# Pregnancy, Lactation, and Weaning Induce a Physiological Redistribution of Bone Mass at Multiple Skeletal Sites with Minimal Impact on Bone Quality

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## Introduction

In addition to its mechanical role, the skeleton also plays an important role in calcium homeostasis. As a result, the increased calcium requirements during reproduction induce substantial changes in maternal bone structure. In rodents, pregnancy and lactation result in significant bone loss, which recovers partially, but not fully, after weaning.[1,2] However, clinical studies have suggested no negative effect of lactation or parity on future risk of osteoporosis or fracture.[3,4] These conflicting results, combined with recent findings that the extent of lactation-induced bone loss varies depending on the skeletal site, led to our hypothesis that the skeletal changes undergone during reproduction represent a physiological redistribution of bone mass with minimal impact on bone quality.[1] The objective of this study was to investigate patterns of reproductive bone loss and recovery and their effects on mechanical integrity of the bone at multiple skeletal sites.

## Methods

*Longitudinal Study:* 4-month-old rats (n = 5) underwent 3 cycles of pregnancy, lactation, and weaning; 6 age-matched, virgin rats were used as controls. Each reproductive cycle consisted of a 3-week pregnancy, 3-week lactation, and 3-6 week post-weaning recovery. Using μCT, the right proximal tibia of each rat was scanned weekly at 10.5μm (vivaCT 40, Scanco Medical) during the first cycle, and every 3 weeks thereafter. The trabecular microstructure in the secondary spongiosa was quantified for each scan, and μCT-based finite element analysis (FEA) was performed to estimate whole-bone stiffness of the rat tibia at the end of recovery after three reproductive cycles and for age-matched, virgin controls.

*Cross-sectional Study:* Rats (aged 6 month at sacrifice) underwent 1 reproductive cycle, and were euthanized at parturition ("Pregnancy", n = 6), after 2 weeks of lactation ("Lactation", n =5), and 2 weeks post-weaning ("Weaning", n= 5). Virgin controls (n = 6) were sacrificed at age 6 months. Each rat was injected with calcein prior to sacrifice for dynamic histomorphometry. At sacrifice, blood was collected for analysis of serum levels of TRAP. Tibiae were harvested, embedded in MMA, and sectioned for histology. L4 vertebrae and femurs were harvested and μCT scanned at 10.5 μm resolution to allow for analysis of trabecular and cortical bone, respectively. Femurs were loaded to failure in 3-point bending to assess stiffness and peak load. Then, the stiffness, μCT-derived moment of inertia, and bone area were used to estimate Young's modulus. [5]

*Statistics:* Repeated-measures ANOVA, adjusted for baseline values, and 1-way ANOVA were used for longitudinal and cross-sectional comparisons, respectively. Bonferroni corrections were applied to all *post hoc* tests. Significant differences were considered when $p < 0.05$.

## Results

During each reproductive cycle, trabecular bone loss at the tibia induced by pregnancy and lactation was partially recovered after weaning. This negative net change accumulated over multiple cycles, resulting in a dramatically altered trabecular microstructure, which persisted even 5 months after the end of the last reproductive cycle (Fig 1). While trabecular bone volume fraction (BV/TV) remained 52% lower for reproductive rats than for controls, there was no difference between groups in cortical bone area. Since cortical bone bears much of the load at the tibia, this resulted in no significant difference between the 2 groups in whole-bone stiffness. Dynamic histology indicated that bone formation rate and mineralizing surface were 581% and 337% greater in the proximal tibiae of rats in the Weaning group as compared to controls, and serum levels of bone resorption marker TRAP were 56% lower at 2 weeks post-weaning than during lactation (Fig 2). Cortical structure of the femur midshaft had deteriorated as early as the end of pregnancy, as illustrated by the 10-12% lower cortical area (Ct.Area) in the Pregnancy, Lactation, and Weaning groups, compared to controls (Fig 3A). However, reproduction had a beneficial effect on bone's material properties, as evidenced by the 32-

