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Skeletal adaptations during mammalian reproduction

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Abstract

Remarkable changes occur in the mammalian skeleton prior to, during and after the reproductive cycle. Skeletal changes occur with ovarian maturation and initiation of menses and estrus in adolescence, which may result in a greater accumulation of skeletal mineral in the female vs the male skeleton. There is also some evidence to suggest an excess skeletal mass in young female experimental animals. In early pregnancy, growth, modeling and perhaps suppressed remodeling promote the accumulation of calcium. Some changes may also occur with the transition from pituitary to placental control of the pregnancy. In later pregnancy, an increase in bone turnover appears to coincide with fetal skeletal mineralization. Rapid and important changes occur in the skeleton and mineral metabolism in the transition from pregnancy to lactation as the mammary gland rather than the uterus draws on the maternal calcium stores. Lactational demands are met at least partially by a temporary demineralization of the skeleton, which is associated with increased bone modeling and remodeling. Endochondral growth almost ceases during lactation, but envelope-specific bone modeling and remodeling are greatly increased. This is generally associated with a loss of skeletal mass and density, more apparent at sites with less of a mechanical role (e.g. central metaphysis regions and the endocortical envelope). The post-lactational period is profoundly anabolic with substantial increases in bone formation, but blunted resorption at almost all skeletal envelopes. Skeletal mass is increased during this period and it is associated with improved skeletal mechanical properties. There are several important observations. 1) The nulliparous animal appears to have an excess skeletal mass to perhaps compensate for maternal metabolic inefficiency of the first reproductive cycle. 2) Changes in growth, modeling and remodeling occur at different times and at different skeletal envelopes during the reproductive cycle. These site-specific, temporal changes appear to be adaptations that facilitate the use of skeletal mineral while preserving mechanical competence. 3) After the first reproductive cycle, modeling and remodeling optimize the existing skeletal mass into a structure that better accommodates the prevailing mechanical environment. 4) The post-lactational period is profoundly anabolic and may provide new strategies for preservation of skeletal mass when reproductive capacity ceases.

Keywords: Pregnancy, Lactation, Reproduction, Bone, Skeleton

Introduction

There are numerous complex but highly coordinated physiological and endocrine events that occur during the mammalian reproductive cycle. The nutritional requirements of the mother greatly increase during pregnancy to ensure adequate growth of the fetus and after pregnancy to sustain lactation. During pregnancy, the mineral needs of the mother increase as the fetus draws from maternal stores, particularly for the mineralization of the developing skeleton. After pregnancy, the demands on the maternal system increase further as the newborn draws mineral from the mother during lactation. The mineral required for the reproductive cycle comes from both dietary and maternal skeletal sources. If dietary sources are limited, then greater relative amounts will be provided from skeletal stores that could possibly compromise maternal skeletal structure and biomechanical competence.

In the course of mammalian evolution, reproductive physiological mechanisms are highly protected and often redundant. This is necessary to ensure survival of the species, often in challenging environments and circumstances. In recent years, some unique skeletal adaptations have been identified that appear to allow the maternal skeleton to supply calcium, yet maintain biomechanical competence. Some of the changes in skeletal and mineral metabolism that occur during the reproductive cycle are the most dramatic and rapid in the life history of the female adult skeleton.

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However, the tissue-level, cellular and systemic (e.g. endocrine) mechanisms involved are poorly described and not well understood. In this overview, we will summarize some of the skeletal changes that occur prior to, during and after the mammalian reproductive cycle. Additional comparisons between experimental studies in shorter-lived (e.g. rodents) and longer-lived (e.g. humans) mammals will be made. We believe that these skeletal changes are important evolutionary adaptations that help ensure successful reproduction and thus survival of the species. Additionally, the loss of these functional adaptations for reproduction results in the involution of the skeletal system with age.

