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A Test of Bone Mobilization Relative to Reproductive Demand: Skeletal Quality Is Improved in Cannibalistic Females with Large Litters

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ABSTRACT

In species with repeated bouts of reproduction, a female’s ability to retain sufficient tissue for self-maintenance is essential to her survival and capacity for future reproduction. Loss of bone mineral content results in bone fragility and the possibility of reduced survival, so females should guard against the overuse of their bone mineral during reproduction. Given these constraints, I predicted that bone mobilization would increase with litter size in mice but plateau before maximum litter size was reached. To test this idea, I manipulated the litter sizes of house mice on the day of parturition to 3, 8, 13, and 18 offspring. At weaning, I euthanized the females and calculated whole-body and bone mineral composition. The total mineral content of females’ femurs dropped as litter size increased to the average litter size for this strain of mouse (13) but surprisingly, femoral mineral content was higher for females assigned the largest litter sizes (18). Seven of the nine females assigned 18 young cannibalized some of their offspring. For females assigned to these larger litters, femoral ash content was not correlated with number of young consumed, suggesting that mineral recycling had little effect on final bone mineral content. However, nursing effort (accounting for young lost to cannibalism) was correlated with maternal femoral ash at weaning. These finding suggest that the high bone mineral content of females assigned the largest litters was associated with a reduction in endogenous mineral allocated to the litter.

Introduction

In iteroparous species, the capacity for future reproduction is dependent on an individual’s ability to retain adequate body condition during previous bouts of reproduction (Clutton-Brock 1991; Stearns 1992). Mammalian females are burdened with supplying the building blocks required for offspring development both in utero and during postnatal dependency. To meet this demand, females adopt one or a combination of strategies, including increasing food intake and mobilizing somatic tissues from their own bodies (Drent and Daan 1980; Speakman 2008). Use of somatic tissue can protect females and their young from unpredictable or predictably poor food availability during pregnancy and lactation (Drent and Daan 1980), but reliance on stored nutrients may affect a female’s residual reproductive value by influencing her probability of survival, the residual amount of stores she has available for the next reproductive bout, and the time required to build new tissue to support subsequent reproductive efforts (Clutton-Brock 1984).

If the residual reproductive value of a female is greater than zero, the allocation of somatic resources to offspring should be governed by priority rules that prevent excessive use of somatic tissue (Zera and Harshman 2001). Numerous studies have shown that mammalian females will forgo reproduction or reduce allocation when they are in poor condition and when they are still developing themselves. For example, Ono et al. (1987) showed that California sea lions transferred less milk to their young when they were in poor condition during an El Niño event, and Landete-Castillejos et al. (2004) showed that primiparous female Iberian red deer prioritize self-maintenance during reproduction while they are still growing by bearing more smaller daughters than larger sons. Negative relationships between litter size and nutrient allocation can also reflect processes that protect maternal condition. Rogowitz and McClure (1995) showed that maternal body mass in lactating cotton rats was comparable between females suckling three offspring and those suckling six. In these examples, priority rules manage and protect the catabolism of maternal tissues.

During uterine and postnatal development, females support the growth of their offspring’s skeletons by increasing intake of calcium and by mobilizing mineral from their bone (Brommage 1989). The skeletal mass of small mammals is low relative to that of larger species (Prange et al. 1979), and thus small species have relatively less bone mineral available for mobilization (Hood et al. 2006). Small mammals produce large volumes of milk relative to their size (Oftedal 1984), while the calcium and phosphorus content of their milk is comparable with that of larger species (Studier and Kunz 1995), suggesting that mineral transfer between females and offspring is relatively high. Yet despite a relatively small somatic resource, bone min-
eral loss during reproduction is proportionally higher in the few small mammals that have been examined relative to that in larger species (Wysolmerski 2002).

