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Pregnancy and lactation, a challenge for the skeleton

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Abstract

In this review we discuss skeletal adaptations to the demanding situation of pregnancy and lactation. Calcium demands are increased during pregnancy and lactation, and this is effectuated by a complex series of hormonal changes. The changes in bone structure at the tissue and whole bone level observed during pregnancy and lactation appear to largely recover over time. The magnitude of the changes observed during lactation may relate to the volume and duration of breastfeeding and return to regular menses. Studies examining long-term consequences of pregnancy and lactation suggest that there are small, site-specific benefits to bone density, and that bone geometry may also be affected. Pregnancy- and lactation-induced osteoporosis (PLO) is a rare disease for which the pathophysiological mechanism is as yet incompletely known; here we discuss and speculate on the possible roles of genetics, oxytocin, sympathetic tone and bone marrow fat. Finally, we discuss fracture healing during pregnancy and lactation and the effects of estrogen on this process.

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1. Introduction

Pregnancy and lactation are challenging situations for the mother's skeletal homeostasis. Significant changes in maternal calcium and bone metabolism must occur to fulfill the calcium requirements to build the fetal skeleton. These processes are regulated by hormonal changes and often lead to physical changes in the mother's skeleton. In addition, lifestyle factors which have an impact on bone health can alter during pregnancy and lactation. Physical activity may be lower than usual in pregnant women, particularly in the third trimester (1), with the most marked deficits in the vigorous activities known to be beneficial to bone homeostasis. In contrast, the 10-15 kg weight gain during a healthy pregnancy (2) as a result of both lean and fat mass gains (3) likely increases bone and joint loading during everyday movements. Whilst nutritional requirements are moderately increased during pregnancy (4), this appears to be counterbalanced by concurrent reductions in energy expenditure from decreased physical activity. Accordingly, there is little change in diet quality and only a small increase in energy intake during pregnancy and early lactation (4,5) without substantial alterations in calcium intake. Despite this, the World Health Organization (WHO) recommends an extra dietary calcium intake of 200 mg/day for pregnant women compared to non-pregnant women (6,7). Here, we review the current knowledge of how the female skeleton physiologically adapts to pregnancy and lactation, how fracture healing is affected in these situations, and the pathophysiology of pregnancy- and lactation-induced osteoporosis (PLO).

2. Physiological adaptation of bone to pregnancy and lactation

A. Hormonal changes during pregnancy

During pregnancy and lactation there is an increased need for calcium in the mother to meet the fetus' calcium requirements (8). In humans, this need is met during pregnancy by increased intestinal calcium absorption, and during lactation by increased calcium resorption from bone. Bone resorption is mediated by parathyroid hormone-related protein (PTHrP). PTHrP is virtually absent in the non-pregnant state, and increases from the first trimester of pregnancy until labor, after which levels drop drastically within hours unless breastfeeding is initiated (9). During pregnancy PTHrP suppresses and replaces parathyroid hormone (PTH), thereby preventing secondary and tertiary hyperparathyroidism. The first 34 amino acids of PTHrP display similarity to the structure of PTH, and therefore PTHrP is also a ligand for the PTH/PTHrP receptor. However, the mid- and terminal regions of PTHrP are distinct, and have additional functions. The mid-molecular region stimulates placental calcium transport in the fetus (10), while the COOH terminal region may also inhibit osteoclast activity (11), in addition to the more generally known indirect activation of osteoclasts by production of receptor activator of nuclear factor-kappaB ligand (RANKL) in (pre)osteoblasts and osteocytes (12,13). PTHrP is released from the placenta and breasts in reaction to estradiol, placental lactogen and prolactin, although other tissues including the parathyroid glands and uterus also contribute (14-16). During pregnancy high estradiol levels largely suppress bone resorption in response to increasing PTHrP. In early pregnancy, increased intestinal calcium absorption appears to largely satisfy fetal calcium demands, whereas higher demands during late pregnancy may additionally increase bone resorption, especially in women with calcium deficient diets (17). However, bone loss cannot be prevented by calcium supplementation (18). Estradiol levels drop during lactation. In combination

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with PTHrP, this drop then stimulates bone resorption (14,15) in a synergistic way (19). Oxytocin also adds to this effect (see below).

Physiology in pregnant and lactating humans differs from that of rodents (14). In rodents, the increase in serum calcium during lactation is not only dependent on increased bone resorption, but also on ongoing increased calcium absorption in the gastrointestinal tract mediated by increased concentrations of PTH.