Skeletal changes prior to the reproductive cycle (puberty)

Changes in skeletal dynamics and mineral metabolism that occur before and during puberty in both males and females influence the magnitude of peak bone mass. In a recent study on calcium accretion, Bailey et al.[1] reported that about 26% of adult calcium is deposited in the skeleton during just 2 years of the peak skeletal growth period ("growth spurt") in adolescents. In their study, this was at about 12.5 years for the girls, about the time of menarche. In calcium balance studies of adolescent females[2], an increased bone retention of calcium was observed with a high calcium intake. This increase in skeletal calcium was attributed to an increase in intestinal absorption and a decrease in bone resorption.

Normative values for whole body and regional bone mineral density were published for a large set of healthy Argentine children and adolescents in 1995[3]. Based upon the mechanostat theory that estrogen lowers remodeling thresholds, Schiessl et al.[4] and Lyritis et al.[5] re-examined the data from the Argentine Children's study with some provocative results. When the whole body bone mineral content (BMC) was plotted as a function of lean muscle mass, this ratio was accelerated in the girls from age 11-12 compared with the boys of the same age and the same muscle mass. These results suggested that with puberty, girls accumulate skeletal mass in excess of that needed for their level of physical activities. It was suggested that the increase in estrogen with puberty lowers the remodeling threshold, resulting in a reduction of remodeling-dependent bone losses, while bone gains from modeling and longitudinal growth continue. This is supported by a recent study demonstrating that males during puberty accumulate bone mostly on periosteal surfaces whereas females add bone on endocortical surfaces[6]. The addition of bone to endocortical surfaces, compared with periosteal surfaces, would have less effect on bone strength. These studies collectively suggest that during puberty, the human female skeleton accumulates extra calcium which may be an adaptation to accommodate the needs of subsequent reproductive cycles. While the maturation of the ovary during puberty and the secretion of estrogen is an obvious endocrine mediator, the possible role(s) of adrenal steroids, including the adrenal androgens should be explored.

Recent experimental studies support the concept that skeletal mass is accumulated in the young, growing female skeleton in excess of that needed for normal mechanical usage[7,8]. For example, the cancellous mass in the nulliparous rat is in clear excess of that observed after the first and subsequent reproductive cycles. It appears that after the first reproductive cycle, a new lower bone mass "steady state" in the skeleton is achieved (Fig. 1). The reduction in bone mass appears to be compensated by an improved skeletal architecture for the prevailing mechanical environment. This excess skeletal mass may be needed to ensure success during the first reproduction period which is generally considered to be "metabolically inefficient"[9].

The accumulation of an excess skeletal mass prior to initiation of reproduction is not unique to mammals. In birds, medullary bone development occurs with maturation of the ovarian follicles and associated increased secretion of estrogens, but prior to initiation of the egg-laying cycle[10], (Fig. 2). This estrogen-dependent bone[11] serves a pure metabolic function of providing a reservoir of calcium for the formation of the eggshell[12] but does not appear to serve any structural function. Thus, the accumulation of skeletal mass prior to reproduction may be a characteristic of all vertebrates, not just mammals.

Skeletal changes during pregnancy

I. Primiparous

The first breeding and first reproductive cycle in the mammals that have been studied is not as successful as future reproductive cycles[9]. This may be in part due to the age of primiparous females. In many cases they are still growing and have not reached adult size; thus, there may be compromises between continued growth and reproduction. In small mammals it has also been found that as first-time breeders

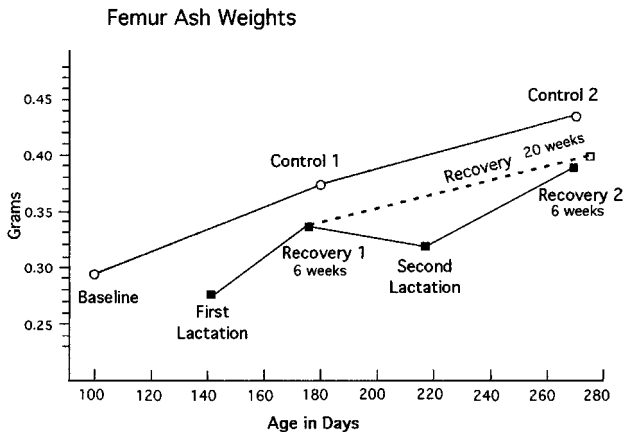

Figure 1. Femoral ash weights during two reproductive cycles of the rat. The amount of bone after the first or second reproductive cycles does not return to that observed in the nulliparous, age-matched animals. This suggests that a new optimal bone mass is achieved after the first reproductive cycle[8].

they require more energy partly due to the less efficient methods of foraging and survival skills.