Several factors may contribute to the total amount of mineral mobilized from the maternal skeleton during reproduction, including offspring demand and mineral ingestion by the female. Loss of mineral from the femur and lumbar vertebrae is positively correlated with litter size in rats (Peng et al. 1988; Tojo et al. 1998). In addition, the total mass of food consumed by house mice, and presumably other rodents, can be limited (Nelson and Evans 1961; Speakman 2008). Specifically, mice suckling large litters are unable to consume more food to compensate for the high nutrient demand of their young (Hammond and Diamond 1994). If litter size crosses a threshold at which food consumption cannot equal the nutrient demand of offspring, then the added mineral demand from more young must come entirely from the female’s skeleton if the same level of allocation per offspring is to be maintained.

Allocation of minerals from the body of the female comes at a cost. There is a negative correlation between the mineral content of a bone and its fragility (Currey 1969, 1988; Boskey and Coleman 2010). Without an intrinsic rule limiting the amount of bone that can be mobilized during reproduction, females with large litters might allocate so much mineral from their own bones that they suffer catastrophic failure.

Lactation is associated with increased bone turnover and remodeling in long bones, such as the femur and tibia, and axial bones, such as the lumbar vertebrae, pelvis, and ribs (Miller et al. 1989; Kovacs and Kronenberg 1997; Vajda et al. 1999b, 2001). With high amounts of calcium partitioned to milk production, an increase in bone turnover leads to a reduction in bone mineral content (Bowman and Miller 2001). Cortical bone can display a reduction in cortical thickness and an increased number of resorptive spaces, and the mineral content of new osteons is reduced (Vajda et al. 1999b). Cancellous bone can display a decrease in number and thickness of trabecular struts (Shahtaheri et al. 1999). Increases in the porosity of cortical bone and reductions in the number and thickness of trabeculae have been shown to negatively affect bone strength (Davison et al. 2006), and as a result, lactating animals have been shown to display reductions in bone strength, stiffness, toughness, and ductility (Peng et al. 1988). There is little evidence of increased fracture risk during reproduction in women (Kovacs and Kronenberg 1997), lending support to the notion that there are physiological mechanisms in place to limit these risks. However, no previous studies have assessed intrinsic limits on the mobilization of bone.

The goal of this investigation is to characterize the pattern of bone mineral loss by females during reproduction relative to litter size, to determine whether there is an intrinsic limit to the amount of bone that females mobilize. Because of its small body size and large litters and a strong body of previous work on food intake and reproductive performance, the ICR laboratory mouse (Mus musculus, Hsd:ICR(CD-1)) was selected for this investigation. I predicted that with increasing litter size and thus increasing cumulative demand, the amount of bone that is mobilized from the maternal skeleton would increase. As a result, females with larger litters would be expected to have less residual bone and less bone strength at the end of the reproduction bout than females with smaller litters. However, I further predicted a physiological cutoff should exist beyond which females will stop mobilizing bone.

Because house mice are so well characterized, I could make explicit predictions. Litter sizes of 11–14 are typical for ICR mice, but litters of 18 and 19 are occasionally observed. The food-intake limit of outbred laboratory lactating mice is reached at 8–9 young, according to studies of MF1 and Swiss-Webster mice (Crl:MF1, Crl:CFW(SW); Hammond and Diamond 1994; Johnson et al. 2001). The experiment was designed to include two groups with litter sizes below those of the food-intake limit (nonreproductive females and females with three pups) and three groups of females at or above the litter size at which food intake can no longer be increased (8, 13, or 18 pups). One of these groups was at the average litter size for this strain of mouse (15 pups), and one was near maximum natural litter size for this strain (18 pups). The inclusion of the latter group was essential for examining the response of females to especially high demand. I focused on bone loss in the femur because the femur is a well-characterized location of bone mobilization during reproduction (Peng et al. 1988; Shahtaheri et al. 1999; Vajda et al. 1999a); in addition, I examined whole-body mineral content to determine whether the body as a whole mimicked these bones. I predicted that the amount of mineral in the specified bones and body would decrease as litter size increased from 0 to 8 pups but then be similar to that for 8-pup litters among other treatment groups. Following the conclusions of previous work, total body fat was also measured for comparison.

Because a reduction in the mineral content of bone in adult animals is usually related to increased risk of fracture (Currey 1988; Boskey and Coleman 2010); Landete-Castillejos et al. 2012, I predicted that the resistance of the femur to fracture (i.e., breaking load) would decrease with litter size up to eight pups and then plateau, the predicted mineral resorption limit.

