Female Reproductive System and Bone

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Abstract

The female reproductive system plays a major role in regulating the acquisition and loss of bone by the skeleton from menarche through senescence. Onset of gonadal sex steroid secretion at puberty is the major factor responsible for skeletal longitudinal and radial growth, as well as significant gain in bone density, until peak bone density is achieved in third decade of life. Gonadal sex steroids then help maintain peak bone density until menopause, including during the transient changes in skeletal mineral content associated with pregnancy and lactation. At menopause, decreased gonadal sex steroid production normally leads to rapid bone loss. The most rapid bone loss associated with decreased estrogen levels occurs in the first 8–10 years after menopause, with slower age-related bone loss occurring during later life. Age-related bone loss in women after the early menopausal phase of bone loss is caused by ongoing gonadal sex steroid deficiency, vitamin D deficiency, and secondary hyperparathyroidism. Other factors also contribute to age-related bone loss, including intrinsic defects in osteoblast function, impairment of the GH/IGF axis, reduced peak bone mass, age-associated sarcopenia, and various sporadic secondary causes. Further understanding of the relative contributions of the female reproductive system and each of the other factors to development and maintenance of the female skeleton, bone loss, and fracture risk will lead to improved approaches for prevention and treatment of osteoporosis.

Keywords

Sex steroids; osteoporosis; bone loss; aging; fractures

1.1 Introduction

The female reproductive system profoundly affects the skeleton during longitudinal and radial growth during growth and development, and during modeling and remodeling throughout adult

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life. Longitudinal and radial growth of the skeleton occurs prior to menarche under the influence of growth hormone, insulin-like growth factors, and other factors.

Onset of estrogen and other sex steroid secretion with menarche at age 11–13 years stimulates rapid skeletal mineral acquisition, as well as further longitudinal and radial skeletal growth, for the next 10 years or so. Women gain about a third of their peak bone mineral density (BMD) within the 4 years around the onset of menarche. The early pubertal rapid increase in BMD is followed by further slower increases in BMD and consolidation of skeletal mineral content during the late teens and early 3rd decade, until peak BMD is achieved at around age 25–35 years.

The female reproductive system subsequently plays a major role in the modeling and remodeling of the skeleton throughout adult life until menopause, when decreased gonadal sex steroid secretion becomes the dominant factor in causation of rapid bone loss in the early postmenopausal period. Continued age-related bone loss in the late postmenopausal period is thought due to sex steroid deficiency as well as vitamin D deficiency and increased parathyroid hormone (PTH) secretion.

1.2 Brief Overview of the Female Reproductive System

The female reproductive system is a complex multi-organ system involving the hypothalamus, pituitary gland, ovaries, uterus (endometrium and cervix), and vagina. A series of cyclic and closely regulated events involving the reproductive organs occur on a regular basis on an average of every 28–32 days in healthy non-pregnant females between menarche and menopause (Figure 1). Menarche normally occurs at age 11–13 years, and is characterized by establishment of new circadian (24-hour) and ultradian (60–90 minute) gonadotropin rhythms, and development of a positive estrogen feedback loop controlling the infradian (monthly) rhythm. Sleep-related increases in gonadotropins and gonadal steroids begin during puberty, and appear to play an important role in pubertal maturation. Menopause occurs on average at age 51–52 years, but may occur normally any time after age 40 years.

The menstrual cycle is caused by a tightly regulated sequence of events that occurs each month (Figure 2). Normal menstrual cycles require cyclic secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus, with subsequent cyclic secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by gonadotropes in the anterior pituitary gland (Figure 3). Regular cyclic secretion of LH and FSH normally results in maturation of one ovum each month, ovulation of the mature egg, and migration of the ovum to the uterine endometrium via the Fallopian tubes. Ovarian secretion of sex steroid hormones causes changes in the uterine endometrium prior to implantation of the fertilized ovum. If the ovum is not fertilized, ovarian secretion of estrogen and progesterone decreases, the endometrium lining breaks down, and menstruation begins.

The normal menstrual cycle begins with the first day of vaginal bleeding, and ends just before the next menstrual cycle starts. The median menstrual cycle duration is 28 days, but normal cycles may occur every 21–40 days. Menstrual cycle duration is most variable in the years after menarche and immediately preceding menopause [1]. Menstrual flow typically lasts 5 ± 2 days, with typical blood loss with each cycle ranging from 30 to 80 mL [2].

The normal menstrual cycle is divided into the earlier follicular, or proliferative, phase, and the later luteal, or secretory, phase (Figure 2). The follicular phase is more variable in duration, whereas the luteal phase consistently lasts about 14 days in most women. Ovulation occurs at the end of the follicular phase, during the transition to the luteal phase, with the ovulatory phase beginning one day prior to the LH surge and continuing until ovulation, typically 16–32 hours after the LH surge.

Circulating FSH begins to increase late in the luteal phase of the preceding menstrual cycle, and remains increased into the early follicular phase, thereby stimulating growth and development of several ovarian follicles [3]. One of these follicles is selected by as yet unknown processes for further maturation into the dominant follicle. Serum FSH levels then begin to decrease and, and except for the marked surge during ovulation, continue to decrease throughout the remainder of the cycle until the late luteal phase.

Serum LH also begins to increase in the late follicular phase of the preceding menstrual cycle, but in distinction to FSH, LH continues to slowly increase throughout the follicular phase until it surges for one to three days at mid-cycle [3]. LH then gradually decreases to its lowest levels in the late luteal phase.

Both LH and FSH are secreted in a pulsatile fashion by gonadotropes in the anterior pituitary gland, in response to pulsatile secretion of hypothalamic GnRH [4]. LH and FSH pulses usually occur 1–4 hours apart, depending on the phase of the menstrual cycle [5]. LH is secreted least during the luteal phase, which is attributed to the feedback effects of progesterone produced by the corpus luteum on the hypothalamus and pituitary gland [6].

Serum estradiol (E2) and progesterone are secreted by the ovaries, along with other gonadal sex steroids and nonsteroidal hormones. Circulating E2 levels are lowest during the early follicular phase, and begin to increase 7–8 days prior to the LH surge. Peak E2 levels of 250–350 pg/mL occur on the day of the LH surge, or the day before [7]. Serum E2 falls quickly as LH peaks, but increases again about 6–8 days after the LH surge. Serum estrone (E1) levels parallel E2 levels, but at lower levels. About 95% of circulating E2 is produced by the dominant ovarian follicle and corpus luteum, whereas serum E1 is produced by conversion from E2 and from peripheral conversion of the adrenal hormone androstenedione.

Androstenedione and testosterone are the main androgens secreted by the ovarian interstitial and theca cells. Androstenedione is the main ovarian androgen, and can be converted in peripheral tissues to testosterone and estradiol. Both androstenedione and testosterone are also secreted in significant quantities by the adrenal glands. Androstenedione and testosterone both peak with the mid-cycle LH surge due to increased ovarian secretion [7,8]. Serum dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S), which are secreted almost entirely by the adrenal glands, do not vary with the phases of the menstrual cycle.

Serum progesterone levels remain low during the follicular phase, and begin to increase just before the LH surge [8,9]. Progesterone levels peak about 6–8 days after the LH surge, and then decrease toward baseline unless the ovum is fertilized. Normal ovulation results in serum progesterone levels of about 10 ng/mL or greater about one week before onset of the next menstrual cycle. Serum 17α-hydroxyprogesterone levels increase at midcycle, before progesterone, and parallel changes in progesterone during the luteal phase.

The ovarian follicular granulosa cells and corpus luteum secrete multiple other peptides and hormones, including activin, inhibin (folliculostatin), follastatin, and others, some of which have feedback effects on the hypothalamus and pituitary gland. Inhibin A is composed of an α-subunit and a β-A subunit, and inhibin B of an α-subunit and a β-B subunit. Serum inhibin A begins to increase during the early and midfollicular phase, increases further during the late follicular phase, and peaks during the luteal phase. However, E2 appears to be the major feedback regulator of FSH secretion by the pituitary gland during the luteal phase [10]. Serum inhibin B peaks during the follicular phase, apparently in response to increasing FSH [11], and is critical for suppressing FSH secretion and causing a plateau in FSH levels in the mid-follicular phase, whereas E2 is responsible for further suppression of FSH secretion [10]. The subsequent rise in LH and fall in FSH in the late follicular phase is thought to be due to more effective suppression of pituitary FSH secretion than LH secretion by E2.