During puberty and adolescence, peak bone mass is achieved and at some sites, adult skeletal architecture is formed. For example, adult human female hip geometry is usually established in the early teenage years[13]. It is possible that reproduction prior to the attainment of peak bone mass and optimal skeletal structure may compromise the maternal skeleton. There are, for example, some recent data that the bone loss that is associated with a normal reproductive cycle in teenage mothers may not recover as fast, if at all, compared with pregnancies in older females [14]. While some investigators are expressing concern over these findings, there is little or no data to date to suggest that reproductive cycles in younger women and adolescents compromise skeletal function or mechanics during or after reproduction.

Most mammals reproduce soon after estrus is established and this may have been the case during the evolutionary history of primates with the initiation of menses. In early human history, the first reproductive cycle likely occurred in younger, adolescent individuals compared with modern man. Thus, it would be expected that significant adaptations evolved to protect the young, growing mammalian female skeleton. The accumulation of excess skeletal mass is one such adaptation, as discussed in the previous section, and the stimulation of modeling-dependent bone gain is discussed in the next section. On the other hand, the consequences of early reproductive cycles may not be manifest until later in life when reproductive capacity has either decreased or ceased (e.g. menopause). The genetic selection for and against the maintenance of the skeleton during the life history of the female is discussed later.

II. Early to mid-pregnancy

Pregnancy or "pseudopregnancy" initiated by cervical stimulation is initially maintained by the pituitary gland. In the rat, pituitary regulation of pregnancy or pseudopregnancy lasts 11-12 days. Throughout the remainder of the pregnancy (e.g. in the rat from about 11 days to parturition at about 21 days) the placenta controls the pregnancy. This initial phase of the pregnancy which is associated with a cessation of the estrous and menstrual cycles may be associated with some changes that may be beneficial for preparation of the maternal skeleton for the ensuing calcium demands. In the rat, some of these changes include increased endochondral growth rates[15], dentin apposition rates[16], bone formation rates and mineral apposition rates with an increase in bone mass[17,18]. Accumulation of calcium in early to mid-pregnancy has also been observed in longer-lived mammals including sheep[19] and humans[20], but the endocrine mechanisms for these changes in longer-lived animals is not known.

The relative changes in endochondral and somatic growth that occur in the rat during early pregnancy and pseudopregnancy are illustrated in Figure 3. The increase in endochondral growth in early pregnancy[15,21] is accompanied by an increase in periosteal apposition and an increase in periosteal diameter[17]. This is an example of modeling-dependent bone gain and because it is occurring on the periosteal surface would have a greater impact on bone biomechanics than if added onto the endocortical surfaces. It has been suggested that these early changes in skeletal tissues are adaptive mechanisms to prepare the maternal skeleton for the ensuing calcium demands of fetal skeletal mineralization and lactation[17]. Envelope-specific changes during and after the reproductive cycle in rats are illustrated in Figure 4. The fact that changes in skeletal and somatic growth and metabolism would occur during pseudopregnancy in the rat indicates that placental factors are not required for these early skeletal changes[21]. While endocrine mechanisms for

