

# Determining the effects of Reproductive Performance on Bone Quality in Female House Mice

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## Abstract

Much of calcium and phosphorus used to support skeletal ossification during prenatal and postnatal mammalian development are derived from the mobilization of maternal bone minerals. As a result, bone loss has been observed in the maternal skeleton during the reproductive cycle. In order to maintain their skeletal integrity, ensure survival, and continue future reproduction, mothers must be able to prevent excessive bone loss. The goal of our research was to evaluate how the number of reproductive bouts and the total number of young produced impacts bone quality in female mice. We evaluated this relationship by quantifying variation in femur morphology and mineral composition of 14 age-matched mice that had the opportunity to breed from reproductive maturity at 2 months until they were 10 months of age. To evaluate femur morphology, we sent one set of femurs to the University of Alabama at Birmingham to quantify the 3D structure of the bones. We quantified the total mineral and calcium content of the bone by inductively coupled plasma spectrophotometry. No significant relationship was established between bone morphology and reproductive performance or between bone mass and mineral content for females that had between zero to seven litters and that vary between weaning 0-44 offspring. These results suggest that female house mice are adept at maintaining bone mineral content and mass throughout their reproductive cycle.

## Introduction

To reproduce successfully, female mammals must be able to provide the necessary nutrients for their offspring's physiological development. Developing vertebrae in offspring requires calcium and phosphorus for successful growth to occur, and most mammalian offspring are dependent on their mother for these mineral resources until they reach weaning (Hood & Hobe-sack, 2015). Evidence from previous studies suggests that increased intestinal calcium absorption during pregnancy provides much of the calcium required by offspring during reproductive development, but that maternal bone loss may still occur in the last months of pregnancy, when rapid mineralization of the

offspring's skeleton occurs (Kalkwarf & Specker, 2002).

It is important to evaluate the tradeoffs associated with reproduction and the mineralization of offspring skeleton to determine the extent to which allocation of nutrients to offspring affects mammalian mothers.

Increasing demand for skeletal mobilization leads to increased bone turnover and reduction in bone mineral and strength. Previous studies suggest that bone resorption is more accelerated during the second half of pregnancy, corresponding with an increased fetal demand for mineral during this time (Avendaño-Badillo et al., 2009). This can lead to substantial bone loss during reproduction. In one study, rats lost 28% of their bone mineral content on a 0.4% calcium diet, and up to 53% of bone mineral on a 0.1% calcium diet (Peng et al., 1988). This study concluded that bone loss during reproduction in these rats was not only accentuated by a calcium-deficient diet, but also aggravated by an increase in suckling pups during lactation (Peng et al., 1988).

Bone loss as a cost of reproduction can lead to decreased skeletal integrity in the maternal mammal and negatively affect future reproductive success. It has been shown that reduction in bone mineral density is correlated with reduction in bone strength (Reilly & Burstein, 1975; Marshall et al., 1996). This means that bone loss as a cost of reproduction corresponds with increased risk of fracture. Bone fracture in wild animals can lead to an inability to acquire food and an increased susceptibility to predators, decreasing their chances of survival. In mammalian females, bone fracture leads to a decreased chance of future successful reproductive bouts. Therefore, availability of maternal skeletal mineral can be viewed as a limit for successful reproduction. Additionally, reproductive bone loss can be viewed as a cost of reproduction because there is a trade-off between maternal skeletal integrity and allocation of mineral to offspring.

For this study, we analyzed bone loss during reproduction in house mice (*Mus musculus*). Due to their

proportionally smaller skeletons (Prange et al., 1979), it has been suggested that less maternal skeletal calcium is available for house mice than for larger animals. This likely corresponds to an increased limitation on bone mineral mobilization (Hood, 2012). For this reason, we expect that the mice will experience statistically significant bone loss during their reproductive cycle. In essence, we predict that increased reproductive output in house mice leads to a corresponding decrease in bone quality.

### Materials and Methods

We obtained the femurs and vertebrae of 14 age-matched mice postmortem that had the opportunity to breed from reproductive maturity at 2 months until they were 10 months of age. All mice were maintained under semi-natural conditions, and thus their skeletons were assumed to have experienced a level of strain comparable to wild mice.

The right femurs were manually cleaned of residual connective tissue after being soaked in an ultrasonic bath for 30 minutes to loosen the tissue. A Soxhlet apparatus was used to extract neutral lipids with petroleum ether and ethanol until the samples reached a constant mass (12 h). The femurs were then ashed in a muffle furnace (Fisher Scientific Isotemp Muffle Furnace, Dubuque, IA, USA) at 550°C for 24 h. The ash content, or total mineral content, of the samples was determined using the change in mass after ashing. The samples were then digested in nitric acid for one hour and diluted in nanopure water for mineral analysis. All concentrations were determined by mass. The calcium and phosphorus content of the samples were determined via inductively coupled plasma optical emission spectrophotometry (Perkin Elmer Optima 7300DV; Waltham, MA, USA) using the following wavelengths: Ca<sup>2+</sup> - 317.933 nm and P - 213.617 nm.

