanced osteoblast proliferation and differentiation, in cell cultures of human-like osteoblasts.[31,32] The mechanism for this effect is postulated to be through a specific nuclear progesterone receptor.[33] In humans, however, the findings related to progesterone and bone mineral density are controversial.[13] In a study that generated a great deal of interest almost 20 years ago, Prior *et al.* found lower bone density of the spine in young women who, despite normal estrogen levels, had a shortened luteal phase of the menstrual cycle.[34] The implication was that decreased concentrations of progesterone associated with a shortened luteal phase accounted for the deficit in bone mineral density. However, in later studies, this finding was not confirmed, that is, as long as estrogen levels were maintained throughout the menstrual cycle, no differences were found in spine bone mineral density between women with normal and those with shortened luteal phases.[35,36] One potential reason for the results reported by Prior *et al.* included a less exacting method of measurement of estrogen than in the later studies. In addition, Prior *et al.*'s study sample was drawn from women runners, who often have inherent sex hormone imbalances, thereby potentially altering the hormone results when compared to nonathletic woman observed in the later studies.

The story is more complicated when it comes to the synthetic derivatives of progesterone, such as progestins. Several progestins have been developed over the past three decades that differ in their molecular lineage. These progestins have wide-ranging biological effects beyond the progestational action that has an impact on several biological systems, including bone.[37] For example, the progestins derived directly

from testosterone, such as norethindrone and norethisterone, possess androgenic properties and are aromatized to estrogen. In contrast, progestins derived directly from progesterone, such as medroxyprogesterone acetate, do not have androgenic or estrogenic properties. Moreover, medroxyprogesterone acetate exhibits glucocorticoid properties, a unique quality distinct from that of progesterone and unique among the progestins. Glucocorticoids administered in pharmacologic doses are a well-known cause of osteoporosis.[14] The negative effect is thought to be through impaired osteoblast function during the bone formation phase of the remodeling cycle.[14] Some preliminary data indicate that medroxyprogesterone acetate occupies and interacts with the glucocorticoid receptor, having an ultimate impact on the osteoblast similar to that of the interaction of glucocorticoids.[38]

Given these differing biological qualities, it is unsurprising that available data from clinical studies on the relationship between the various progestins and bone are conflicting.[39–47] The general impression among the relevant clinical studies is that progestins derived from testosterone have a more positive impact on bone than does medroxyprogesterone acetate. Moreover, with the high doses of medroxyprogesterone acetate used for contraception, in addition to the negative glucocorticoid effect on bone, the hypothalamus-pituitary-ovarian axis is suppressed. This suppression causes estrogen insufficiency with consequent loss in bone mineral density.[48–51] However, overall, more research needs to be done to tease apart the differing biological actions on bone of these aforementioned progestins , as well as others that have been more recently developed.

Androgens

Interesting case reports in the early 1990s of men with no biological effect from estrogen, because of either estrogen receptor defect[52] or aromatase deficiency,[53] revealed poor skeletal maturation, tall stature, and low bone mineral density. These findings underscored the critical role that estrogen plays in bone among men as well as women. However, several lines of evidence indicate that androgens have an important impact on the skeletal health in both sexes that is independent of estrogen.[13]

A major discovery that supports the contention that androgens exert a direct effect on bone is that of androgen receptors first in cell culture,[54] and then in human bone.[55] Specific androgens for which receptors have been identified include testosterone, dihydrotestosterone, and dehydroepiandrosterone (DHEA).[1] These receptors have been found on osteocytes, osteoblasts, and osteoclasts at different sites of the skeleton. Through interaction with the receptors, androgens appear to enhance bone formation by decreasing apoptosis of osteoblasts, thereby increasing their life span.[12,13]

The second line of evidence in support of androgens' having an impact on bone that is distinct from that of estrogen is the different specific areas of the skeleton on which each appears to have its greatest effect. In animal models, androgens promote chondrocyte maturation, metaphyseal ossification, and growth of long bone.[13] In humans, the most prominent example is that testosterone, in particular, stimulates periosteal expansion. Given lower circulating concentrations of testosterone in the female, this effect is much less prominent and explains why bones are smaller in the female than in the male.[12]

Previous work has shown that women who have increased circulating concentrations of androgens (e.g., polycystic ovary syndrome) have higher bone mineral density than do women with normal endogenous levels of androgens.[56,57] Similarly, postmenopausal women treated with both androgens and estrogen have higher bone mineral density than those treated with estrogen alone.[58] The positive effects of androgens in both these clinical circumstances could have been through androgen aromatization to estrogen and the consequent positive effect of estrogen on bone mineral density. However, nonaromatizable androgens appear also to enhance bone formation by increasing osteoblast proliferation and differentiation, again suggesting an independent positive effect of androgens on the skeleton.[59]