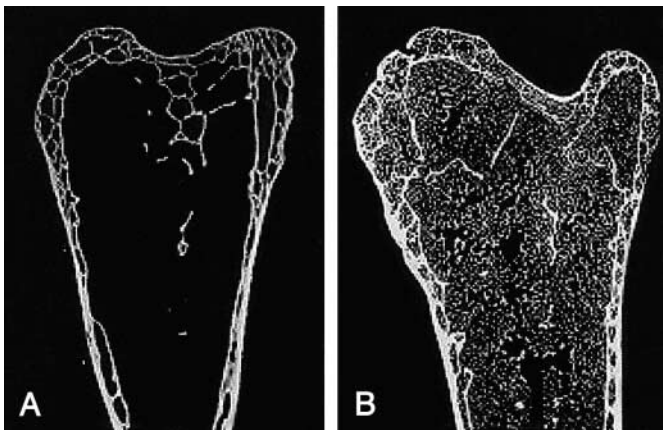

Figure 2. Medullary bone development occurs during maturation of the ovary and secretion of estrogen. A. A pigeon that is not in an egg-laying cycle. B. A pigeon that is ready to enter the egg-laying cycle. Note the presence of medullary bone in the marrow cavitiy. Magnification x 4.

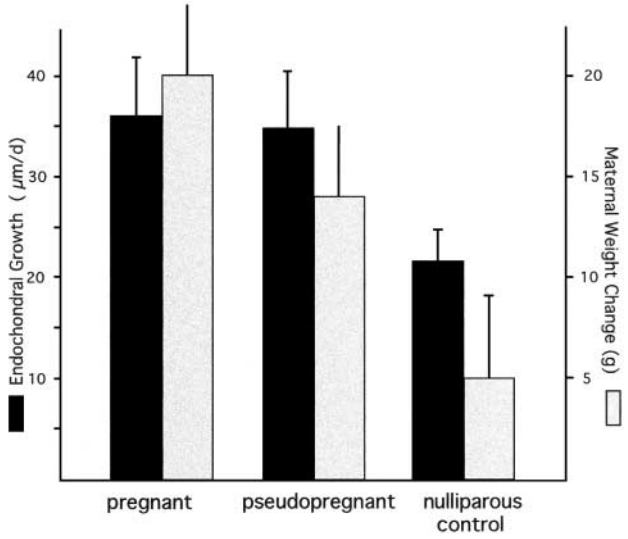

Figure 3. Endochondral (distal femur growth plate) and somatic growth of rats during the early part of a normal pregnancy compared with a pseudopregnancy. The stimulation of endochondral growth by a neuroendocrine response mediated through the pituitary gland is an example of modeling-dependent bone gain[21].

these early skeletal responses are not understood, it has been suggested that the changes in the relative ratios of estrogen and progesterone may be involved[21]. When pseudopregnancy, which is a state of low estrogen with high progesterone, was compared with ovariectomy, a state of low estrogen and progesterone, significant differences in skeletal metabolism were noted suggesting a role for progesterone in some of these initial skeletal changes in pregnancy[22].

Insulin-like growth factors (IGF) are also known to have roles in the stimulation of skeletal growth. Circulating levels of IGF-1 increased in early pregnancy in the rat and then decreased between 12 and 15 days of gestation[23,24]. This would coincide with the increased growth that occurs in mid-pregnancy in rats[17]. In humans, IGF-1 concentrations rise throughout pregnancy[25]. Mechanical factors may also play a role as the maternal mechanical loads increase due to the increasing weight of the developing fetus and somatic growth of maternal organs and tissues associated with pregnancy.

III. Mid to late pregnancy and the transition into lactation

The maternal need for calcium increases as pregnancy progresses, coinciding with mineralization of the fetal skeleton. In the rat, maternal calcium needs increase from < 0.5 mg/day in early pregnancy to about 12 mg/fetus on day 17 of pregnancy to parturition[26]. The rat exhibits several adaptations for the increased calcium needed during pregnancy. Besides the early increase in bone mass occurring early to mid-pregnancy[17], the rat also has increased intestinal absorption of calcium[18] associated with elevations in 1,25-dihydroxyvitamin D_3 (1,25-OH_2-D_3)[27] and parathyroid hormone (PTH)[28].