PTH rises during pregnancy in rodents and the subsequent secondary hyperparathyroidism causes both augmented intestinal absorption by increased activation of cholecalciferol into calcitriol, and resorption of the skeleton (14). This is in contrast to the suppression of PTH by increased PTHrP in humans. Besides PTH (rodents) and PTHrP (human), vitamin D metabolites, prolactin and placental lactogen are key regulators of calcium metabolism in mammals (14). Together they are responsible for increased calcium levels; by increasing bone resorption, stimulating intestinal calcium absorption via synthesis of calcitriol, and reducing urinary calcium waste. Calcitriol is elevated during pregnancy in both humans and rodents, and while it declines during lactation in humans, it remains elevated in rodents and stimulates intestinal calcium absorption. Calcitonin is thought to protect the skeleton from excess resorption during lactation, although few data exist for the role of calcitonin and FGF23 in this process (14).

To summarize, as already reviewed beautifully by Kovacs (14,15), increased calcium needs during pregnancy are fulfilled by almost doubling intestinal calcium absorption. Meanwhile, during lactation, increased PTHrP levels act synergistically with low levels of estrogen to increase bone resorption. In rodents, increased intestinal uptake of calcium continues during lactation.

Mechanisms of pregnancy-induced bone changes are informed by the study of non-mammalian models. Birds that lay hard-shelled eggs in part fulfill their calcium requirements by resorbing calcium from medullary bone present in the marrow cavities of long bones (20). The mechanism of medullary bone turnover is identical to bone turnover in mammals. However, the process is triggered by a distinct mechanism, particularly well characterized in the seasonally reproducing Japanese quail (*Coturnix coturnix japonica*). Long day length triggers expression of thyroid stimulating hormone (TSH) in the *pars tuberalis* of the pituitary (21). This induces expression of the type 2 iodothyronine deiodinase (DIO2) in the mediobasal hypothalamus (22), converting thyroxine prohormone (T_4) to bioactive 3,5,3'-triiodothyronine (T_3). T_3 actions on gonadotrophin-releasing hormone (GnRH) nerve terminals trigger release of gonadotrophins from the pituitary, increasing gonad size and allowing reproduction. During the egg production cycle, hypocalcemia in the mother during shell calcification increases PTH levels, indirectly triggering bone resorption and providing calcium for eggshell calcification (23). Resorption of medullary bone in avian models therefore represents a model of rapid and pregnancy-related bone turnover.

B. Whole-bone changes during pregnancy and lactation

Pregnancy

The endocrine and associated metabolic changes which occur during pregnancy and lactation result in effects on the skeleton at both the microstructural and whole-bone level. At the microstructural level, results of human iliac crest bone biopsies suggest increased bone resorption in early pregnancy (8-10 weeks), whereas at term, resorption is normalised and markers of increased bone formation have been observed (24). Bone loss through trabecular thinning and

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reduced trabecular connectivity in early pregnancy appear to be regained through the addition of new trabeculae by late pregnancy (25). At the whole bone level, bone mineral density (BMD) assessed by clinical dual-energy X-ray absorptiometry (DXA) has been shown to decrease by up to 5% during pregnancy in the lumbar spine (3,5,26-29), with several reports of smaller losses in the hip. Results at other skeletal sites are more inconsistent; these between-study discrepancies may be explained in part by differences in calcium intake, although variations in the timing of BMD measurement will also contribute. Quantitative computed tomography (QCT, or pQCT for peripheral scanners) gives information on cortical and trabecular bone in addition to detailed bone geometry. However, few studies have employed these techniques to examine pregnancy-related changes, and results from the few studies available have been inconsistent. QCT/pQCT studies have found no change in cortical, trabecular or total BMD at the radius or tibia (30), distal radius cortical BMD (31) or lumbar spine trabecular BMD following pregnancy (5), respectively. In contrast, substantial variation in distal radius trabecular BMD changes between individuals were observed in one study, with annual losses of up to 21% (median 1.6%), and with individuals with lower baseline BMD values experiencing the greatest losses (31). One recent study has examined changes in periosteal and endocortical circumferences and bone microstructure using high-resolution pQCT (HR-pQCT) to characterise the moderate and site-specific bone losses occurring during pregnancy in detail. The authors found deterioration of trabecular microarchitecture and evidence of periosteal and endocortical expansion in women aged 30-45 years across pregnancy compared to non-pregnant controls (32).

Lactation

Lactation is associated with decreases in bone mass (33-35), particularly at trabecular-rich sites such as the spine and hip (33). These rates of bone loss are pronounced, with a mean 4% loss of lumbar spine BMD reported after only 3 months of feeding (33-35). In contrast, these changes are smaller or not evident in formula-feeding mothers or non-pregnant, non-lactating women (33-35). Unfortunately, to our knowledge no studies have used pQCT to examine changes in endocortical and periosteal geometry during lactation. Changes at the microstructural level can also be assessed *in vivo* using high-resolution pQCT (HR-pQCT). Longitudinal studies of breastfeeding women observed increases in cortical porosity and decreased cortical thickness, BMD and mineralisation of new bone (36,37). In the same studies, trabecular number and bone volume decreased, whilst contrasting findings with regards to trabecular thickness were observed. The latter findings could relate to methodological issues, with trabecularisation of cortical bone influencing reported values.