Activin is a dimer composed of two β subunits that does not vary during the menstrual cycle [12]. Follistatin is concentrated 100-fold in the follicular fluid, and appears to regulate activin action by binding the hormone. FSH and prostaglandin E2 stimulate follistatin production in granulosa cells [13], but it is not known whether follicular fluid levels of follistatin vary with the menstrual cycle.

A number of other hormones that are not thought to be directly related to ovulation, including insulin-like growth factor-I (IGF-I), growth hormone (GH), prolactin, ACTH, cortisol, PTH, calcitonin, and estrogen-sensitive neurophysin also peak at mid-cycle. IGF-I is secreted in small amounts by the ovary [14], although mostly produced by other tissues elsewhere, including the liver [15]. Serum IGF-I reaches a nadir during the menses, and peaks during midcycle and the luteal phase [16]. Serum IGF-I levels decrease with age [17], and its actions are regulated by IGF binding protein-3 (IGFBP-3), which binds IGF-I and decreases the amount of bioactive IGF-I available in the circulation. GH levels correlate with E2, and peak during midcycle [18]. GH potentiates the stimulatory effects of FSH and LH on ovarian follicles, and stimulates progesterone production by the corpus luteum, with actions largely mediated by IGF-I. The physiological contribution of the other hormones to the menstrual cycle is not clear.

Circulating estrogen and to a lessor extent, progesterone, levels directly benefit the skeleton by effects mediated by estrogen and progesterone receptors on bone cells. Estrogen suppresses bone resorption, and increases bone formation, in women from puberty to menopause. Progesterone has not been demonstrated to have antiresorptive activity after menopause, but it may stimulate bone formation when used as a co-therapy with estrogen [19]. These hormones cause a marked increase in bone mineral density starting at menarche, which peaks at peak bone density by age 25–35 years. The physiological effects of other female reproductive system hormones on the skeleton are not as clear.

1.3 Skeletal Growth and Development Before Menarche

The skeleton of both females and males becomes dependent at birth on intestinal absorption of minerals and other nutrients for longitudinal and radial growth [20]. Milk intake is usually low for the first several days after birth, and body weight decreases. By the end of the first week, however, milk intake is usually sufficient to promote weight gain and growth. Birth causes a transient decrease in neonatal serum calcium, with a consequent increase in parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D levels [21]. PTH-related Peptide (PTHrP) rapidly loses the dominant role in calcium metabolism that it occupied during fetal life. Bone formation markers decrease in the first few days after birth, whereas bone resorption markers increase, but both types of markers subsequently increase over the next several weeks to months and for the remainder of growth and development [22].

Volumetric BMD (vBMD) decreases by as much as 30% in skeletal long bones during the first six months of life, as the bone marrow cavity expands by endosteal resorption [23]. Cortical vBMD also deceases during the first few months of life, but overall bone strength remains stable, unlike later in life, when similar changes during postmenopausal life cause decreased bone strength. These changes in vBMD result in a shift of endocortical and intracortical bone to the periosteal surface during rapid periosteal growth, with rapidly increasing bone mass and areal BMD (aBMD) during the first six months of life [24].

Continued growth during childhood causes significant increases in skeletal bone mass, as well as changes in skeletal size, shape, geometry, and material properties of bone [25]. The rate and magnitude of changes that occur during growth and development vary by skeletal site, and within the cortical and trabecular bone compartments [26,27]. Genetic factors determine 60–80% of the variability in skeletal development, but diet, lifestyle, and acquired illness have significant impacts on skeletal growth, modeling, and remodeling. A variety of osteoblast-

related genes, including estrogen receptor 1 (*ER1*), collagen type 1α2, osteocalcin, parathyroid hormone receptor 1 (*PTHR1*), transforming growth factor-beta 1 (*TGFβ1*), vitamin D receptor (*VDR*), and lipoprotein receptor-related protein 5 (*LRP5*) have been identified as a candidate genes for regulation of bone mineral density in children in recent genome-wide association (GWA) studies [28]. A recent GWA study in children (the Avon Longitudinal Study of Parents and Children study) showed that common variants in the region around the gene *Osterix* are associated with bone mineral density and growth in childhood [29]. Acquired illness may have greater or lesser effects on the skeleton, depending on the age of onset.

Normal prepubertal skeletal development results in more rapid bone growth and mineral acquisition in the appendicular skeleton than the axial skeleton. Longitudinal and radial bone growth occurs more rapidly than mineralization through the peripubertal period, with 7 year-old girls achieving 80% of their final adult stature on average, but only 40% of their projected peak bone mass [30]. Transiliac bone biopsies from healthy children older than one year show that metabolic activity within bone appears to decrease gradually with increasing age [31]. Bone remodeling decreases in both cortical and trabecular bone with increasing age, but bone formation remains higher in cortical bone, and remodeling is more active on the endocortical surface than on the periosteal surface of cortical bone.

1.4 Effects of Menarche on the Skeleton

Early and mid-puberty stimulates rapid axial skeletal growth, whereas late puberty results in slowing of both axial and appendicular growth [30]. Because peak rates of mineral accrual lag about 8 months behind peak height velocity, peripubertal life is associated with relative undermineralization of bone and increased fracture risk [32]. Girls have onset of puberty earlier than boys [33], and blacks have earlier onset of puberty than whites [34]. Women achieve a final adult height that averages 13 cm less than men. Between ages 6 and 16 years, total body bone mineral content (BMC) increases 2.5-fold, and aBMD at the spine increases roughly 2-fold [35]. Increasing bone size accounts for much of the increase in total body BMC and aBMD, but vBMD also increases at certain skeletal sites during puberty [34,36]. Cortical mid-tibial bone samples taken from young adult women 17–46 years old have similar tissue stiffness, strength, toughness, and damageability as cortical mid-tibial biopsies from men, even though the women have narrower tibial diameter for body size than men [37].

Although bone size and mass increase significantly throughout the skeleton during adolescence, apparent vBMD assessed by DXA increases at the lumbar spine only [34,38]. One study showed that while aBMD increased by 55% in women between ages 9 and 20 years, vBMD increased by only 20% [34]. Other studies have shown that vBMD did not change in the femoral neck or midshaft [34,38], whereas other studies showed increased femoral mid-shaft vBMD during late puberty [29]. Studies using quantitative CT (QCT) scanning have shown that apparent vBMD increases only in the axial skeleton during adolescence [36]. Both cortical and trabecular vBMD increase in girls [36], with increased trabecular vBMD attributed to increased trabecular thickness rather than trabecular number [26]. However, femoral mid-shaft or radial cortical vBMD did not change throughout adolescence [39,40]. Studies using peripheral QCT, however, have shown that distal radial and distal tibial cortical vBMD increases during puberty [41–43]. Radial diaphyseal vBMD increased by 48%, cross-sectional area by 50%, and BMC by 111%, in females between age 6 and 40 years in one study [41]. After matching with developmental stage and cortical width for men in the study, women had 3–4% greater radial diaphyseal vBMD than men in late puberty [42]. Total and cortical vBMD, but not trabecular vBMD, increased in the women in this study between age 15 and adulthood [43]. Girls had greater tibial cortical vBMD than boys from pre- to late puberty, suggesting decreased intracortical remodeling and less porous cortical bone in girls [44,45].

Previous study of the second metacarpal bone in girls and boys showed that girls had narrower metacarpal bones and decreased marrow cavity size compared to boys. These findings were attributed to the differential effects of sex steroids on the skeleton [46]. Subsequent studies have largely confirmed these results, although some studies have shown similar results in pre-pubertal children, suggesting that skeletal sexual dimorphism may not be due only to differences in sex steroid levels. DXA studies have shown that cortical width increases in girls at puberty [28]. QCT studies have shown smaller cross-sectional area in vertebrae in girls than boys matched for height and weight, but vertebral height and vBMD were similar, and no differences were seen at the femoral mid-shaft [47]. Tibial pQCT studies have shown pubertal girls to have decreased periosteal expansion, bone diameter, total bone area, and cortical area compared to boys, with decreased calculated bone strength as a result [45,48]. Muscle cross-sectional area correlated with tibial bone strength [49]. No evidence was seen for increased tibial endosteal apposition, even in late puberty [48]. Radial pQCT studies have shown decreased endocortical diameter in girls in mid- to late puberty, suggesting increased endocortical apposition [50]. Increased estrogen levels are correlated with smaller medullary cavities, greater cortical thickness, and increased total vBMD in pubertal girls. A femoral shaft MRI study in healthy girls and young women aged 6–25 years showed that total bone area, cortical bone area, and medullary area all increased between prepuberty and adulthood [51]. Some of the differences seen may reflect different biomechanical forces at weight-bearing skeletal sites such as the distal tibia, compared to non-weight-bearing sites such as the radius.