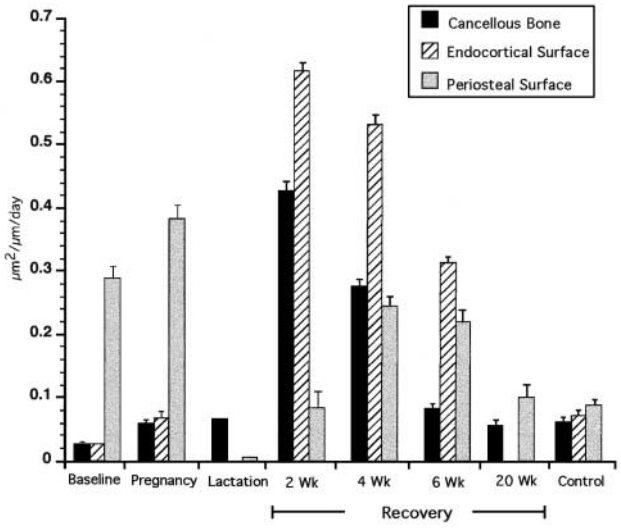

Figure 4. Envelope-specific changes of the proximal tibial cancellous bone and tibial cortical bone during and after the reproductive cycle in rats. The greatest periosteal formation rates occurred during pregnancy while the greatest endosteal and endocortical formation rates occurred after lactation, during the recovery period (2 wk, 4 wk and 6 wk). One group of rats was allowed to "recover" for 20 weeks after the first lactation (20 wk).

Calcium needs increase in later pregnancy in all mammals due to progressive mineralization of the developing fetal skeleton. In humans, the normal amount of calcium in a fetus is about 21 g of which about 80% is accumulated during the third trimester. Some adaptations in calcium metabolism differ among mammals (e.g. rat vs human) due to differences in gestation time and number of fetuses. In humans, there is an increase in calcium absorption associated with increases in 1,25-OH_2-D_3 during early pregnancy, but PTH generally remains at normal to low levels. Interestingly, parathyroid hormone-related protein (PTHrP) was found to increase in late pregnancy in hypoparathyroidism, suggesting compensatory mechanisms for PTH involvement during later pregnancy in humans[29].

There is emerging evidence in humans that bone resorption and turnover is increased in later pregnancy[30,31] and may be associated with small decreases in BMD[32,33]. The losses in BMD at the end of pregnancy are reported to be greater in adolescents than adult women[34]. There are few reports, however, detailing the envelope-specific changes that occur in skeletal modeling and remodeling during later pregnancy.

Skeletal changes during lactation

Following parturition there is a rapid transition in the maternal system as the calcium required for fetal development changes to accommodate lactation and milk production. In rats, there is a virtual cessation of endochondral growth[8] (Fig. 5) and a marked bone loss as lactation progresses. This loss of cancellous bone mass is most evident during the first lactation (Fig. 6, A and C), but also occurs in subsequent lactations. Bone loss during lactation has been documented

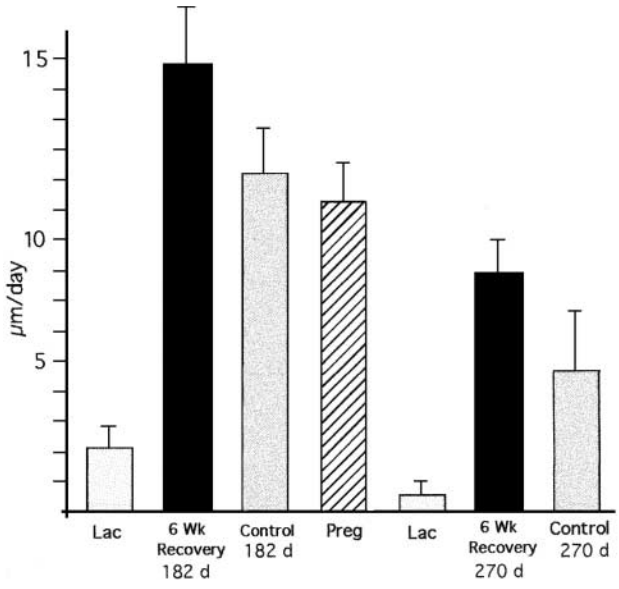

Figure 5. Longitudinal growth during the reproductive cycle in the rat[8]. Growth was measured at the proximal tibial epiphyseal plate and the data are expressed as longitudinal growth per day. The smallest growth rates were observed during lactation.