The left femurs were sent to the University of Alabama at Birmingham (UAB). There, the 3D structure of the bones was quantified, including cortical thickness, trabeculae thickness, and trabeculae density. UAB used microcomputed tomography to obtain these values. We then used the obtained values in regression analysis to determine if any significant relationships could be established.

### Results

There were no significant relationships between bone mass and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 1). No significant relationships were established between total mineral content and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 1). No significant relationships could be established between percent calcium and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 1). Additionally, there were no significant relationships between percent phosphorus and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 1).

We then tested our variables against Ca/P ratios to see if there was any evidence of a significant relationship. However, we could not show any significant relationships between Ca/P ratio in bone and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 1). Overall, we found no evidence of significant relationships between bone mass and parity or mineral content of bone for females that had between zero to seven litters, and that vary between weaning zero to 44 offspring.

No significant relationships were established between femur trabecular number and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 2). There was no significant relationship between femur trabecular spacing and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 2). There was no evidence of significant relationships between vertebral trabecular number and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 2). Further, there were no significant relationships established between vertebral trabecular spacing and total number of litters per female aged up to 1 year, average litter size at weaning, or total number of pups throughout life (Table 2). Note that despite the presence of visible variability in the morphological structure of the bones in Figure 1,

**Table 1.** Comparison of Maternal Characteristics and femoral bone morphology. Results of linear regression are given.

Maternal Characteristic	Bone Characteristic	F*	df*	P*	Interpretation
Total number of litters	Bone mass	0.26	12	0.6184	Not significant
Average litter size at weaning	Bone mass	0.43	12	0.5266	Not significant
Total number of pups throughout life	Bone mass	0.77	12	0.3994	Not significant
Total number of litters	Total mineral content	0.27	12	0.6139	Not significant
Average litter size at weaning	Total mineral content	0.42	12	0.5369	Not significant
Total number of pups throughout life	Total mineral content	0.74	12	0.4066	Not significant
Total number of litters	Ca %	0.03	12	0.8668	Not significant
Average litter size at weaning	Ca %	1.86	12	0.2000	Not significant
Total number of pups throughout life	Ca %	0.33	12	0.5780	Not significant
Total number of litters	P %	0.26	12	0.6182	Not significant
Average litter size at weaning	P %	0.43	12	0.5269	Not significant
Total number of pups throughout life	P %	0.77	12	0.3994	Not significant
Total number of litters	Ca / P ratio in bone	0.00	12	0.9917	Not significant
Average litter size at weaning	Ca / P ratio in bone	0.10	12	0.7602	Not significant
Total number of pups throughout life	Ca / P ratio in bone	0.02	12	0.8886	Not significant

\* F= F value, df= degrees of freedom, P= P value

regression analysis using vertebral trabecular number and spacing, and femur trabecular number and spacing determined that this morphological variability was not statistically significant. Overall, no significant relationships were established between bone morphology or mineral content of bone for females that had between zero to seven litters, and that vary between weaning zero to 44 offspring.

### Discussion

We predicted that increased reproductive output would correspond to a decrease in bone mineral. Results of this experiment failed to support this prediction. There was no significant difference between the mineral content and bone morphology of mothers with zero to 44 offspring and zero to 7 litters. These results suggest that female house mice are adept at maintaining bone mineral content and mass throughout their reproductive cycle.

These results contradict our hypothesis that a small mammal with a much smaller skeleton would experience bone loss with an increase in reproductive output if bone mobilization occurred. In a previous study on

female white-footed mice (*Peromyscus leucopus*), there was an observed net bone loss that indicated female white-footed mice trade off skeletal condition for reproduction (Schmidt & Hood, 2014). A separate study on female mice suggested that when faced with increasing litter sizes, the amount of mineral mobilized from the maternal reserves increased correspondingly (Hood, 2012). The results of these studies suggest bone loss on a significant scale during reproduction. However, it is possible that the laboratory conditions of the aforementioned studies affected the experimental results if diminished strain on the skeleton limited the need to maintain bone density.