Material and Methods

Animal Care

All live-animal methods described here were approved by Auburn University’s Institutional Animal Care and Use Committee (2008-1471). Fifty-five 8–9-wk-old ICR mice were obtained from Harlan Laboratories (Prattville, AL; 43 females, 11 males). Mice were maintained on a 14L : 10D cycle at 24° ± 1°C. Females were paired in 30 × 30-cm boxes maintained on a ventilated rack. All animals were fed Teklad rodent breeder diet 8626 ad lib. (Teklad Diets, Madison, WI; 3.5 kcal/g metabolizable energy, 21.3% protein, 10.5% fat, 3.2% crude fiber, 1.1% calcium, 0.98% phosphorus). Food intake of all animals was quantified weekly by recording the mass of food offered and residual mass of food 1 wk later. Weekly intake was converted to daily intake by dividing by 7. Females were randomly assigned to one of four treatment groups before breeding. The
of weaning in lab mice, all females and their litters were euthanized with carbon dioxide. This occurred approximately 3 days after food was removed from the box in an effort to reduce the incidence of cannibalism because it is not known whether the female killed the pup or whether the pup died of natural causes before the female consumed it. On day 21 postpartum, the typical day of weaning in lab mice, all females and their litters were euthanized with carbon dioxide.

To achieve the assigned litter sizes, pups were added or removed from each litter on the day of parturition. Each pup was assigned a number, and then a random-number generator was used to select pups for removal. Cross fostering was inevitable to achieve the target litter sizes. The effects of cross fostering on females was controlled for by including cross-fostered pups in all treatment groups. Following the manipulation, one-third of each litter was assigned to a different female. On three occasions, only one female gave birth within a 24-h period. The size of these litters was adjusted without cross fostering. Pups that were not assigned to a litter on the day of parturition were euthanized with carbon dioxide.

Six randomly selected pups from each litter (all pups for litter sizes of three) were marked for identification, retrieved briefly to measure body mass and tail length on days 1, 7, 14, and 21 postpartum, and analyzed for nutrient composition at weaning (hereafter "select pups"). The number of offspring in each litter was recorded daily to account for deaths and incidents of cannibalism. Cannibalism is common among captive and free-ranging mice (Labov et al. 1985), and thus this natural process was allowed to occur by leaving dead pups in the box for up to 24 h after they were first observed. Pup carcasses that showed signs of discoloration indicative of rotting were removed. The disappearance of pups is described as an incidence of cannibalism because it is not known whether the female killed the pup or whether the pup died of natural causes before the female consumed it. On day 21 postpartum, the typical day of weaning in lab mice, all females and their litters were euthanized with carbon dioxide. This occurred approximately 3 h after food was removed from the box in an effort to reduce the effect of intestinal fill on the final body mass of the animals.

After euthanasia, the animals were weighed, the carcasses were desanguinated, and dissections were completed within 30 min. The major internal organs and both femurs were removed from the adults shortly after euthanasia. The femurs were removed by pulling the skin from the lower half of the body, carefully bisecting the muscle and connective tissues surrounding the head of the femur, dislocating the head of the femur from the acetabulum, removing the bulk of the muscle tissue from the femur, and finally bisecting the ligaments surrounding the knee until the lower half of the limb could be freed from the femur. The pups were frozen immediately at −20°C and saved for later dissection. The six select pups from each litter were dissected and prepared for compositional analyses at a later date. Residual digesta in the stomach and intestine of adults and pups was cleared by manual expression at the time of dissection.

## Laboratory Analyses

All tissues, excluding the removed bones, were dried to a constant mass in a forced-convection oven (Binder drying oven FED 115-UL, Binder, Great River, NY) at 60°C. All dried tissues from the same individual were combined and homogenized. The homogenate was subsampled (0.5 g), and fat extractions were completed in duplicate. Neutral lipids were extracted with petroleum ether in a Soxhlet apparatus until samples reached constant mass (12 h). The total fat content of each replicate was determined on the basis of the difference in mass between the pre- and postextracted samples.