in other species including sheep[19], dogs[35], pigs[36] and monkeys[37,38]. Rats produce about 70 ml of milk per day with a calcium content of about 300 mg/ml[39]. It has been estimated that each rat pup reduces the mother's bone mineral density in the lumbar vertebrae by about 6.7 mg/cm^2 [40]. In humans, the maternal loss of calcium during 9 months of lactation is fourfold higher than during pregnancy[41]. The daily loss of calcium in breast milk is 280-400 mg but has been reported as high as 1000 mg[42].

The loss of cancellous bone during lactation in both rats and dogs is associated with greatly elevated rates of cancellous bone turnover, with increases in bone resorption exceeding increases in bone formation[17,35]. We have also recently reported increased intracortical bone turnover rates in both a weight-bearing and non-weight-bearing bone during lactation in the dog[43]. Histomorphometric data are not available for humans; however, the serum and urine markers for resorption were increased 2 to 3 times during lactation and were higher than values at late pregnancy[44-46].

In the rat, there is preferential and rapid loss of cancellous bone mass and density during lactation, particularly in the metaphyseal spongiosa regions of the long bones[7,8,17,47] (Fig. 6), but loss of cortical bone mass is also observed[17]. During the first lactation, for example, more than half of the metaphyseal bone mass of the proximal tibia and distal femur is resorbed[7]. Although ovariectomy has been used extensively as a model for bone loss[48], the bone loss during a normal lactation is even more severe. For example, the trabecular bone volume in the rat proximal tibia decreases from 30% in a nulliparous control to about 19% at 3 weeks post-ovariectomy but to 6 % at 3 weeks of lactation[7]. The loss of skeletal mass is also influenced by other factors such as calcium content of the diet and the number of pups[49]. Increasing dietary calcium does not prevent the bone resorption associated with lactation. Numerous studies supplementing diets with various levels of calcium during lactation show little difference in the magnitude of bone loss in both experimental animals[39,50,51] and women[44,52-54].

Most studies in humans also report some loss of bone mineral density during lactation, particularly at cancellous bone sites. For example, a 7.1% decrease in the lower forearm bone mineral density has been reported in lactating women compared to non-lactating women[45]. Other human studies have shown loss of bone mineral density of 5.1% in the lumbar spine and 4.8 % in the femoral neck after 6 months or more of breastfeeding[55]. Changes in bone mineral density during the human reproductive cycle were reviewed by Sowers[56].

The endocrine environment during lactation is estrogen deficient due to the lack of a regular menses or an estrous cycle. Associated with the decrease in estrogen and progesterone is an increase in prolactin and PTHrP. Deficient estrogen, which results in reduced bone mass following menopause[57], is likely involved in the bone loss during lactation. The secretion of PTH, which normally acts to increase serum calcium concentrations, has been found to be unchanged or slightly lower during lactation in women[53,54]. In the rat, PTH does increase during lactation and similar to the response in late pregnancy, a mild hyperparathyroidism develops. There is evidence, however, from parathyroid-ectomized, lactating rats demonstrating that PTH is not essential for mineral resorption and calcium availability during lactation[58]. As PTH does not appear to be solely regulating the calcium release from bone during lactation, PTHrP has been suggested as having a role in this process. PTHrP has been found to be elevated in lactating women[59] and present in high concentrations in the lactating mammary gland[60] and in breast milk[61].