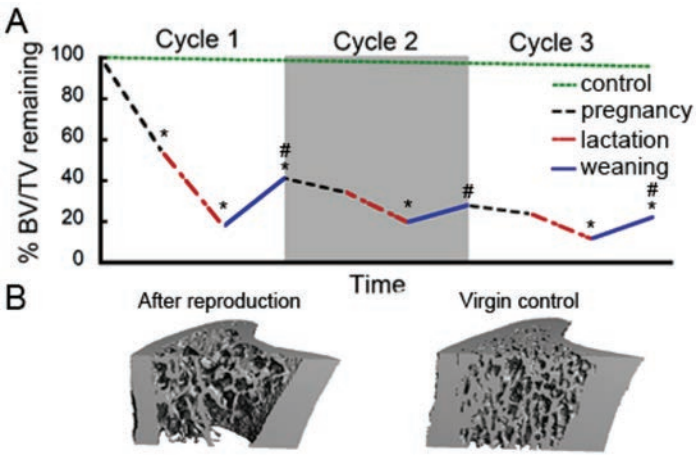


**Figure 1.** (A) A profile plot indicating changes in the percentage of the baseline trabecular BV/TV that remains at the proximal tibia at ea·ch reproductive stage,and (B) representative renderings of the proximal tibia of reproductive and virgin control rats, made 5 months after the end of the third reproductive cycle. *: different from measurement made 3 weeks prior ($p < 0.05$). #: different from pre-pregnancy measurement of the same cycle (< 0.05).

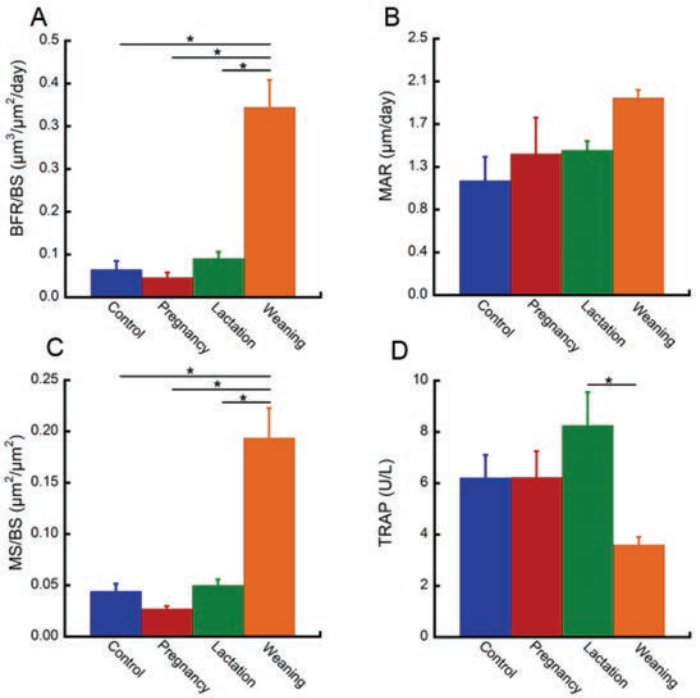


**Figure 2.** Effects of reproduction on bone remodeling parameters, including (A) BFR/BS, (B) MAR, (C) MS/BS, and (D) serum TRAP.

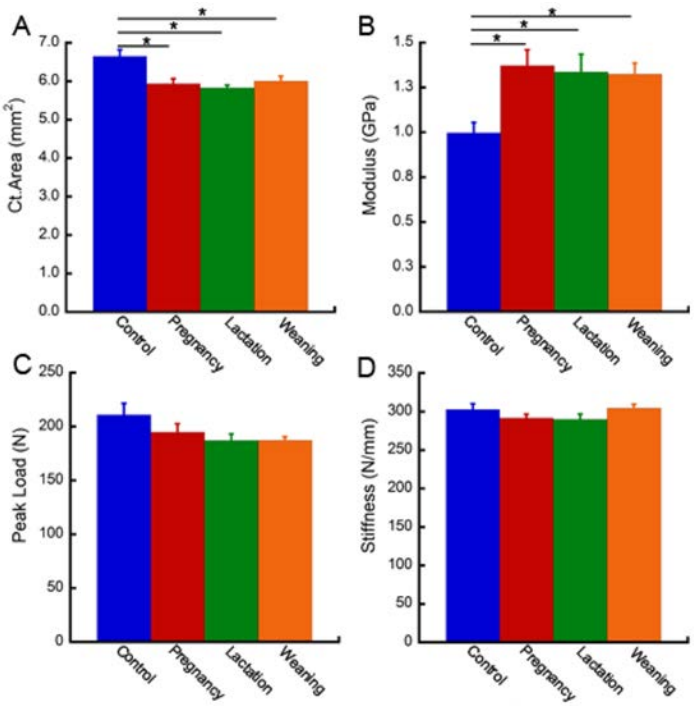


**Figure 3.** Effects of reproduction on (A) Ct. Area, (B) Young's modulus, (C) Peak load, and (D) Stiffness at the femur midshaft. *: $p < 0.05$.