In order to better define changes in bone structure during puberty, Kirmani and colleagues [52] recently studied healthy 6 to 21 year-old girls (n = 66) and boys (n = 61) using high-resolution peripheral quantitative computed tomography (voxel size, 82 micrometers) at the distal radius. Subjects were classified into 5 groups by bone-age: Group I (pre-puberty, 6–8 yrs), Group II (early puberty, 9–11 yrs), Group III (mid-puberty, 12–14 yrs), Group IV (late puberty, 15–17 yrs) and Group V (post-puberty, 18–21 yrs). Compared to Group I, trabecular parameters (bone volume fraction [BV/TV], trabecular number [Tb.N] and thickness [Tb.Th]) did not change in girls, but increased in boys from late puberty onwards. Cortical thickness and density decreased from pre- to mid-puberty in girls, but were unchanged in boys, before rising to higher levels at the end of puberty in both sexes. Total bone strength, assessed using micro-finite element models, increased linearly across bone age groups in both sexes, with boys showing greater bone strength than girls after mid-puberty. The proportion of load borne by cortical bone, and the ratio of cortical to trabecular bone volume, decreased transiently during mid- to late-puberty in both sexes, with apparent cortical porosity peaking during this time. This mirrors the incidence of distal forearm fractures in prior studies. The authors concluded that regional deficits in cortical bone may underlie the adolescent peak in forearm fractures. Whether these deficits are more severe in children who sustain forearm fractures or persist into later life warrants further investigation. None of the biochemical or hormonal variables assessed in this study were significantly associated with BV/TV, Tb.N, Tb.Th, or trabecular separation (Tb.Sp) in the girls; in boys, greater BV/TV and Tb.Th were related to higher serum testosterone levels, and Tb.N and Tb.Sp were most closely associated with higher IGF-I. Cortical thickness and cortical vBMD were inversely associated with bone turnover (serum procollagen type I N-terminal propeptide [PINP] and C-terminal cross-linked telopeptides [CTX]) in girls and with PTH in boys. Periosteal and endosteal circumference were most closely associated with IGF-I levels in the girls and testosterone levels in the boys. Overall bone strength was associated with serum E_2 in girls and testosterone in boys, whereas the percent load carried by cortical bone was negatively associated with CTX and IGF-I levels in girls, and with IGF-I and PTH in boys. The cortical porosity index, on the other hand, was positively associated with CTX and IGF-I in girls, and with IGF-I in boys.

The female reproductive system affects the skeleton significantly at puberty, but these effects occur in the context of genetic inheritance, diet, and physical activity. Candidate genes linked

to the skeleton affect bone size, efficiency of calcium absorption, response to biomechanical forces, and future risk of osteoporosis and fractures [53]. Short stature and delayed puberty affect assessment of the skeleton by DXA. Skeletal loading by weight-bearing exercise effectively stimulates bone mineral acquisition during childhood and adolescence [54], and increases bone diameter and total and cortical bone area in elite racket sports players [55]. Dietary calcium intake correlates well with DXA BMD in many studies [56]. Low calcium intake in childhood and adolescence, whether due to lactose intolerance, dietary preferences, cultural patterns, or other factors, is associated with increased risk of osteoporotic fractures in older women [57]. Avoidance of milk intake is associated with childhood fractures [58]. Calcium intake in children and adolescents in one study appeared to increase DXA BMD only by 1.7% at the forearm, and to not affect BMD at the spine or hip [59]. The beneficial effects of calcium appear limited to improvement in bone mass at cortical sites by reducing bone remodeling.

Physical activity may affect the skeleton differently at different pubertal stages. Skeletal sensitivity to mechanical loading is increased in the presence of estrogen, indicating that early and mid-puberty may be optimal times for exercise-induced skeletal benefit [54]. Pubertal girls have greater ratios of vBMD and cross-sectional area of cortical bone to muscle cross-sectional area, suggesting that skeletal calcium acquisition is increased in response to physical activity [41–43]. Some studies have shown that the combination of increased calcium intake and physical activity have greater effects than either alone at certain skeletal sites.

Fractures are associated with bone size and mass in children, as they are in adults. Forearm fractures occur most commonly in peripubertal girls at age 8–12 years [60], and are increasingly common [61]. Pubertal girls with fractures commonly have lower BMC, aBMD, and estimated vBMD compared to controls [62]. Lower total body BMD and bone area for height are the best predictors of fracture in pubertal girls [63].

1.5 Pregnancy and Lactation

The female reproductive system significantly strengthens the skeleton in women by stimulating acquisition of bone mass and biomechanical strength at puberty, but also presents significant challenges to the skeleton during pregnancy and lactation. The fetus and placenta take up significant calcium from the maternal circulation during pregnancy to mineralize the fetal skeleton, and the nursing infant requires significant calcium from breast milk to promote continued skeletal growth [64]. The physiological adjustments made by pregnant or lactating women to accommodate the baby's need for calcium are quite different [65].

1.5.1 Pregnancy

The developing fetal skeleton normally acquires about 30 grams of calcium by birth, with about 80% of this calcium acquired during the last 3 months of pregnancy, when the fetal skeleton is rapidly mineralizing. This demand appears to be largely met by doubling maternal intestinal calcium absorption beginning as early as 12 weeks of gestation, largely mediated by increased maternal renal production of serum 1,25-dihydroxyvitamin D, although the placenta, decidua, and fetal kidney also contribute small amounts [64]. Renal 1α-hydroxylase activity is upregulated during pregnancy by PTHrP, estrogen, prolactin, and placental lactogen, rather than PTH, because PTH levels are usually low- to mid-normal during pregnancy, at least in North American and European women with sufficient calcium intake. PTHrP is produced by many tissues in the fetus and mother during pregnancy, but it is not clear that there is a dominant source. PTHrP increases 1,25-dihydroxyvitamin D and suppresses PTH during pregnancy, and may help regulate placental calcium transport [66] and protect the maternal skeleton during pregnancy. PTHrP C-terminal fragments have been shown to inhibit osteoclast-induced bone resorption [67].

One study showed that markers of bone resorption increased gradually during normal pregnancy through the 28th week, whereas markers of bone formation remained stable through the 28th week, and then increased markedly by the 36th week [68]. These findings suggest that bone remodeling is uncoupled during the first two trimesters of pregnancy, with a marked increase in bone resorption, and that bone formation does not increase until the third trimester.

Assessment of changes in maternal BMD during pregnancy is confounded by changes in body composition, weight, and skeletal volume. DXA BMD studies during pregnancy have been limited by radiation exposure to the fetus, but several studies have been done with measurements taken shortly before and after pregnancy [65]. One study showed no changes in lumbar spine BMD before conception and 1–2 weeks after delivery, whereas other studies have shown that lumbar spine BMD decreased by 4–5% when the second measurement was taken 1–6 weeks after delivery. The studies that have shown bone loss may be confounded by the fact that lactation causes lumbar spine bone loss of 1–3% per month beginning at birth. Longitudinal calcaneal ultrasound studies have shown slow progressive bone loss during pregnancy. None of the available studies have addressed whether skeletal BMD increases during the first trimester in preparation for losses during the third trimester.

Rare women have been reported to sustain osteoporotic fractures during or shortly after pregnancy, and cases of transient osteoporosis of the hip have been reported during pregnancy. Osteoporosis and fractures during pregnancy likely reflect low bone mineral density or other skeletal abnormalities before pregnancy, usually unrecognized, with the superimposed effects of the changes of pregnancy on the skeleton. Uncoupling of bone turnover during the first two trimesters, with increased bone resorption and stable bone formation, may increase the risk of fracture during pregnancy. In spite of the adverse skeletal consequences of pregnancy, most epidemiological studies of osteoporotic or osteopenic women have not shown an association between parity and BMD or fracture risk [69].

1.5.2 Lactation

Nursing mothers typically lose 280–400 mg of calcium each day through their breast milk, although losses as high as 1,000 mg have been reported. Most of this calcium appears to come from temporary skeletal calcium losses, possibly mediated by PTHrP, with decreased estrogen levels during lactation resulting from prolactin-mediated suppression of GnRH, LH, and FSH, but not by changes in PTH or 1,25-dihydroxyvitamin D. Studies using DXA BMD assessment show losses of 1–3% per month in lactating women, with cumulative losses of 3–10% over 2–6 months at trabecular sites such as the lumbar spine, hip, and distal radius [64,69]. These very rapid losses compare to losses of 1–3% per year in early postmenopausal osteoporotic women who are losing bone density rapidly. Calcium supplementation during lactation does not prevent bone loss during lactation [70].