Skeletal changes after lactation (recovery)

Lactating rats may lose as much as 35% of their skeletal mineral or femoral ash weight[39]. Weaning or the cessation of breastfeeding appears to initiate a profound anabolic response in the skeleton with greatly increased rates of bone formation and accumulation of skeletal mineral. This anabolic period enables the skeleton to recover bone mass[8,62] and mechanical competence[62]. We have found in rats rapid increases in bone formation within 2 weeks of the cessation of lactation (Fig. 4). The increased bone formation is first seen on endosteal and endocortical surfaces. The periosteal surface responds more slowly but also has increased bone formation. The increased anabolic activity shows signs of

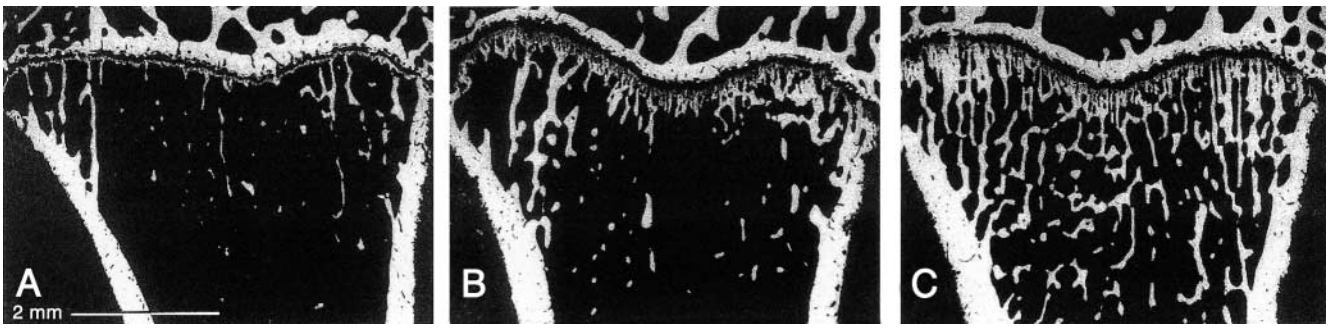

Figure 6. Changes in the cancellous bone in the proximal tibia of the rat at the end of a first lactation (A), the end of a 6-week recovery (B) and a nulliparous age-matched control (C). Note the reduction in bone during lactation (A) compared with the control (C). By 6 weeks after lactation, increased bone mass, particularly increased trabecular thickness, is apparent (B). Magnification x 10.

decreasing by 6 weeks post-weaning. The increase in bone formation following lactation occurs even when the lactation is immediately followed by pregnancy. An early study by Ellinger (1952), followed 3 successive pregnancies in rats and found the reduction in long bone ash weights during lactation were followed by new bone formation during pregnancy[50].

There are some important differences in the rat skeleton following a first-time reproductive cycle and recovery. There are significant changes in the cancellous bone structure[7,62]. The majority of the trabecular bone in the proximal tibia metaphysis is resorbed during lactation. As bone formation is increased and endochondral growth resumes during recovery, new bone is added to the metaphysis but it never attains the mass of the nulliparous animal. This may be evidence for an optimization of the skeletal structure for the prevailing mechanical loads. An example of this is evident in Figure 6. The lateral metaphyseal trabeculae are preserved over the trabeculae in the less loaded central metaphysis.

The amount of bone mass regained during the 6 weeks following the first lactation was similar to that gained after the second lactation. The absolute gain in bone ash following the first lactation was 0.0598 g and following the second, 0.0594 g [8]. The total femur ash weight of the nulliparous animals was still greater than femur ash weight at the end of either recovery. After the loss of bone during the first lactation, though there were mineral increases, the bone mass never quite attained the amount of mineral equivalent to the nulliparous animals. This would suggest that after lactation, the skeleton has adapted a new set point that is slightly lower than that of the nulliparous skeleton. This lower bone mass "set point" following a reproductive cycle is also evidence for excess skeletal mass in the nulliparous animal.