Among the various androgens, testosterone, as a marker of ovarian androgen production, and DHEA (along with the sulfate compound DHEAS), as markers of adrenal androgen production, have received the most research attention as to their relationship to bone health in women. Although studies in human females have shown that both ovarian and adrenal androgens are associated with spine and hip bone mineral density in adult women across the age span, only testosterone appears to maintain an independent relationship with bone mineral density after adjustment for pertinent clinical and hormonal confounding influences.[60,61] However, more research needs to be conducted to refine our understanding of the individual androgens' contributions to bone metabolism.

In summary, evidence to date indicates that androgens, particularly testosterone, exert a clinically significant, positive impact on bone not only through aromatization to estrogen, but also through a direct effect via androgen receptors that results in increased bone

formation, manifested especially through periosteal expansion.

Follicle-Stimulating Hormone

An intriguing new candidate that recent research suggests may contribute directly to bone metabolism is the follicle-stimulating hormone (FSH). Elevated serum levels of FSH have traditionally been used as an early indicator of menopause, the classic model of estrogen insufficiency. Thus, in clinical conditions of estrogen insufficiency, high circulating levels of FSH go in tandem with low levels of estrogen. To date, as described above, the major mechanism for bone loss in menopause is attributed to lack of estrogen. However, new evidence suggests that that mechanism may be masking a direct negative impact of high circulating levels of FSH on bone.

The breakthrough study in this area was published by Sun and colleagues, although some previous work in rats, a decade earlier, suggested that an intact pituitary may be needed for the full negative impact on bone that occurs with gonadectomy.[62,63] Using mice in whom the ovaries and FSH receptors had been removed, Sun *et al.*, found that "areal and volumetric bone mineral density at both trabecular and cortical sites were indistinguishable from those in controls."[64] In contrast, the mice who had had their ovaries removed, but had intact FSH receptors, experienced a 15% decrease in lumbar spine areal bone mineral density after eight weeks. Furthermore, these investigators located FSH receptors on mice and human osteoclasts and demonstrated increased osteoclastogenesis in response to stimulation with FSH. These effects were felt to be independent of the effects of estrogen.

Two reports of thousands of pre- and perimenopausal women from the study entitled Women's Health Across the Nation (SWAN) also lend support to the contention that FSH may be directly involved with bone metabolism. One study found that, although there was no significant relationship between serum estrogen levels and lumbar spine bone mineral density, serum log-transformed levels of FSH were significantly inversely related to lumbar bone mineral density.[65] Furthermore, bone mineral density values were around 0.5% lower for each higher FSH quartile. However, it should be noted that all hormone levels were obtained during the early follicular phase of the menstrual cycle, which would likely result in much less variation in estrogen levels than in the late follicular or luteal phases, thereby increasing the likelihood of type 2 error.

The second study, conducted by the same team of investigators, also using a study population from SWAN, conducted five annual examinations of bone mineral density and sex hormone predictors. These researchers found that baseline FSH levels, along with the annual FSH levels, significantly predicted bone loss over the period of observation.[66] Although estradiol levels <35 pg/mL were significantly associated with lower bone mineral density, annual estrogen measures did not predict bone loss. The authors noted that, during the time of the perimenopause, FSH may simply serve as a "proxy" for ovarian estrogen productivity. The wide variation in estrogen levels typical of this hormonal phase may also predilect to a type 2 error.

In conclusion, if FSH is directly involved in producing bone loss in estrogen-deficient conditions, this insight would not only have an interesting, and perhaps profound, impact on our understanding of hormonal mechanisms in bone metabolism, but also may carry important clinical implications. The data described above offer initial compelling evidence that FSH may be intimately involved in skeletal health. However, a great note of caution should be issued that this particular research is still in its infancy. Further research is required that will elucidate the role of FSH in bone health.

Future Directions

From this review of the literature, it is evident that more research efforts are needed to more accurately define the relationship between fluctuations in sex hormones through a normal, ovulatory menstrual cycle and changes in bone metabolism. Such evidence will advance our understanding of the mechanisms of action of sex hormones on bone in women with ovulatory and in women with anovulatory cycles. In addition, although estrogen's impact on bone metabolism has been studied in great detail, more research attention needs to be directed to the role(s) of progesterone (and the synthetic progestins), androgens, and FSH on bone health.

Conflicts of Interest

The author declares no conflicts of interest.

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