Weaning results in rapid regain of BMD at 0.5–2.0% per month, with most women regaining BMD to baseline several months after completion of lactation [64,69,71]. The mechanisms behind this rapid regain in BMD are not yet clear. Most epidemiological studies of premenopausal and postmenopausal women have not shown an adverse effect of lactation on peak bone mass, BMD, or hip fracture risk.

1.6 Postmenopausal Bone Loss and Fractures in Women

Significant bone loss occurs with normal aging in women [72]. Development of osteoporosis, usually in old age, is the natural consequence of age-related bone less if left untreated. Multiple population-based cross-sectional and longitudinal studies over the last 25 years using aBMD assessed by DXA have helped define the general pattern of bone loss with normal aging (Figure 4). Women lose aBMD at relatively slow rates starting at around age 40, with women losing

aBMD more rapidly with onset of menopause in their late 40s or early 50s. Postmenopausal women lose trabecular BMD rapidly in their vertebrae, pelvis, and ultradistal wrist. There is less rapid cortical bone loss in the long bones and vertebrae after the menopause. About 8–10 years after menopause, slower age-related bone loss becomes prominent, and continues for the rest of life.

However, because DXA BMD is not able to differentiate changes occurring in trabecular and cortical bone with age, and because DXA BMD cannot assess age-related changes in bone geometry and/or size, more recent studies have utilized QCT scanning [73] to assess bone loss in greater detail. Both peripheral and central QCT, with new image analysis software [74], have been used to better define the age-related changes in bone volumetric density, geometry, and structure at multiple skeletal sites.

Riggs et al. [73] reported large decreases in lumbar spine vBMD with normal aging in a cross-sectional study of women aged 20 to 97 years in Rochester, Minnesota, predominantly due to vertebral trabecular bone loss beginning in the third decade. Lumbar spine vBMD decreased by 55% in women. The rate of bone loss appeared to increase in middle age in women, accounting for the greater decrease in vBMD seen with aging in women (Figure 5). Assessment of changes in radial cortical vBMD at the wrist showed that cortical bone loss did not begin until middle age in women. After middle age, there was a 28% linear decrease in cortical vBMD in women. Normal aging was associated with increases in cross-sectional area at the femoral neck and radius because of continued periosteal apposition with normal aging. The bone marrow space increased more rapidly than cross-sectional area due to continued endosteal bone resorption. Because the rate of periosteal apposition was slower than the rate of endosteal resorption, cortical area and thickness decreased with aging. However, because periosteal apposition increased bone diameter, the ability of bone to resist biomechanical forces increased, partially offsetting the decrease in bone strength resulting from decreased cortical area.

Khosla et al. [75] subsequently showed that the structural basis for bone loss in the ultradistal radius with aging in women is thinner trabeculae in young adulthood, and primarily decreased trabecular number and increased trabecular spacing as they get older. These changes result in greater microstructural damage with aging in women, which likely explains the increased risk of wrist fractures seen in women. Khosla et al. [76] then demonstrated that decreased sex steroids were the major hormonal determinants of trabecular microstructure in elderly women. In a subsequent study, Riggs et al. [77] showed that the late onset of cortical bone loss is temporally associated with sex steroid deficiency. However, the early-onset, substantial trabecular bone loss in women during sex steroid sufficiency is unexplained, and indicates that current paradigms on the pathogenesis of osteoporosis are incomplete.

These studies showed that these age-related changes in bone density and structure correlated with the observed increased fracture risk seen in women. Previous studies had shown that distal forearm (Colles') fractures increased rapidly in women after menopause, and then remain constant from about 10 to 15 years after menopause until the end of life (Figure 6). In contrast, vertebral fractures increase more slowly after menopause, but continue to increase exponentially during later life. Hip fractures in women increase more slowly than vertebral fractures after menopause, but continue to increase throughout life, and increase rapidly in later life.

Based on these and other studies, it is estimated that 40% of Caucasian women aged 50 years or older will develop a vertebral, hip, or wrist fracture sometime during the remainder of their lives, and that this risk increases to about 50% if non-clinical vertebral fractures detected by radiological imaging are included in the estimate [78]. The risk of these fractures is somewhat

lower in non-Caucasian women. It is estimated that osteoporotic fractures cost the U.S. between $12.2 to $17.9 billion each year, as measured in 2002 dollars [79].

1.7 Pathophysiology of Age-Related Bone Loss in Women

1.7.1 Accelerated Postmenopausal Bone Loss Due to Gonadal Sex Steroid Deficiency

Menopause is associated with the onset of rapid bone loss in women, due largely to decreased ovarian function, leading to decreased estrogen secretion. This rapid bone loss can be prevented by estrogen or hormone replacement [80,81]. During the menopause transition, serum 17β-estradiol levels decrease by 85–90% from the mean premenopausal level, and serum estrone levels decrease by 65 to 75% from premenopausal levels [82]. Serum estrone has one-fourth the biological effect of serum 17β-estradiol. Serum testosterone also decreases following menopause, but to a lesser extent, because testosterone continues to be produced by the adrenal cortex and interstitial cells in the ovary [83]. Some longitudinal clinical studies show that increased bone turnover in perimenopausal women correlates with elevated serum FSH, as well as serum estradiol [84]. The perimenopausal rise in FSH is due to a selective decrease in ovarian inhibin B. Decreases in inhibin levels across the menopause transition are associated with increasing bone turnover, independent of changes in sex steroids or FSH [85].

Bone resorption increases by 90% after menopause, as assessed by markers of bone resorption, whereas bone formation also increases, but only by 45% as assessed by markers of bone formation [86]. The difference between bone resorption and formation favors greater bone resorption, which leads to accelerated bone loss during the first 8–10 years after menopause. Increased bone resorption leads to an efflux of calcium from the skeleton into the extracellular pool, but compensatory increased renal calcium excretion [87], decreased intestinal calcium absorption [88], and partially suppressed parathyroid hormone secretion [89] prevent development of hypercalcemia.

The cellular and molecular mechanisms by which estrogen deficiency leads to bone loss are increasingly well understood (Figure 7 and Table 1). Estrogen deficiency increases receptor activator of nuclear factor kappa B ligand (RANKL) [90], leading to increased osteoclast recruitment and activation and decreased osteoclast apoptosis. RANKL is the final key molecule required for osteoclast development, and is normally expressed by bone marrow stromal/osteoblast precursor cells, T-lymphocytes, and B-lymphocytes [91,92]. RANKL binds to its receptor RANK on osteoclast lineage cells [93], and is neutralized in the bone microenvironment by its soluble decoy receptor osteoprotegerin (OPG), which is produced and secreted by osteoblast lineage cells [94]. Combined in vitro and in vivo studies have shown that estrogen normally suppresses RANKL production by osteoblastic cells and T- and B-lymphocytes [91,92], and increases OPG production by osteoblastic cells [95,96], so that estrogen deficiency leads to an increase in the RANKL/OPG ratio that favors bone resorption.

Estrogen also modulates production of additional cytokines by bone marrow stromal mononuclear cells and osteoblasts, thereby controlling osteoclast activity by paracrine action [96]. Estrogen is thought to suppress production of bone-resorbing cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, macrophage colony-stimulating factor (M-CSF), and prostaglandins by the appropriate cells [97]. Estrogen-deficient model systems have shown that increased IL-1 and M-CSF levels [98,99] can be reduced by specific antagonists to these molecules [100–103]. The bone-resorptive activity of TNF-α is able to be inhibited by soluble type I TNF receptor [104]. Estrogen deficiency is associated with increased IL-6 levels [105,106]. In vivo, however, it is likely that estrogen suppresses production and activity of multiple cytokines in premenopausal women that would otherwise act cooperatively to cause bone loss. With estrogen deficiency, each cytokine likely accounts for only part of cytokine-mediated age-related bone loss. It is not yet clear that there is a dominant cytokine

contributing to estrogen-deficiency associated bone loss. Estrogen also normally increases the production of transforming growth factor (TGF)-β by osteoblast precursor cells [107]. TGF-β induces apoptosis of osteoclasts [108].

Estrogen also directly stimulates apoptosis of osteoclast precursor cells, and decreases osteoclast precursor differentiation by blocking RANKL/M-CSF-induced activator protein (AP)-1-dependent transcription by reducing c-jun activity [109,110]. C-jun activity is reduced by decreasing c-jun transcription and decreasing phosphorylation. Estrogen is also capable of inhibiting the activity of mature osteoclasts by direct, receptor-mediated mechanisms [111].