In a study conducted by Hood & Hobensack (2015), it was shown that when female mice were given the opportunity to climb, and thus experience greater strain on their skeleton, they did not experience a drop in bone mass or mineral content during reproduction. Despite some predicted recovery after weaning, we anticipated a cumulative loss of bone in the mice in our study because they endured more reproductive cycles than those in Hood and Hobensack's study (1 bout per females). Our study did not yield results that support

**Table 2.** Comparison of maternal characteristics and femoral bone morphology. Results of Linear regression are given.

Maternal Characteristic	Bone Characteristic	F*	df*	P*	Interpretation
Total number of litters	Femur trabecular number	0.56	9	0.4770	Not significant
Average litter size at weaning	Femur trabecular number	0.43	9	0.5266	Not significant
Total number of pups throughout life	Femur trabecular number	0.91	9	0.3684	Not significant
Total number of litters	Femur trabecular spacing	0.51	9	0.4947	Not significant
Average litter size at weaning	Femur trabecular spacing	0.89	9	0.3738	Not significant
Total number of pups throughout life	Femur trabecular spacing	0.83	9	0.3899	Not significant
Total number of litters	Vertebral trabecular number	0.95	9	0.3581	Not significant
Average litter size at weaning	Vertebral trabecular number	0.72	9	0.4196	Not significant
Total number of pups throughout life	Vertebral trabecular number	2.03	9	0.1921	Not significant
Total number of litters	Vertebral trabecular spacing	0.98	9	0.3505	Not significant
Average litter size at weaning	Vertebral trabecular spacing	0.95	9	0.3574	Not significant
Total number of pups throughout life	Vertebral trabecular spacing	2.29	9	0.1687	Not significant

\* F= F value, df= degrees of freedom, P= P value

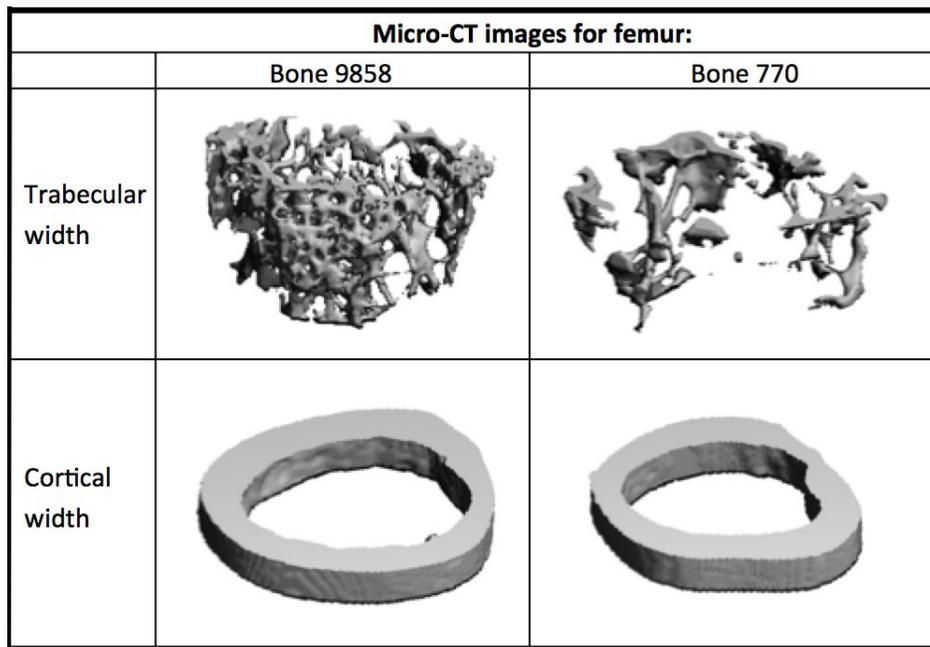
this hypothesis. This is likely because under natural conditions, mice experience sufficient strain to stimulate full compensation for the bone loss experienced during the reproduction. Because the mice in this study were kept in semi-natural conditions, the stress and strain experienced by the animals would have been greater than mice kept in a small rodent box. Therefore, the results of this study are predicted to represent the responses of mice in the wild.

Overall, our results suggest that wild-derived house mice in semi-natural conditions experience no loss of female skeleton integrity after reproduction, suggesting the skeleton experiences no cost of reproduction. It is important to note that although no significant relationships were established in this study indicating bone loss throughout the reproductive cycle, visible variability in bone morphology did occur (Figure 1). If bone loss does occur, skeletal maintenance may be compensating for the increase in bone mobilization during reproduction. If these mice can maintain their skeletal integrity during reproduction or quickly recover their bone quality postpartum, they decrease

the risk of bone fracture and susceptibility to predators. This further increases their chances of future reproductive success. By studying wild-derived animals, our results have broader application than experiments conducted under laboratory settings. Future studies should evaluate whether focus on skeletal self-maintenance of the maternal mice had impacts on offspring bone quality. Future studies should also evaluate which variables affect reproductive bone loss and to what extent. These variables may include exercise, dietary mineral intake, and presence of disease. Replications of this experiment should emphasize utilization of a larger sample size.

### Acknowledgments

I would like to thank Maria Johnson in the Animal Phenotyping lab at University of Alabama at Birmingham for completing the microcomputed tomography measurements, Dr. Paul Cobine for his assistance with the ICP spectrophotometer, and Auburn University for funding this project through my Undergraduate Research Fellowship.



**Figure 1.** Microcomputed tomography imaging of trabecular and cortical femoral bone.

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