The right femur of each mouse was cleaned of residual muscle tissue after being soaked in nanopure water in an ultrasonic bath for 32 min. Femoral data for two individuals were based on the left femur because the right femur was damaged or lost. Fat was extracted from cleaned bones in the Soxhlet as described above. Although many investigators use a combination of polar and nonpolar solvents and break bones to remove fat from the marrow, only 1.05% ± 0.37% (n = 10) more fat was extracted with a 2:1 petroleum ether–acetone solution than with petroleum ether alone. There was no difference in the amount of fat extracted between bones that were broken and those that were left intact (one-tailed t-test: t = 0.45, P = 0.34). Thus, for consistency with other tissues, all extractions were completed with 100% petroleum ether while keeping the bones intact to avoid inadvertent loss of mineralized tissue. Any re-

<table>
<thead>
<tr>
<th>Assigned litter size</th>
<th>N</th>
<th>Litter size at birth</th>
<th>Litter size at weaning</th>
<th>Proportion of females cannibalistic</th>
<th>Mean number of pups cannibalized ± SEM (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9</td>
<td>13.1 ± .6</td>
<td>3.0</td>
<td>0/9</td>
<td>0</td>
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<tr>
<td>8</td>
<td>10</td>
<td>12.2 ± .6</td>
<td>8.0</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>13.0 ± .4</td>
<td>12.8 ± .3</td>
<td>1/8</td>
<td>.3 ± .3 (0–2)</td>
</tr>
<tr>
<td>18</td>
<td>9</td>
<td>11.8 ± .9</td>
<td>15.4 ± .7</td>
<td>7/9</td>
<td>2.0 ± .7 (0–6)</td>
</tr>
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</table>

*Accounts for cannibalized pups and pups that died but were not consumed.
sidual tissue remaining on the bone after ether extraction was removed before ashing.

All fat-extracted bone samples and whole-body homogenates (0.5 g) were ashed in a muffle furnace (Fisher Scientific Isotemp Muffle Furnace, Dubuque, IA) at 550°C, with the bones ashed for 24 h and whole-body samples ashed for 12 h. The percentage of ash in each sample was calculated as the ash content of the sample/fat-free dry mass of the sample before ashing. Ashed samples were digested in 70% trace metal-grade nitric acid with one of two methods because of an instrument malfunction. The adult femur samples were digested in a microwave digestion unit (Speedwave MWS-2, Berghof, Eningen, Germany). The microwave temperature was increased gradually to 200°C over 15 min, held at 200°C for 15 min, and then decreased to room temperature over the final 15 min. The remaining samples were also digested in 70% nitric acid but were heated on a dry-block heater at 100°C for 1 h. Finally, digests were diluted for final mineral analysis. All concentrations were determined by mass. The calcium concentration of the whole-body and bone samples was measured as an indication of the degree of bone calcification (Currey 2002), and the sodium content of the whole-body and bone samples was also measured. Sodium can also be stored in bone (Widdowson and Dickerson 1964), and thus we evaluated this element because it has been shown to be limiting in herbivorous species (Belovsky and Jordan 1981; Christian et al. 1993; Grasman and Hellgren 1993). Calcium and sodium concentrations were determined by inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima 7300DV, Waltham, MA; wavelengths: Ca²⁺ = 317.93 nm; Na⁺ = 589.52 nm). The mineral contents of whole-body samples run in duplicate (n = 5) were compared among digestion methods; the results of these analyses were statistically equivalent (two-way ANOVA, partial F_{method} = 0.000, P = 0.99). Nevertheless, within any given statistical test, the method of digestion was similar. All total-body-composition data were corrected for the composition of the removed femurs (2 × the mineral content of one femur).

Bone strength was measured for the left femurs. The strength of a bone is largely dependent on two effects: structural variables, such as cortical thickness and number of trabeculae, and mechanical quality of the material (Currey 2002; Davison et al. 2006). In small bones, such as those of mice, it is impossible to test intrinsic mechanical properties. Thus, as a composite measure of mechanical performance of the bone, a whole-femur bending test was employed (Turner and Burr 1993) using a Mini Bionix Mechanical Testing System (model 858, MTS Systems, Minneapolis, MN). All bones were wrapped in phosphate-buffered saline-soaked gauze for at least 3 d before testing the load required to break each bone (hereafter “breaking load”; Turner and Burr 1993). The gauze and overlying muscle tissue were removed before testing. Three-point bending tests were completed with a 100-N load cell. Each bone was centered on two points 9.5 mm apart (gauge length). Force was applied to the midshaft of the bone at 0.05 mm/s. Minimum force required to break the bone was recorded.