38% greater Young's modulus in rats from the Pregnancy, Lactation, and Weaning groups, compared to controls (Fig 3B). Additionally, reproduction had no effect on femoral stiffness or peak load (Fig 3). A comparison of the effects of reproduction at two different trabecular sites (the L4 vertebra and proximal tibia) indicated that although both sites underwent decreases in BV/TV as a result of pregnancy and lactation, the structural adaptations of L4 were dramatically different from those of the tibia (Fig 4). BV/TV recovered more fully at L4, and there were minimal changes in trabecular number (Tb.N) and connectivity density (Conn.D) at L4 as compared to the tibia (Fig 4), while both sites underwent similar, reversible changes in trabecular thickness (Tb.Th).

## Discussion

The combined effects of pregnancy and lactation resulted in dramatic changes in bone structure, which persisted long after weaning. Similar to previous studies, these changes and their duration varied based on the skeletal site that was assessed.[1] This study also investigated the effects of reproduction on mechanical competence of the bone: FEA of the tibia resulted in a measurement of structural stiffness, whereas 3-point bending of the femur allowed measurement of the mechanical consequences of both structural and material properties of the bone. The effects of reproduction on the mechanical properties of the spine are currently being investigated. Results demonstrate that the mechanical integrity of the load-bearing regions of bone, such as the vertebral trabecular bone or the cortical bone in the femur midshaft, appeared to be preserved either through maintenance of structural integrity (as in the vertebrae) or improved material properties (as in the femur). On the other hand, trabecular bone microarchitecture in the proximal tibia plays a less crucial role in mechanical function, as the cortex of the long bones bears the majority of the bone's load. This may explain why reproduction caused greater calcium release from tibial trabecular bone, resulting in dramatic and irreversible deterioration. These results indicate that reproduction induces a shift in bone structure, which persists long after weaning.The lack of change in tibial

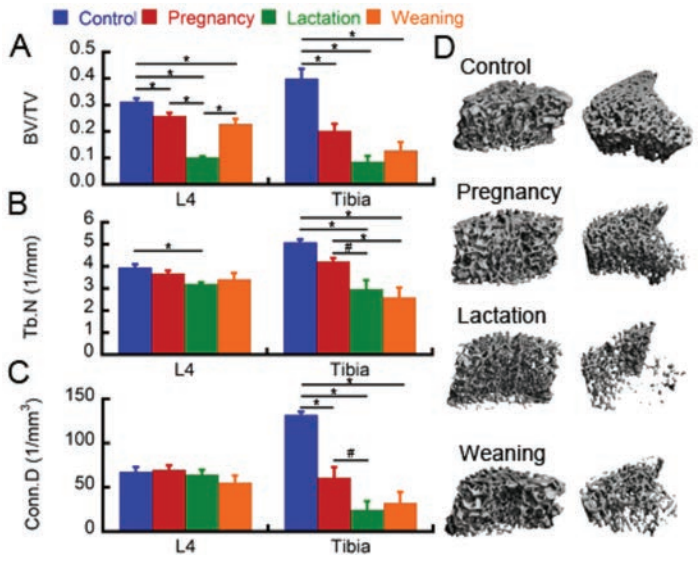


**Figure 4.** Effects of reproduction on (A) BV/TV (B) Tb.N, and (C) Conn.D at the L4 vertebra and proximal tibia. (D) Representative 3D renderings of the trabecular compartment of L4 (left) and the tibia (right) are shown at each time point. *: $< 0.05$, #: $p < 0.1$.

whole-bone stiffness and femoral peak load and stiffness, combined with previous clinical studies which showed that reproduction has no adverse effect on osteoporosis risk, suggest that reproduction does not diminish the mechanical integrity of bone.[3,4] Instead, it appears that the body responds to increased calcium demands during pregnancy and lactation by selectively degrading bone structure, resulting in an alternate skeletal composition where bone structure may be optimized at the more load-bearing regions to allow maintenance of similar quality to non-reproductive bone.

## Significance

Although reproduction constitutes a physiological process that has minimal impact on bone quality, dramatic, irreversible changes in bone structure and composition do occur. By characterizing these changes and assessing the mechanical integrity of the post-reproductive skeleton, this study establishes reproductive bone as a new model of an altered skeletal status where bone quality remains intact.

## Acknowledgements

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