Lactation-related bone losses vary substantially, with 38% of women losing >5% spine BMD (38). Magnitude of bone loss is positively associated with the amount of breast milk produced (33). Furthermore, rates of bone microstructural change were greater during exclusive rather than intermittent feeding (36), and increased with the duration of lactation (37,39). Taken together, these results suggest that increased calcium demand associated with exclusive breastfeeding, or increased volume or duration of feeding results in greater bone loss. Given the modest changes in calcium intake during pregnancy and the high incidence of vitamin D insufficiency in both northern and southern latitudes in pregnant women, it could be suggested that dietary insufficiency of these nutrients would contribute to bone changes in pregnancy. However, the majority of studies suggest that vitamin D and calcium levels are not related (3,33,40) or only weakly associated to bone turnover markers or bone loss during pregnancy and lactation (41), and

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calcium supplementation has been shown to have small (18,30) or transient (34) effects on bone loss in pregnancy and lactation (42-46) . Other mechanisms must thus explain these observations.

Following lactation, there appears to be full recovery and even improvements in bone mass (34,35,47,48), although this appears incomplete in adolescents (49). There is some suggestion that recovery at the trabecular-rich spine is quicker than in other regions. In addition, return of regular menses and use of the progestin-only contraceptive pill were associated with improved bone recovery during and after lactation (35,48,49). Lactation-induced changes in bone microstructure did not resolve over a longitudinal study with median follow-up period of 3.6 years (36) such that cortical porosity was 0.6 SD higher, and mineralisation density and trabecular number 1.3 SD lower in lactating women than controls. We note that this study did not control for postpartum use of hormonal birth control which may have influenced results. Overall, lactation appears to be associated with a series of changes in bone at the macro and microstructural level. Changes at the microstructural level do not appear to be fully resolved with time, although it is unclear whether compensatory changes at the macrostructural level may preserve strength comparable to the increase in periosteal diameter in non-pregnant aging elderly with severe bone loss (50).

Pregnancy in Adolescence

Another area of interest is in adolescent mothers, as it has been hypothesised that pregnancy during growth may negatively affect bone accrual. A DXA-based study (four cases, twelve matched controls) found 9-10% lower total body and hip BMD (51) in late adolescent mothers at follow up at 19-21 years of age, although lactation was not controlled for. A pQCT-based study found a 1% decrease in radius cortical BMD and a 4% decrease in total body BMC in new teenage mothers (mean age 18 years) (52) although both studies were limited by small numbers of participants. However, these studies focused on later adolescent mothers where pregnancy occurs

after peak growth, and this limit understanding of the effects of early post-pubertal pregnancies. One study which did examine earlier pregnancies found impaired recovery of post-weaning lumbar spine and total body BMD in mothers aged 14 and 15 years compared to those aged 16 or 17 years (53).

Long-term Effects of Pregnancy and Lactation

The long-term effects of pregnancy and lactation on bone characteristics in later life have also been examined, and overall small, site-specific benefits to BMD have been observed (54). Whilst no strong evidence for differences in BMD was observed between paired parous/nulliparous twins, in a large cross-sectional study parity was associated with both greater lumbar spine BMD and total body BMC than in other female relatives, whilst breastfeeding was associated with higher total body BMC and hip BMD (55). No association between gravidity, parity and BMD was observed in women aged 40-80, but parity was associated with greater total body and femoral neck bone area (56). Similarly, cumulative duration of lactation was associated with increased femoral neck and tibia bone cross sectional area (CSA) in women 16-20 years after their final pregnancy (57). Therefore longer-term effects of pregnancy may primarily relate to altered size rather than density (56). These associations appear site-specific, as gravidity and parity were not associated with vertebral shape or size at age 46 in a Finnish cohort (58). This may relate to the suppression of estrogens (which inhibit periosteal apposition) during lactation, with similar expansion of the axial skeleton observed in both eumenorrheic and amenorrheic athletes with lower estrogen levels (59). The long-term effects of adolescent pregnancy are unclear, having been associated with higher BMD (60,61) and lower BMD at multiple sites (62). In terms of long-term clinical consequences, parity and lactation were not found to be associated with increased fracture risk over 16-year follow-up (63).