Loss of these multiple estrogen-induced restraining actions on osteoclast bone resorption leads to rapid upregulation of bone loss shortly after onset of menopause. It is likely that the major direct effect of estrogen deficiency is mediated by alteration in the ratio of RANKL to OPG in the bone microenvironment, thereby leading to increased osteoclast activity and bone resorption. However, the multiple other changes induced by estrogen deficiency also likely play a significant role in causation of early postmenopausal bone loss. The rapid phase of bone loss is sustained for 8–10 years before gradually subsiding. Why rapid bone loss gradually subsides after this duration is not yet clear, but it may be that estrogen deficiency alters biomechanical sensing of skeletal mechanical loading by osteocytes within the bone [112]. It is hypothesized that for a given level of skeletal mechanical loading during estrogen deficiency, bone mass is sensed as being excessive by the osteocytes, which then signal osteoclasts to resorb more bone and/or the osteoblasts to form less bone, leading to net rapid bone loss. Once enough bone is lost, however, it is thought that the proportionately increased skeletal mechanical loading on remaining bone is sufficient to limit further rapid bone loss.

Some evidence suggests that increased serum FSH may cause bone loss independent of decreased serum estradiol levels [113]. While intriguing, this evidence remains controversial, and is not supported by all experimental evidence.

1.7.2 Age-Related Bone Loss Due to Secondary Hyperparathyroidism

While gonadal steroid deficiency may be a major cause for postmenopausal bone loss, other important factors also play a role. During the rapid phase of early postmenopausal bone loss, there is mild suppression of PTH secretion, but during the slower phase of later postmenopausal bone loss there is gradually increasing PTH secretion, with corresponding increasing markers of bone resorption. The increases in serum PTH and markers of bone turnover correlate with each other. Transient suppression of PTH secretion by an intravenous 24-hour calcium infusion in younger premenopausal and elderly postmenopausal women is associated with suppression of markers of bone turnover, strongly suggesting that increased serum PTH was the proximate cause of the increased bone turnover [114].

The reason for gradually increasing levels of PTH secretion with age is likely multifactorial. Vitamin D deficiency is common in postmenopausal women [115], and is associated with increased serum PTH levels. Longstanding estrogen deficiency also leads to chronic negative calcium balance because loss of estrogen causes decreased intestinal calcium absorption [88, 116] and renal tubular calcium reabsorption [117,118]. Unless these changes leading to negative calcium balance are compensated for with adequate calcium supplementation, secondary hyperparathyroidism inevitably develops, leading to age-related bone loss.

1.7.3 Bone Loss Due to Decreased Bone Formation

Postmenopausal and age-related bone loss is due not just to accelerated bone resorption, but also to decreased bone formation. Decreased bone formation results, in part, from changes in the differentiation potential of mesenchymal stem cells with aging. Bone mesenchymal stem

cells give rise to both osteoblasts and adipocytes, but aging induces changes that favor adipocyte differentiation over osteoblast formation, which leads to decreased potential to form new bone. This change in differentiation potential is poorly understood, but known to be influenced by the type 1 cannabinoid receptor (CB1) [119] and Wnt signaling pathway. Lineage commitment by mesenchymal stem cells depends on activation of the cyclic AMP/CREB pathway, which downregulates expression of the osteoblast-specific transcription factor Cbfa-1 [120], and upregulates the expression of adipocyte-specific transcription factors CEBPβ and PPARγ [121]. Thiazolidinedione drugs used to treat type 2 diabetes mellitus also favor differentiation of mesenchymal stem cells toward the adipocyte lineage by this mechanism.

Decreased bone formation has generally been attributed to decreased paracrine production of growth factors [122], and/or decreased GH [123,124] and IGF-1 levels [125–127]. Estrogen has been shown to directly stimulate bone formation via an estrogen receptor-dependent mechanism in mice [128], so it is likely that estrogen deficiency may also directly result in bone loss. Impaired bone formation is detectable in early menopause [129]. Estrogen increases production of IGF-1 [130], TGF-β [107], and procollagen synthesis by osteoblast precursor cells in vitro [130], and increases osteoblast life span by decreasing osteoblast apoptosis [131,132]. Khastgir and colleagues provided direct evidence that estrogen can stimulate bone formation after cessation of skeletal growth by evaluating iliac crest bone biopsies from 22 elderly women of mean age 65 years before, and 6 years after, percutaneous administration of high doses of estrogen [133]. They found that cancellous bone volume increased by 61%, and trabecular wall thickness by 12%. Tobias and Compston reported similar results [134]. It is not yet clear whether these results are due to the pharmacological doses of estrogen used, or augmentation of physiological effects that are normally not of sufficient magnitude to detect. Accumulating data implicate estrogen deficiency as a contributing cause of decreased bone formation with aging, but there is not yet a clear consensus on whether estrogen directly stimulates osteoblast function, and if it does, what the relative magnitude of increased proliferation or decreased apoptosis are.

1.7.4 Other Factors Contributing to Age-Related Bone Loss in Women

Age-related bone loss may be driven primarily by gonadal sex steroid deficiency and physiological secondary hyperparathyroidism, but multiple other factors also contribute to this process. Vitamin D deficiency, widely prevalent in postmenopausal women with osteoporosis, and common among children and adults without osteoporosis in many countries around the world, worsens age-related physiological secondary hyperparathyroidism [115]. Age-related decreased bone formation may be due in part to sex steroid deficiency, but other sex steroid-independent factors likely also contribute to decreased bone formation.

After menopause, ovarian production of estrogen is markedly diminished, and peripheral aromatization of androgens to estrogen remains important as the major source of serum estradiol. The presence of low but detectable levels of serum estradiol after menopause prevents more rapid and severe bone loss that would otherwise occur. The importance of low but detectable levels of estradiol after menopause is demonstrated by the rapid bone loss that occurs with use of aromatase inhibitors such as exemestane and letrozole in treatment of postmenopausal women with estrogen receptor-positive breast cancer. These potent inhibitors of the aromatase enzyme cause marked reduction in serum estradiol levels, which leads to rapid bone loss unless treatment with antiresorptive agents is initiated prophylactically [135].

Other changes in endocrine function with aging likely also contribute to the physiology of bone loss. The less potent adrenal androgens DHEA and DHEA-sulfate both decrease by about 80% with aging, with much of the decrease occurring in young adults [136], whereas cortisol secretion remains relatively constant throughout life. The decrease in adrenal androgens early

in adult life may therefore change the ratio of adrenal catabolic/anabolic hormone activity. This change may contribute to younger adult trabecular bone loss.

Lower peak bone density at 25–35 years of age also contributes to risk of osteoporosis and fractures later in life. Individuals with lower peak bone density for whatever cause will develop low bone density or osteoporosis sooner than those with higher peak bone density, assuming the rate of bone loss is equivalent as they age [137].

It has been suggested that bone strength is homeostatically adapted to habitual skeletal loading conditions and that bone loss could, therefore, result simply from age-related reductions in physical activity and muscle mass. In a stratified random sample of Rochester, Minnesota, women and men 21–97 years of age, Melton et al. [138] estimated indices of bone strength, flexural rigidity, and axial rigidity from central QCT measurements at the femoral neck and lumbar spine, and pQCT measurements at the ultradistal radius. Habitual skeletal loading was assessed using lean body mass, total skeletal muscle mass, body weight, and physical activity. This study showed that there was not a close correspondence between changes in habitual load and changes in bone strength, nor any consistent pattern. Moreover, inter-individual variation in the strength-to-load ratios was substantial. These data suggest that the hypothesis that reduced skeletal loading is the primary basis for age-related bone loss may be oversimplified.

Numerous sporadic secondary causes of bone loss play a role in age-related bone loss by various mechanisms. It is estimated that about 40% of women have an identifiable sporadic cause of bone loss [136], such as glucocorticoid therapy, subtle or clinically evident malabsorption, anorexia nervosa, idiopathic hypercalciuria, or behavioral factors such as excess alcohol intake, cigarette smoking, high-caffeine or high-sodium diet, or physical inactivity.

Age-related bone loss and sarcopenia proceed in parallel. It has been suggested that age-related muscle loss is the main factor causing age-related bone loss [137,138]. Although not yet proven, it is likely that age-related muscle loss, causing decreased muscle loading on the skeleton, contributes significantly to age-related bone loss in women.