Calculations and Statistics

All statistical analyses were completed in SAS (ver. 9.1.3, SAS Institute, Cary, NC). Food intake was compared among treatment groups on day 8 of lactation with ANOVA. Day 8 was selected for comparison because it was near peak lactation but still before pups consumed solid food, ensuring that food intake reflected only intake by the adult female. Because pups first consume solid food on day 15 of lactation (Hammond and Diamond 1992) and food intake was based on a 7-d average, it was necessary to compare groups 7 d before day 15.

All nutritional analyses were completed in duplicate; the average of these replicates was used for all statistical comparisons. Data were excluded when the coefficient of variation among replicates exceeded 10%, resulting in a small variation in sample size among analyses. All comparisons of maternal, pup, and litter characteristics among treatment groups were initially made with ANOVA, with litter size at birth included as a covariate. When litter size at birth was not significant, it was removed from the model and results based on an ANOVA were presented. All tests distinguished groups with a Tukey’s test for multiple comparisons. Maternal characteristics included percentage of body fat, percentage of total body ash, whole-body concentration of Ca²⁺ and Na⁺, mass and ash content of the right femur, and the Ca²⁺ and Na⁺ concentrations of the femurs. Quantifying mineral allocated to the litter was complicated by cannibalism. Disappearance of the cannibalized bodies made it impossible to directly measure the mass and body composition of these young. Thus, both minimum and maximum mineral allocation are given for each litter. Maximum mineral allocation was estimated on the basis of assigned litter size (an overestimate where cannibalism occurred), and minimum mineral allocation was estimated on the basis of the litter size at weaning (an underestimate for litters reduced by cannibalism). To determine litter mass at weaning, the sum of the body masses of all pups within each litter was determined at the termination of the study (21 d postpartum). When cannibalism occurred, total litter body mass was estimated on the basis of the assigned litter size by adding the estimated mass of the consumed young to the total litter mass; the estimated mass of the consumed young was based on the average of all other young in the litter. The total ash content of the litter was based on the average of the six select pups multiplied by the assigned litter size. Independently, the same calculations were performed on the basis of litter size at weaning. Proportional data do not display a normal distribution; thus, proportional data were normalized with an arcsine transformation (Zar 2010).

In nearly all instances of cannibalism, most (less a foot or short length of the tail) or all of the offspring was consumed (W. R. Hood, personal observation). To determine whether cannibalism improved maternal skeletal condition, the relationship between the number of pups consumed and the femoral ash content and femoral breaking load of females at weaning was assessed with linear correlation. This analysis and all subsequent analyses were limited to females assigned 18 young
because cannibalism was common only in this group. Differences in offspring skeletal size and degree of ossification at the time each pup was consumed were also expected to affect how much mineral females could potentially recover. To examine this relationship, offspring size at the time of cannibalism was estimated, and maternal femoral ash content was correlated with the sum of the skeletal sizes of the young consumed. Because tail length displayed a strong linear relationship with age before weaning, tail length could be used as a proxy for skeletal size (W. R. Hood, personal observation). Six pups per litter were measured on days 1, 7, 14, and 21 postpartum. Pup growth rates differed among litters but not between male and female pups (repeated-measures ANCOVA with pup ID nested within maternal ID: overall $F_{13,130} = 60.2$, $P < 0.001$; maternal ID: partial $F_{5} = 11.5$, $P < 0.001$; sex: partial $F_{1} = 2.79$, $P > 0.09$); therefore, linear-regression equations for tail length were determined independently for each litter (all regressions $r^2 \geq 0.90$). To determine whether cannibalism enhanced femoral mineral by reducing the cumulative mineral demand of the litter, femoral ash content of females at weaning was correlated with nursing effort, the sum of the number of pups suckled for each day of lactation (e.g., if 18 pups were suckled for 21 d, nursing effort = $18 \times 21 = 378$, but if three pups were consumed on day 11, nursing effort = $\Sigma [(18 \times 10) + (15 \times 11)] = 345$). And finally, to determine whether organic material (primarily collagen in the fat-extracted bone; Campo and Tourtellotte 1967; Miller and Martin 1968) was being mobilized from bone, the mass of organic material (fat-free dry mass of the femur − mass of the ash in the femur) was compared among groups by means of ANOVA.