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3. Pathophysiology during pregnancy and lactation

As described in part 2, it is known that bone loss during pregnancy and lactation is a result of the hormonal changes that are made to fulfill the increased calcium requirements for fetal and neonatal development (14,64,65). If intestinal calcium absorption is insufficient to meet calcium demands, calcium is mobilized from the maternal skeleton by either osteoclast activity or osteocytic osteolysis mechanisms (14,66), which in turn cause a decrease in bone mass. It is not yet understood why some mothers exhibit pregnancy- and lactation-associated osteoporosis (PLO) and fracture whilst others do not, despite both having low vitamin D and low estrogen status (65). Moreover, at pre-clinical levels it has been shown that maximizing calcium and vitamin D physiology through calcitriol supplementation is not sufficient to restore specific bone loss during pregnancy and lactation (42-44,46). More recently it has been shown that calcium restriction during this period has minimal effects on post-weaning bone metabolic changes (45). These studies may explain why some clinical trials failed to demonstrate a full prevention of bone loss during pregnancy and lactation with calcium supplementation (18,67). Since it has not been demonstrated that differences in inactivity levels can explain PLO, and since pathologically raised PTHrP levels are rare (16), other factors including genetic polymorphisms, sympathetic nervous system activity, secretion of oxytocin, and bone marrow fat may contribute to bone loss and disturbance of microarchitecture during pregnancy and lactation (summarized in Figure 1). This may explain some of the differences between physiological and pathophysiological bone loss, i.e. PLO. We will discuss each of these in the following paragraph.

A. Genetics

Early reports have indicated that hereditary factors may play a role in PLO. Osteoporotic fractures at younger age more often have a genetic cause (68,69), in contrast to osteoporotic fractures at older ages which are often due to simple postmenopausal osteoporosis. Remarkably, a high prevalence of fractures has been reported in cases of mothers with PLO (70). Other findings included lack of recovery of bone density in the mother during years of follow-up after delivery and osteopenia in the children when screened at a young age (71). Finally, a series of five index cases with PLO patients revealed osteoporosis, as defined by low BMD, in over half of their relatives (72).

Multiple cases have been published which describe patients in whom a genetic cause of PLO has been identified. Osteogenesis imperfecta is the best-known form of monogenic osteoporosis, and indeed, heterozygous *COL1A1* or *COL1A2* mutations have been found in several cases of PLO (73) (74). These autosomal dominant mutations lead to qualitative or quantitative defects in the formation of collagen type I (75). Other cases have been attributed to mutations in the gene encoding the LRP5 (low-density lipoprotein receptor-related protein 5) cell-surface protein receptor, a key player in intracellular signaling pathways including the Wnt pathway (74). Simple and compound heterozygous *LRP5* mutations have been described in PLO specifically (74,76). Osteoporosis may be associated with visual impairments, such as osteoporosis pseudoglioma syndrome/familial exudative vitreoretinopathy, in these patients. This is because LRP5 has been shown to be essential for the development of blood vessels in the eye (77) as well as bone accrual. The underlying genetic mechanisms may be more complex in some patients, as illustrated by the report of a patient with a heterozygous *LRP5* mutation together with homozygous

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polymorphisms in the *MTHFR* gene encoding methylenetetrahydrofolate reductase, an enzyme involved in homocysteine metabolism (78). Intriguingly, osteoporosis did not cosegregate in this patient's family with the *LRP5* mutation, the homozygous *MTHFR* polymorphism, or even the combination of the two, implicating additional genetic or non-genetic factors in PLO.

Consideration of a genetic cause for PLO is recommended for patients with a family history of osteoporosis and fragility fractures, or a severe phenotype such as a history of fractures before pregnancy and severely reduced BMD (Z-score < -2.0 SD). This is especially critical in cases in which BMD does not recover in the months after pregnancy and/or weaning (79). Screening for an underlying monogenetic bone disorder has been further proposed in patients in whom PLO occurs together with one of the following features: joint hypermobility and blue sclerae (indicative of osteogenesis imperfecta), congenital blindness, or severely reduced vision (as associated with osteoporosis pseudoglioma syndrome). Nowadays, genetic diagnostics are often offered in multi-gene test panels labeled "osteogenesis imperfecta and related conditions" which may include additional variants derived from familial osteoporosis, osteogenesis imperfecta, and genome-wide association studies, studies (80-82). The indication for genetic testing should be seriously considered, because a diagnosis has significant implications for the patient as well as their relatives. Similar to the systematic genetic studies performed for BMD and fractures in general, there is a need for genome-wide association studies for PLO (83-86).