1.8 Summary

The female reproductive system plays a major role in regulating the acquisition and loss of bone by the skeleton from menarche through senescence. Onset of gonadal sex steroid secretion at puberty is the major factor responsible for significant skeletal longitudinal and radial growth, as well as significant gain in bone density, from the early teenage years through the early 20s. Multiple factors affect acquisition of peak bone density in addition to the female reproductive system. Gonadal sex steroids then help maintain peak bone density until menopause, including during the transient loss and regain of skeletal mineral associated with pregnancy and lactation. At menopause, decreased gonadal sex steroid production normally leads to rapid bone loss. The most rapid bone loss associated with decreased estrogen levels occurs in the first 8–10 years after menopause, with slower age-related bone loss occurring during later life. Age-related bone loss in women after the early menopausal phase of bone loss is caused by ongoing gonadal sex steroid deficiency, vitamin D deficiency, and secondary hyperparathyroidism. Other factors also play key roles in age-related bone loss, including vitamin D deficiency, intrinsic defects in osteoblast function, impairment of the GH/IGF axis, reduced peak bone mass, age-associated sarcopenia, and various sporadic secondary causes. Further understanding of the relative contributions of each of these factors to development and maintenance of the female skeleton, bone loss, and fracture risk will lead to improved preventive and therapeutic approaches for osteoporosis.

Acknowledgments

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Final Menstrual Period
(FMP)

| Stages:	-5	-4	-3	-2	-1	0	+1	+2
Terminology:	Reproductive			Menopausal Transition			Postmenopause	
	Early	Peak	Late	Early	Late*		Early*	Late
				Perimenopause				
Duration of Stage:	variable			variable		(a) 1 yr	(b) 4 yrs	until demise
Menstrual Cycles:	variable to regular	regular		variable cycle length (*>7 days different from normal*)	≥2 skipped cycles and an interval of amenorrhea (*≥60 days*)	Amen x 12 mos	none	
Endocrine:	normal FSH		↑ FSH	↑ FSH			↑ FSH	

Figure 1.
The STRAW staging system, showing the relation between alterations in cycle regularity and
endocrine changes across the various stages of reproductive aging. (*Reproduced from* Soules
MR, Sherman S, Parrott E, et al. Executive summary: Stages of Reproductive Aging Workshop
(STRAW). Fertil Steril 2001;76:874–878; with permission.)
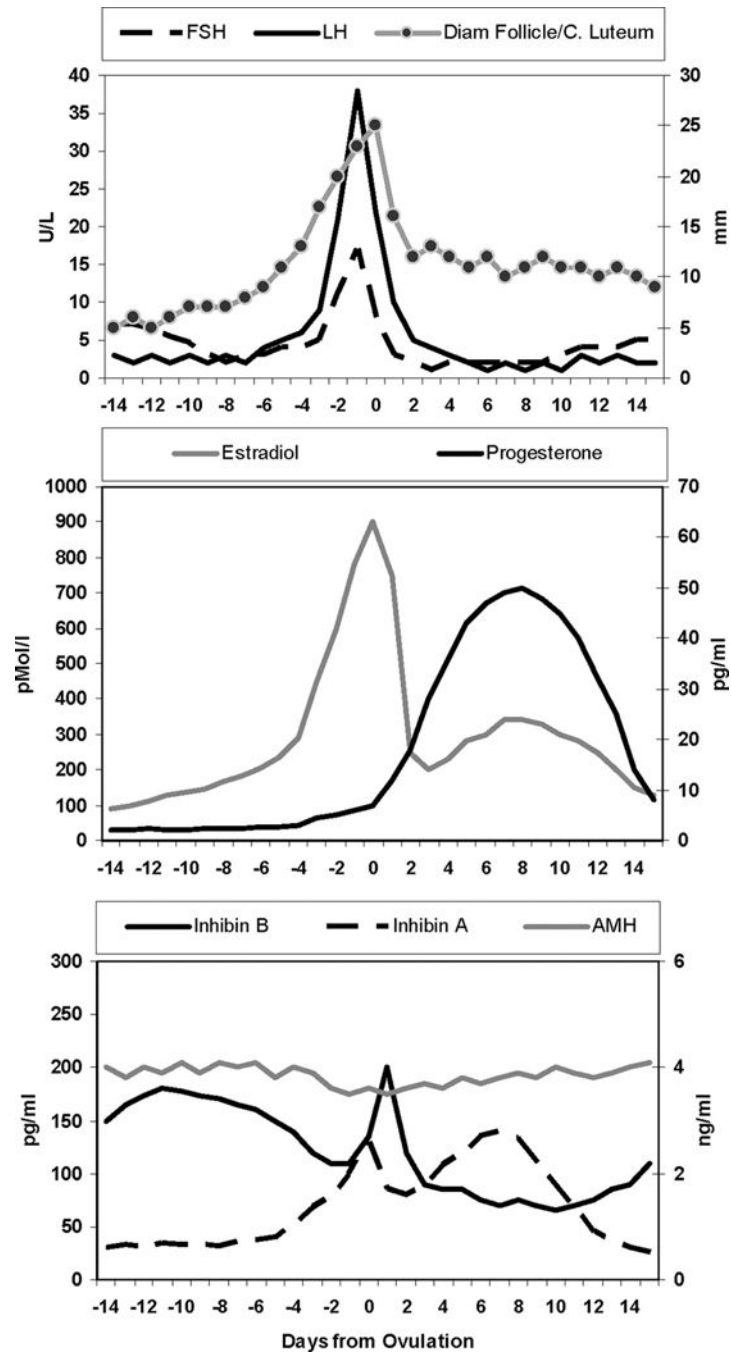
Figure 2.
Endocrine fluctuations and follicle growth in the menstrual cycle. (*Reproduced from*
Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences.
Endocrine Rev 2009;30:472; with permission.)