In the post-lactational period in the rat, the periosteal, endocortical and endosteal bone envelope all show substantial increases in bone formation activities, but differ in their temporal responses and magnitude of response. The cancellous and endocortical surfaces increase formation activity within 2 weeks following weaning. Interestingly, the bone formation rate on the endocortical surface is greater than at any other time or site during the reproduction cycle. The periosteal surface also increases formation rates but responds more slowly[8] (Fig. 4).

Some longitudinal studies in humans demonstrate some recovery of bone mineral density following lactation. For example, Sowers et al.[55] reported spinal BMD recovered to baseline values from 6 to 12 months after lactation. Loss of BMD during lactation in the forearm was also demonstrated to recover to control levels within 4-6 months after weaning[45]. In a study of women who were either pregnant or lactating for most of their lives ("grand multiparous women"), repeated pregnancy and lactations without a "recovery period" was not associated with lowered BMD[63]. In this case, the ensuing pregnancy may have initiated responses in the skeleton (as discussed earlier) that may have, in essence, served as the "recovery period".

The extent of recovery of BMD after lactation in humans may depend on some other factors including the site measured, differences in cortical and cancellous bone, diet, mechanical loading, initiation of menses and the age of the mother. For example, there is some recent data indicating that younger, adolescent mothers may not fully reconstitute skeletal mass after lactation[14]. However, this may be a case where the excess skeletal mass that is accumulated prior to the first reproductive period is used and the remodeling and modeling of the skeleton during the reproductive cycle improves skeletal architecture such that a new lower bone mass "steady state" can be achieved, as observed by us in experimental studies[7,8,62].

Genetic selection for reproduction, long-term consequences for skeletal tissues

Maximization of maternal efficiency for reproduction is an evolutionary trait necessary for successful species propagation. Thus, the evolution of modeling and remodeling of bone has likely been optimized for reproductive success by genetic selection. The multiplicity and redundancy of some endocrine pathways, for example, likely developed during evolution to optimize and protect reproductive capacity in a diversity of environments. While these traits might be beneficial, indeed essential, for reproduction, they may also have deleterious effects later in life after reproduction has slowed or stopped. For example, the remodeling dynamics that are essential for reproductive success may become dysfunctional in later life, resulting in an imbalance that would lead to osteopenia. The genetic determinants of traits that manifest after reproduction has ceased are not selected against and may persist. These traits are called "antagonistic pleotrophies" and it has been proposed that type I osteoporosis may be an example of the expression of these traits[64]. Because these traits are not selected against, they may actually increase in the population and this may be one reason for the prevalence of age-related osteoporosis, particularly with the increases in life expectancy that have occurred over the last century. The understanding of the skeletal mechanisms that optimize reproductive success may provide new paradigms for the treatment of age-related disorders, when reproductive capacity has ended.

Conclusions

In recent years a number of significant skeletal adaptations have been recognized that are involved in and perhaps essential for the mammalian reproductive cycle. Some of these include:

- Changes in growth, modeling and remodeling occur at different times and at different envelopes during the reproductive cycle. These changes appear to promote the biomechanical properties of bone during a period when bone mass may decrease.
- There is an increase in bone modeling during early to mid-pregnancy, particularly evident on periosteal surfaces but also on endocortical and endosteal surfaces.

- There are substantial increases in endosteal and intracortical bone remodeling during lactation.
- Lactation is associated with a cessation of endochondral growth, marked bone loss at some skeletal envelopes and decreased mechanical strength.
- There is a dramatic "anabolic" period that commences soon after the end of lactation. This includes greatly increased rates of bone formation and is associated with improved biomechanical properties of both cortical and cancellous bone.
- The transient decreases in bone strength during lactation suggest that the female skeleton during the period of reproductive capacity is "overbuilt" for the prevailing mechanical environment.
- The reconstitution of bone mass after the first lactation does not restore the mass to that of the nulliparous animal. However, skeletal architecture is substantially changed. In subsequent reproductive cycles, the bone mass that is lost is essentially regained in the post-lactational period.
- Modeling and remodeling optimize the existing skeletal mass into a structure that better accommodates the prevailing mechanical environment. This is particularly apparent during the first reproductive cycle.

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