B. Oxytocin

Plasma levels of oxytocin peak during late pregnancy and lactation. These periods coincide with rapid fetal skeletogenesis and neonatal bone modelling; both processes that require a high intake of calcium ions for mineralization, which is delivered by the mother (87). Oxytocin

originated as a highly conserved nanopeptide for electrolytic homeostasis in primitive vertebrates over 400 million years ago (88). Although it is well known that oxytocin facilitates parturition, this hormone is not indispensable for this function (89). In fact, oxytocin knock-out mice ($OT^{-/-}$) can give birth normally but are unable to feed their pups because of the lack of milk ejection reflex. This defect is completely reversible by peripheral injection of oxytocin, suggesting that its primary role in mammalian lactation is mediated by a peripheral action rather than a central mechanism (89). Conversely, the regulation of maternal and sexual behavior, social memory, and penile erection and ejaculation in males, are mediated by a central action of oxytocin (90-93).

In addition to its action on social behavior, milk ejection, and uterine contraction, receptors for oxytocin are expressed on osteoblasts, osteoclasts, and their precursors (94,95). Furthermore, bone marrow osteoblasts synthesize oxytocin, suggesting the existence of an oxytocin/oxytocin receptor system in the bone milieu regulated by autocrine and paracrine interactions (95,96). Oxytocin stimulates osteoblast differentiation (97), and $OT^{-/-}$ mice as well as mice lacking the oxytocin receptor ($OTR^{-/-}$) display reduced bone mass, mainly due to a bone-forming defect (95). Systemic injections of oxytocin into wild-type rodents increase bone mass and improve osseointegration of titanium implants (98) (99). At the same time, oxytocin stimulates differentiation of pre-osteoclastas (Figure 1) by increasing the ratio of RANKL and osteoprotegerin (OPG), while inhibiting bone resorption by triggering cytosolic Ca^{2+} release and nitric oxide synthesis. More specifically, oxytocin increases the expression of the Ca2+ sensitive NOS isoform (eNOS) and triggers a time-dependent increase in the production of nitric oxide, as a mechanism to inhibit bone resorption (95). Altogether these results are consistent with the hypothesis that oxytocin is responsible for maintaining a high rate of cell activity in bone, stimulating the proliferation of both forming and resorbing cells, while at the same time controlling

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the amount of bone resorbed. The inhibitory effect of oxytocin on mature osteoclasts may serve as a checkpoint for bone resorption that would otherwise be unrestricted following stimulation of osteoclastogenesis

PTHrP, indispensable for the development of the mammary gland, and pro-resorptive during breastfeeding (100), is only partly responsible for the intergenerational calcium transfer. It has been suggested that this complex process, fundamental for skeletal morphogenesis, is also driven by oxytocin. The anabolic effect of oxytocin may facilitate the restoration of the maternal skeleton while its pro-osteoclastogenic action may contribute to the intergenerational transfer of calcium. As evidence of this, pregnant $OT^{-/-}$ mice have reduced markers of bone formation, and $OT^{-/-}$ pups displayed hypo-mineralized skeletons (101). Furthermore, pregnant wild type mice exhibited increased plasma levels of C-telopeptide, a marker of bone resorption, and osteoclast cultures obtained from the bone marrow of these mice showed higher numbers of TRAP-positive cells than non-pregnant mice (101). Intraperitoneal injections of oxytocin thrice weekly for 5 weeks in non-pregnant mice, as well as the addition of oxytocin to bone marrow cell cultures obtained from control mice, mimicked this pregnancy-induced increase in osteoclastogenesis (101). A similar result has been obtained in twelve healthy women with spontaneous vaginal deliveries at term. The number and size of osteoclasts generated *in vitro* from peripheral blood of these women were significantly higher than age- matched controls (102).

Overall, these findings show that the enhanced osteoclast formation can be explained by elevated oxytocin levels during late pregnancy (103). Consistent with this hypothesis, pregnant mice lacking oxytocin showed a ~80% reduction in *ex vivo* osteoclast formation compared with wild type pregnant littermates, suggesting that the osteoclastogenesis triggered in pregnant mice is, at least in part, dependent upon an intact oxytocin axis. It is therefore possible that the increase

in serum oxytocin levels during pregnancy is responsible for the increase in osteoclast formation which tends to mobilize maternal calcium for fetal skeletogenesis to occur. Additionally, considering that oxytocin mainly stimulates osteoblastogenesis (95), it has been investigated whether osteoblast formation *ex vivo* was enhanced in pregnant wild type mice, and whether this response was reduced in oxytocin-deficiency. As expected, the formation of alkaline phosphatase-positive colonies (CFU-f) was increased during pregnancy in mice, as mimicked by intraperitoneal oxytocin injection, thrice weekly, in non-pregnant mice, indicating that oxytocin might be responsible for inducing osteoblast formation (101). The uncoupling of bone remodeling from net skeletal loss during lactation has been mainly ascribed to decreased levels of estrogen (100). However, estrogen deficiency during menopause causes irreversible bone loss, while PLO is mostly reversible in both rodents and humans (104). Nevertheless, maternal bone loss would be continuous in the absence of a mechanism that would inhibit excessive osteoclastic resorption. Therefore, it has been proposed that oxytocin, which also inhibits resorption by mature osteoclasts (95), provides one of these mechanisms for the self-regulation of oxytocin-induced osteoclastogenesis and bone loss. Overall, these studies indicate that oxytocin, which is also a mammary gland-specific peptide (89), is important for the regulation of intergenerational calcium transfer and is possibly also mechanistically involved in PLO in humans.