Hypothalamus

GnRH +

Estrogen -

Pituitary

Progesterone -

Inhibin -

LH and FSH +
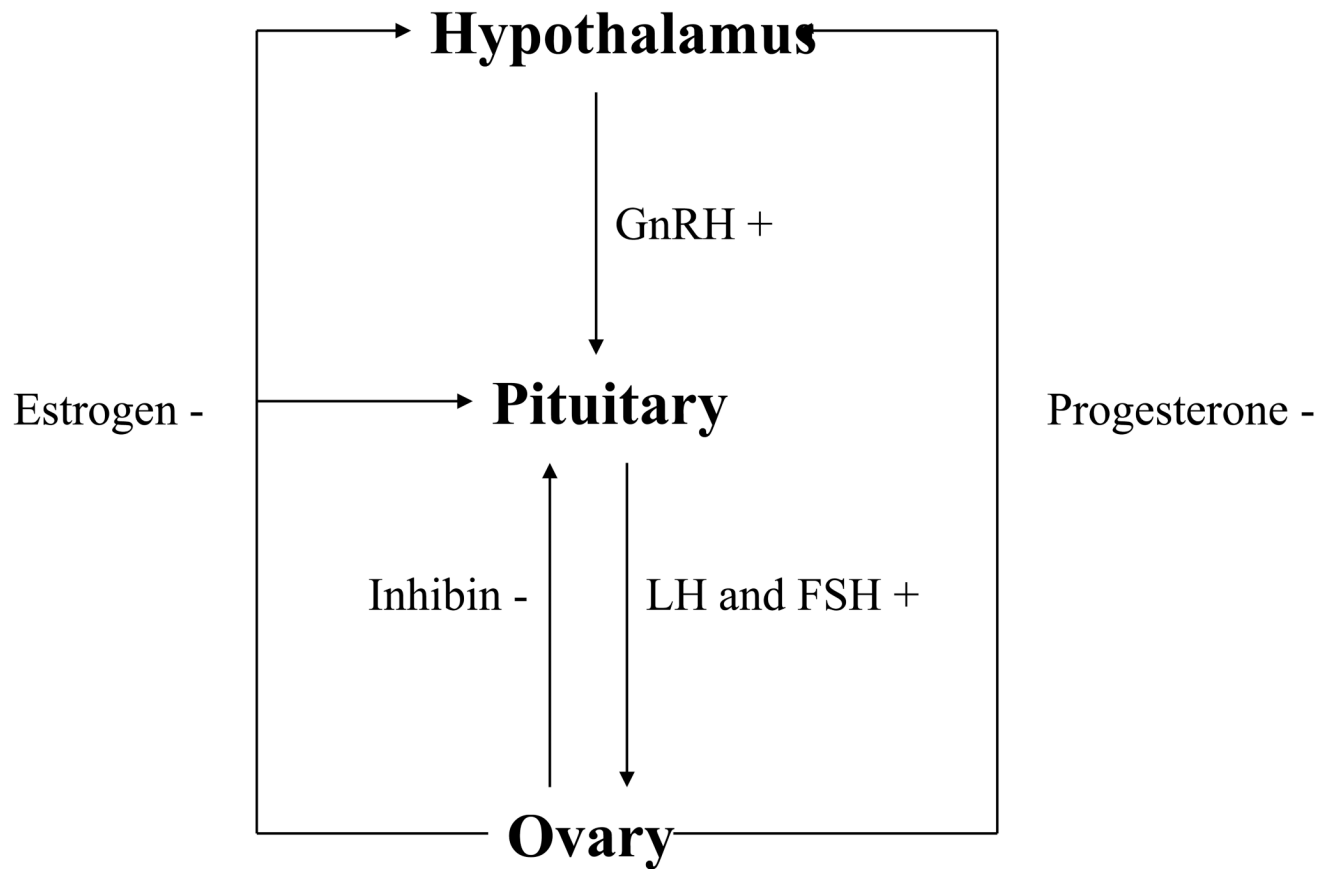
Ovary

Figure 3.
Diagrammatic representation of how the various hormones interact at the ovary, pituitary, and hypothalamus, with feedback loops.
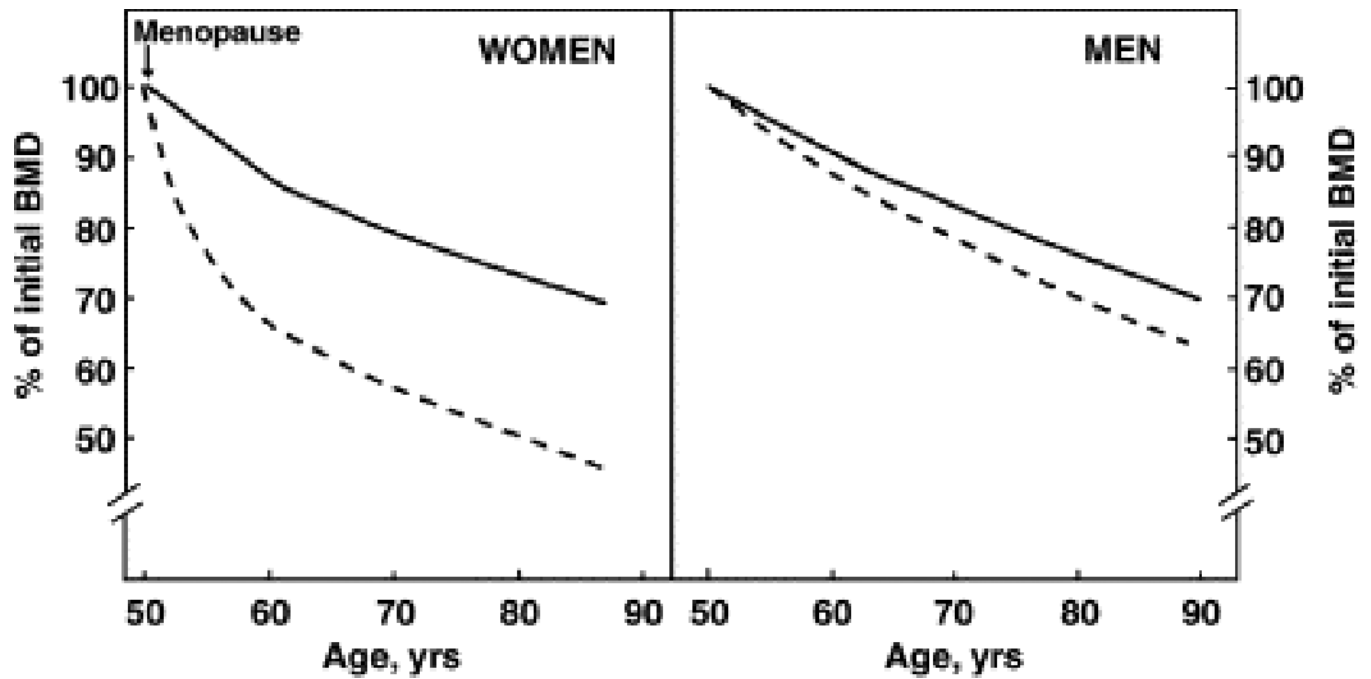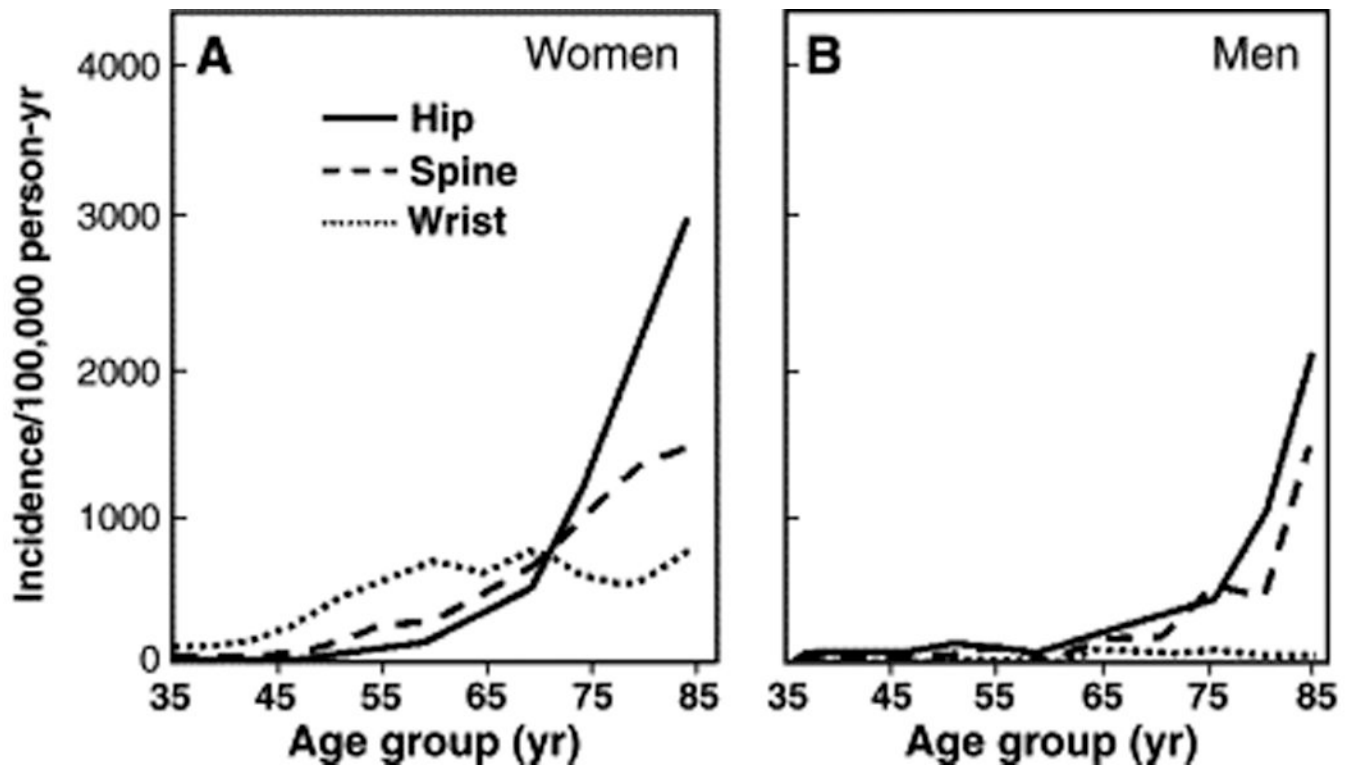
Figure 4.
Patterns of age-related bone loss in women and men. Dashed lines represent trabecular bone
and solid lines, cortical bone. The figure is based on multiple cross-sectional and longitudinal
studies using DXA. (*Reproduced from* Khosla S, Riggs BL. Pathophysiology of age-related
bone loss and osteoporosis. Endocrinol Metab Clin N Am 2005;34:1017; with permission.)
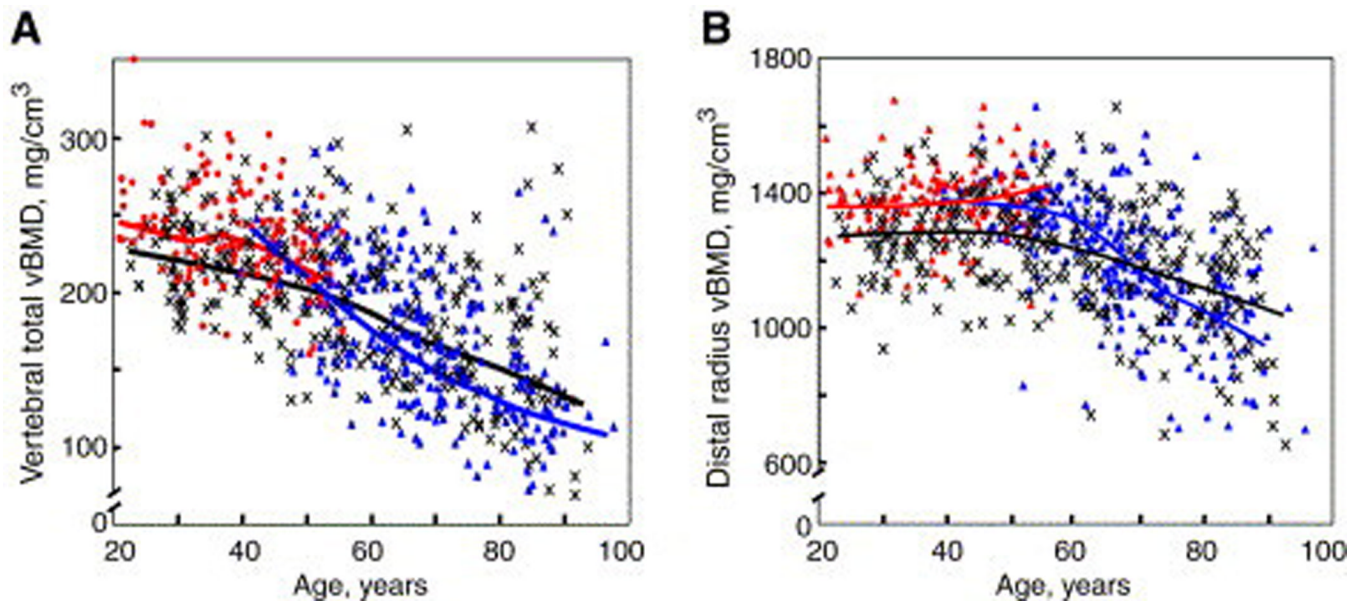
Figure 5.
(A) Values for vBMD (mg/cm^3) of the total vertebral body in a population sample of Rochester, Minnesota, women and men between the ages of 20 and 97 years. Individual values and smoother lines are given for premenopausal women in red, for postmenopausal women in blue, and for men in black. (B) Values for cortical vBMD at the distal radius in the same cohort, with color code as in (A). All changes with age were significant ($P <.05$). (*Reproduced from* Riggs BL, Melton LJ 3rd, Robb RA, et al. A population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 2004;19:1950; with permission.)