Results

Food intake of females on day 8 of lactation increased with assigned litter size to eight offspring ($F_{3,38} = 45.9$, $P < 0.001$). However, there was no difference in intake among groups assigned 8, 13, or 18 young (fig. 1).

Litter size at birth had a significant effect on litter size at weaning (partial $F_1 = 9.7$, $P = 0.004$) but had no effect on maternal body composition or bone strength ($P > 0.22$, all maternal comparisons). The results of ANOVA suggest that maternal body fat ($F_{3,38} = 6.03$, $P = 0.002$) but not total body ash of females ($F_{3,38} = 1.09$, $P = 0.374$) varied with assigned litter size (fig. 2A, 2B). Total body fat was statistically similar between nonreproductive females and females assigned three and eight young (fig. 2A), although means suggest a decrease in residual fat with litter size of 13 young. Body fat did not differ between females assigned 13 and 18 offspring (fig. 2A). Total-body concentration of calcium was higher in nonreproductive females than in females assigned 18 young, but all other pairwise comparisons were similar ($F_{3,38} = 8.24$, $P = 0.001$). Total body sodium did not differ among groups ($F_{3,38} = 0.46$, $P = 0.761$; fig. 2C).

Treatment had several significant effects on bone characteristics. The mass of the femurs decreased with increasing litter size ($F_{4,38} = 5.31$, $P = 0.002$; fig. 3A), whereas the concentration of ash in the femurs decreased with increasing assigned litter size to 13, but females assigned 18 pups displayed ash concentrations greater than those of females with 13 pups and similar to those of all other groups, including females who did not reproduce ($F_{3,38} = 5.63$, $P < 0.001$; fig. 3B). Changes in the concentration of ash in the femurs reflect changes in the mineral and not the organic content of the bone because the mass of ash in the femur followed a pattern similar to ash concentration ($F_{3,38} = 8.94$, $P < 0.001$), while the organic content of bone did not differ among groups ($F_{3,38} = 1.94$, $P = 0.12$).

Following femoral ash, concentrations of bone Ca$^{2+}$ declined with increasing litter size to 13 and then increased again in females assigned 18 young ($F_{3,38} = 4.66$, $P = 0.004$; fig. 3C). There was no difference among groups in the Na$^+$ content of the bone ($F_{3,38} = 0.27$, $P = 0.897$; fig. 3C). Bone breaking load differed significantly among treatments, with the load required to break the femur decreasing with increasing litter size up to 13 young, but there was no difference between females assigned 13 and 18 young (ANOVA, $F_{3,38} = 5.30$, $P = 0.002$; fig. 4).

Nine of 36 (25%) reproductive females cannibalized offspring. The number of offspring cannibalized was significantly greater among females with an adjusted litter size of 18 than in all other groups (ANOVA, $F_{3,38} = 8.60$, $P = 0.002$). No females assigned 3 or 8 pups consumed offspring, and only one female in the group with 13 pups consumed pups (table 1). The number of young cannibalized per female ranged from 1 to 6. Four dead pups were found in the boxes of females with 18 young. One of these was missing the next day, and the other three dead pups were removed from the box the day after they were observed. Despite cannibalism, litter sizes remained different among groups (ANOVA, $F_{3,38} = 337$, $P < 0.001$; table 1), and litter size at birth had a significant effect on final litter size (partial $F = 9.7$, $P = 0.004$).