C. Sympathetic nervous system

Genetically and pharmacologically induced over-activity of the sympathetic nervous system (SNS) has been well demonstrated to be deleterious for bone mass and structure (105-108). This is explained by an increase in bone resorption through enhanced activating transcription factor 4(ATF4)/RANKL secretion by osteoblasts (109), in combination with decreased

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osteoblastogenesis (105). In line with this, a recent randomized clinical trial demonstrated that patients treated with β-adrenoreceptor-selective blockers had better bone microarchitecture than nonusers (110). It has been demonstrated that SNS activity is important for regulation of pregnancy and lactation (111,112), but to our knowledge, the direct contribution of the SNS to PLO has never been investigated or reported.

During pregnancy (113), the increase in neural activity is likely to manifest as an increase in the release of neurotransmitters such as norepinephrine (NE) and neuropeptide Y (114,115), resulting in an early increase in blood pressure within the first few weeks of conception (116,117). Since both neurotransmitters are linked to bone metabolism (118-120) we speculate that they could be involved in PLO, although this has not been studied to date. *During lactation*, levels of both NE and serotonin (5-hydroxytryptamine, 5HT) in the anterior cerebral cortex, hippocampus, and cerebellum have been shown to increase, particularly from late pregnancy to early postpartum period, and continue to increase in the postpartum period (121). This implicates NE and serotonin in the regulation of hormone secretion and bone metabolism changes during these periods. The increased SNS activity during lactation is magnified during post-partum depression, which occurs in 25-35% in pregnant women after delivery (122). Interestingly, a connection between depression, bone loss and SNS activity increase has been demonstrated (123). Moreover, modulation of the SNS could also control other tissues such as adipose tissue (124) which is well known to be associated with bone remodeling levels (125,126). However, as stated, the role of SNS in PLO remains highly speculative.

D. Bone marrow fat

Bone marrow adipocytes appear in the bone marrow directly after birth and continue to expand throughout the skeleton during growth. In humans, at the age of 25 years, most of the marrow of the long bones is occupied by adipocytes, whereas in the axial skeleton the marrow remains mostly hematopoietic. However, bone marrow adipocytes accumulate with aging, and there is a clear inverse association between bone marrow adiposity and bone mass, which is accentuated in diseases characterized by increased fracture risk such as osteoporosis. This is hypothesised to be due to a shift in the lineage allocation of the skeletal stem cell, favoring adipogenesis over osteoblastogenesis. However, situations of 'beneficial' increased bone marrow adiposity have also been described, such as hematopoietic regeneration following radiation or chemotherapy. In this situation bone marrow adipocytes are hypothesised to be a source of energy by supplying fatty acids to hematopoietic and bone cells (127).

Studies of bone marrow adiposity during pregnancy and lactation have not been performed in humans. In rats, bone marrow adipocytes, as assessed by histomorphometry of the vertebrae, decrease both in number and volume during and after pregnancy, in lactating and non-lactating mothers, compared to non-pregnant controls (128). The changes in bone marrow adipocytes were negatively correlated with changes in bone formation parameters, although the decrease in bone marrow adiposity was accompanied by a decrease in bone volume. In mice, bone marrow adiposity, as assessed by osmium staining of the tibia, also decreases significantly during lactation and this was accompanied by a decrease in trabecular bone volume (129). These two studies show that bone marrow adiposity decreases during pregnancy and lactation, and this is accompanied by a decrease in bone volume. A possible hypothesis is that the bone marrow adipocytes undergo lipolysis to provide fatty acids as an energy source for the increased bone resorption due to the increased calcium demands both for fetal skeletal development and for maternal milk production.

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Interestingly, the disappearance of adipocytes during lactation has also been described in the mammary gland of mice (130). Mammary adipocytes were shown to undergo de-differentiation during lactation and re-differentiation during weaning. Whether bone marrow adipocytes could also undergo this de- and re-differentiation remains to be investigated.