Figure 6.
Age-specific incidence rates for proximal femur (hip), vertebral (spine), and distal forearm
(wrist) fractures in Rochester, Minnesota, women (A) and men (B). (*Adapted from* Cooper C,
Melton LJ. Epidemiology of osteoporosis. Trends Endocrinol Metab 1992;3:225; with
permission.
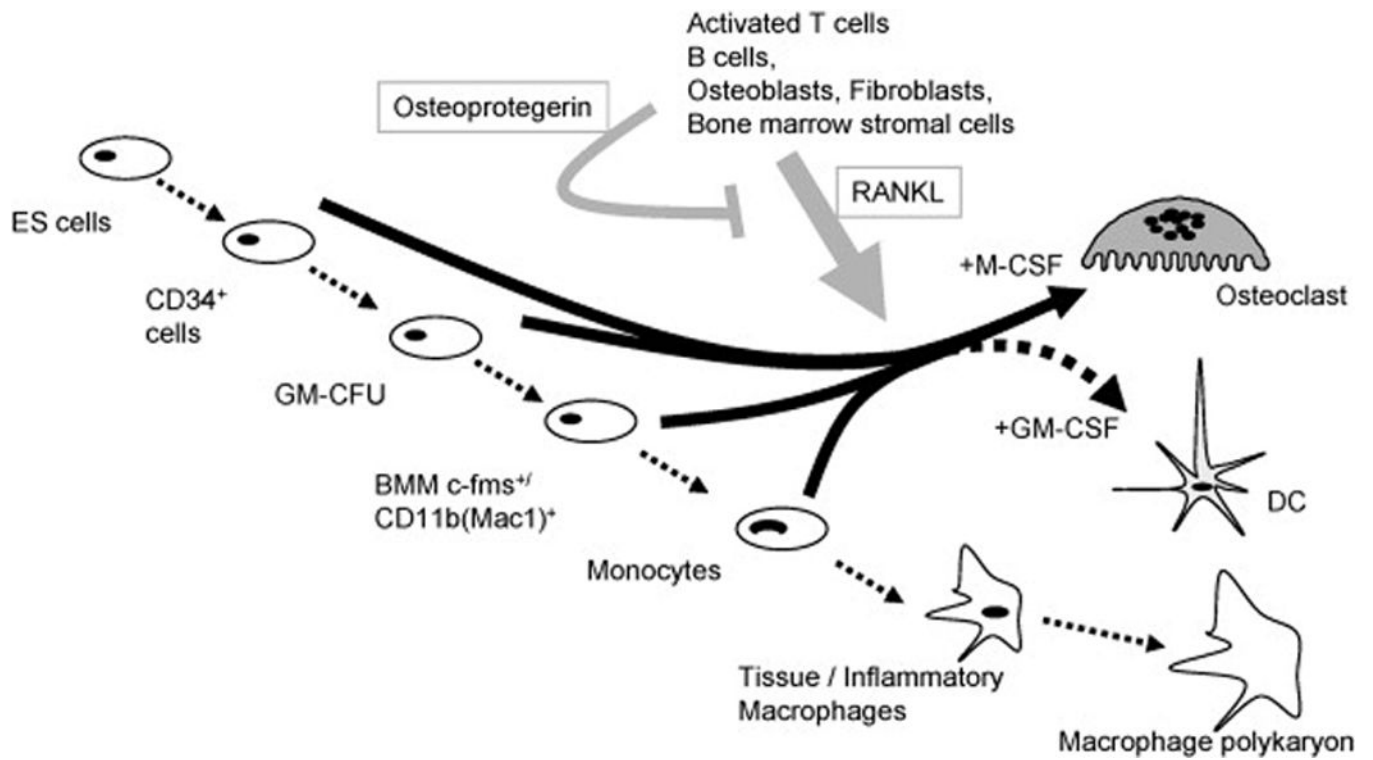
Figure 7.
Summary of stimulatory and inhibitory factors involved in osteoclast development and apoptosis. (*Reproduced from* Quinn JMW, Saleh H. Modulation of osteoclast function in bone by the immune system. Mol Cell Endocrinol 2009;310:42; with permission.)

Table 1

Potential Mechanisms by Which Estradiol Deficiency Causes Bone Loss in Postmenopausal Women

Physiological Changes Caused by Estrogen Deficiency	Potential Mechanism(s) Causing Bone Loss
Increased Receptor Activator of Nuclear Factor Kappa B (RANK) Ligand Expression by Osteoblasts and T- and B-Lymphocytes	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Decreased Osteoprotegerin Production by Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Decreased Osteoclast Precursor Apoptosis and Increased Osteoclast Differentiation	Increased Osteoclast Recruitment and Activation
Decreased Inhibition of Osteoclast Activity Mediated by Estrogen Receptors	Increased Osteoclast Activity
Increased Interleukin-1 (IL-1) by Bone Marrow Stromal Cells and Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Increased Interleukin-6 (IL-6) by Bone Marrow Stromal Cells and Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Increased Tumor Necrosis Factor-alpha (TNF-α) by Bone Marrow Stromal Cells and Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Increased Macrophage-Colony Stimulating Factor (M-CSF) by Bone Marrow Stromal Cells and Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Increased Prostaglandins by Bone Marrow Stromal Cells and Osteoblasts	Increased Osteoclast Recruitment and Activation, Decreased Osteoclast Apoptosis
Decreased Transforming Growth Factor-β (TGF-β) by Osteoblast Precursors	Decreased Osteoclast Apoptosis
Decreased Intestinal Calcium Absorption	Decreased Calcium for Bone Formation
Decreased Renal Tubular Calcium Reabsorption	Decreased Calcium for Bone Formation