E. PLO profile and treatment

PLO is rare, and its prevalence is unknown. The occurrence of fragility fractures appears mostly in the third trimester and lactation period (131), typically presenting in the first pregnancy with acute back pain from (multiple) vertebral fragility fractures and subsequently determined low BMD. Since premenopausal women rarely undergo DXA scanning, the pre-pregnancy BMD in these women is mostly unknown. Bone loss, predominantly from trabecular bone sites, in combination with the increased weight bearing and the lordotic posture of pregnancy, is assumed to lead to these spontaneous vertebral fractures. Decreased BMD prior to pregnancy in such premenopausal women is mostly caused by osteoporosis secondary to anorexia nervosa, endocrine diseases (e.g. Cushing's disease, hyperthyroidism or primary hyperparathyroidism), inflammatory diseases (e.g. rheumatoid arthritis or inflammatory bowel diseases) or medication (e.g. glucocorticoids or cancer treatments) (15).

Vertebral fractures presenting with back pain should be treated with appropriate pain medication and advice on weight bearing and mobility. Quality of life and capacity to work should also be taken into account, although long-term prognosis for PLO is good (132). In general, pharmacological treatment for low bone mass is not indicated, except calcium and vitamin D supplements, since bone mass recovers fully in the year following weaning and the onset of the menstrual cycle (3). Several studies have shown that parity and lactation are not associated with

increased long-term risk of postmenopausal osteoporosis and fractures, and may even protect against hip fractures (133,134). Moreover, PLO typically presents in first pregnancy, with limited evidence for increased fracture risk in future pregnancies (17,135). In contrast, Kyvernitakis et al have shown that the refracture risk in subsequent pregnancies is 20%, and the refracture rate after 6 years is 25%, with risk positively related to the number of fractures during the pregnancy (136). Therefore, it remains important to counsel women with PLO on the fracture risk associated with lactation and further pregnancies. Treatment with anti-osteoporosis medication such as the anti-resorptive bisphosphonates, the anabolic PTH anaologue teriparatide, and the human monoclonal RANKL antibody denosumab has been shown to be effective in increasing BMD, although there are no data on fracture outcomes and the reported studies did not include a placebo-treated control (17). In addition, the possible fracture reduction following treatment with bisphosphonates that are retained in the bone (137) should be weighed against a possible negative effect on skeletal development of the fetus in following pregnancies and other pregnancy related complications such as low birth weight. Although the limited available evidence in humans has not indicated that bisphosphonate treatment causes major congenital anomalies, the number of patients exposed is not sufficient to draw any conclusions on the safety profile of these medications (138). Moreover, use of teriparatide and denosumab are contraindicated during pregnancy and lactation and should therefore be used with caution in patient groups of fertile age.

F. Fracture healing during pregnancy

Although severe fractures are rather rare during pregnancy, several clinical case reports have described fracture healing during pregnancy (139-142). These studies suggest that fracture healing is accelerated during pregnancy, based on earlier bone bridging of the fracture callus and reduced time until bony union. This appears to be independent from the stage of pregnancy at which the

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fracture occurred. Accelerated bone regeneration may be due to the positive impact of several pregnancy-associated growth factors (such as pregnancy-associated plasma protein-A and placental growth factor) on fracture healing, which was demonstrated in experimental studies (143,144). Increased estrogen levels may also account for improved bone regeneration, as estrogen application was shown to accelerate fracture healing (145). In line with this, several experimental studies in rodent models of estrogen-deficiency demonstrated delayed fracture healing. Ovariectomised (OVX), estrogen-deficient rats and mice displayed decreased mechanical properties and bone formation in the late fracture callus (146-148). The number of osteoclasts was significantly increased during callus development (149). This might be explained by the effects of estrogen in increasing osteoclast and decreasing osteoblast apoptosis (150-152), and by stimulating the recruitment, proliferation and differentiation of skeletal progenitor cells (153,154). Analysis of the intermediate phase of endochondral fracture healing in OVX mice further demonstrated a decreased cartilaginous callus area (145) and a reduced expression of cartilage markers (155) and angiogenic factors (156). Therefore, bone regeneration appears to be disturbed by estrogen-deficiency both during the middle and late phases of healing, with changes in angiogenesis and the formation of the cartilaginous and the bony callus. The molecular mechanisms underlying these findings remain unclear. However, as it is known that postmenopausal females display a chronic low-grade inflammatory phenotype and the inflammatory response to injury is altered under estrogen-deficiency (157-159), estrogen may also modulate the inflammatory response after fracture. Indeed, recent studies demonstrated an imbalanced immune response to fracture in OVX mice and postmenopausal fracture patients (160,161). In particular, the molecule midkine (Mdk) appears to play an important role in this process. Mdk is known to be both a proinflammatory cytokine regulated by estrogen (162) and a negative regulator of bone formation (163) and fracture

healing (164). Indeed, antibody inhibition of Mdk reduced the negative effects of serum from postmenopausal fracture patients on the osteogenic differentiation of human MSCs. Overall, accelerated fractured healing during pregnancy might be due to the positive effects of increased estrogen levels on bony and cartilaginous callus formation as well as on the inflammatory response after fracture.

Conclusion

Reproduction brings about changes in the endocrine-mediated processes of calcium, mineral and bone metabolism. This applies to pregnancy in humans and other mammals, as there is a need for building material for the fetal skeleton. Moreover, physical changes occuring in pregnant and lactating women such as excessive weight on the lumbar spine region cause physiological and pathophysiological changes which are not yet fully elucidated. We have discussed the possible contribution of oxytocin, genetics, bone marrow fat and the SNS to pathophysiological changes such as PLO. Finally, fracture healing appears to be improved during pregnancy, and this is possibly due to the positive effects of estrogen on bone regeneration.

Glossary of abbreviations

ATF4: Activating Transcription Factor 4

COL1A1: COLlagen Alpha1

DIO2: type 2 iodothyronine deiodinase

FGF23: fibroblast Growth Factor 23

GnRH: Gonadotrophin-Releasing Hormone

5HT: 5-HydroxyTryptamine or serotonin

LRP5: Low-density Llipoprotein Receptor-related Protein 5

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Mdk: Midkine, also known as neurite growth-promoting factor 2 (NEGF2)

MTHFR: Methylene TetraHydroFolate Reductase

NE: norepinephrine, also called noradrenaline

OPG: Osteoprotegerin, also known as osteoclastogenesis inhibitory factor

OT: OxyTocin

PRL: Prolactin, also known as luteotropin

PTH: ParaThyroid Hormone

PTHrP: ParaThyroid Hormone-related Protein

RANK: Receptor Activator of Nuclear factor κ B

RANKL: RANK-Ligand

TRAP: Tartrate-Resistant Acid Phosphatase

TSH: Thyroid Stimulating Hormone, also known as thyrotropin

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Figure legend

Figure 1. Proposed model for the control of osteoclastogenesis and resorptive function of osteoclast (OCs) during adaptations of the skeleton to the demanding situations of pregnancy and lactation. Parathyroid hormone-related protein (**PTHrP**), synthesized in the placenta, breast tissue, parathyroid glands and the uterus, is the main actor of increased bone resorption. The increased production of oxytocin (**OT**) from the posterior pituitary stimulates osteoclastogenesis by acting on the osteoclast precursor (preOCs). The over-activity of the sympathetic nervous system (**SNS**), which increases release of neurotransmitters such as norepinephrine, stimulates bone resorption through an enhanced RANKL secretion by osteoblasts (OBs). In the bone marrow adipose tissue

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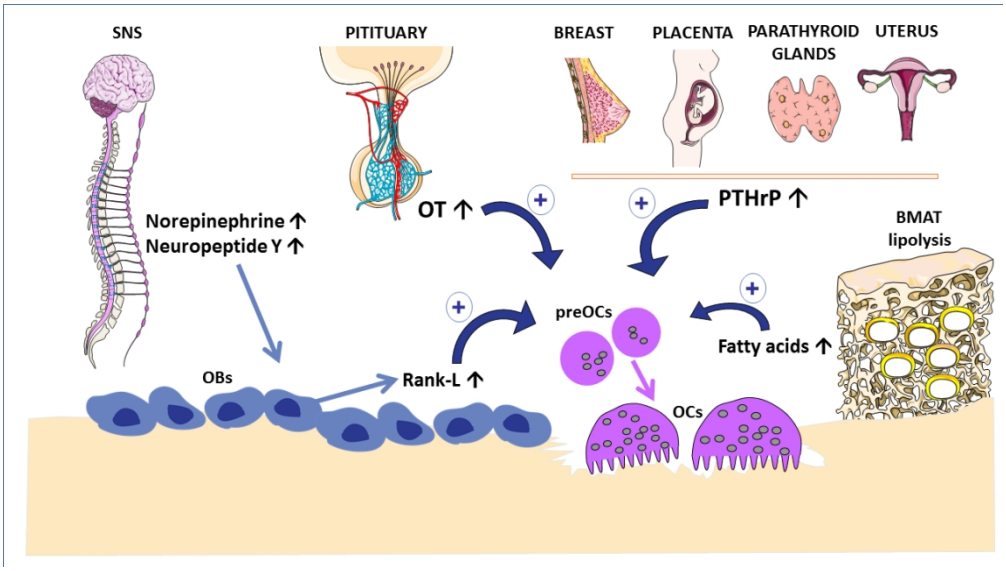
(**BMAT**), adipocytes undergo lipolysis to provide fatty acids as an energy source for the increased bone resorption.



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338x190mm (96 x 96 DPI)