The timing of cannibalism varied among individuals. All but one cannibalistic individual consumed pups during early lactation (days 1–8), including the one female with an adjusted...
litter size of 13 that cannibalized offspring. For half of these females, the only incidence of cannibalism occurred during early lactation, while half of the females also consumed young at peak lactation (days 9–17, as defined by König et al. 1988; Hammond and Diamond 1992; Speakman and McQueenie 1996); only one pup was consumed during late lactation. Three pups assigned to litters of 18 died but were not cannibalized; these pups died during peak (n = 2) and late (n = 1) lactation.

Pup mass decreased with assigned litter size (ANCOVA: overall: $F_{3,51} = 76.9, P < 0.001$; partial: $F_p = 89.4, P < 0.001$; fig. 5A), whereas litter mass increased with assigned litter size up to 13 young, but no difference was found in these variables between adjusted litters of 13 and 18 (ANOVA: $F_{3,1} = 77.9, P < 0.001$;
Discussion

When female mice were challenged with larger litter sizes, they increased the amount of fat and minerals that they mobilized from reserves. This trend in resource allocation occurred only up to a litter size of 13, above which females were faced with demands that may have exceeded their ability to further increase allocation. Female mice with litters of 18 young adopted a pattern of nutrient allocation that limited the loss of their own fat and bone mineral reserves. Cannibalism of young was common among females with these large litters; this behavior improved the femoral mineral content and thus the skeletal condition of these females that had faced an especially high demand.

Somatic Tissue Mobilization

The mineral mobilization imposed by the average litter size for this strain of mouse (13) approximated a limit to the amount of mineral that females would mobilize for their reproductive effort, at least under the conditions of this experiment. In laboratory mice, peak lactation occurs before weaning (9–17 d postpartum; König et al. 1988; Hammond and Diamond 1992; Speakman and McQueenie 1996). Because the nutritional demand of milk production declines when peak lactation precedes weaning (Oftedal and Iverson 1995), recovery of some somatic tissue before weaning is possible. Thus, femoral ash concentration displayed by females with 13 young may have been lower at peak lactation. Factors such as relative maternal age, number of previous reproductive bouts, availability of dietary calcium, and disease may contribute to differences in the relative amount of bone loss that is tolerated (Peng et al. 1988; Bowman and Miller 1999; Odiere et al. 2010). Because young virgin mice were used for this study and mineral loss from bone during reproduction decreases with parity in rats (Bowman and Miller 1999), it is likely that the patterns described here were more pronounced than would be observed in older, experienced females.

Femoral breaking load decreased with increasing litter size but was not statistically different among females with the largest litter sizes. Thus, the greater bone mineral in females with 18 young may not translate to a functional increase in bone strength. Reduced bone mineral contents and densities and reduced bone strength in females with large litters have been described previously (Peng et al. 1988; Tojo et al. 1998).

Unlike the femur, the whole-body mineral (ash) content of females was similar among groups. Total-body and femoral sodium contents were also similar, suggesting that sodium availability was not limiting for females in this study. Total-body calcium content differed only between females that did not reproduce and those with 18 young, with the latter having significantly less calcium. Error alone could obscure reproductive losses when mineral mobilization is localized. For example, with only 2%–3% of the total calcium in a mouse’s body stored in the femur (Ca$^{2+}$ mass in femur/total body Ca$^{2+}$), changes in femoral mineral could be hidden with <2% error in whole-

Figure 4. Femoral bone breaking load (N) relative to assigned litter size. Treatment groups that were statistically similar are labeled with the same letter within each bar. Adult females that did not reproduce are included as NR. SE bars are given.
Figure 5. Allocation of nutrients by females to individual young and the full litter. Data include the body mass, ash content, and calcium and sodium content of individual pups (A, B, and C, respectively) and of the full litter (D, E, and F, respectively). The amount of nutrients that females allocated to dead young and allocated to and recovered from cannibalized young could not be determined. Thus, allocation to the litter was estimated two ways: (1) as if all pups had survived (black bars; i.e., assigned litter size × average values for pups in litter) and (2) as if the female did not invest in dead or consumed pups (gray bars; i.e., litter size at weaning × average values for pups in litter). Treatment groups that were statistically similar are labeled with the same letter within each bar. Adult females that did not reproduce are included as NR. SE bars are given.

Body analyses. Losses of mineral during lactation are common in several appendicular bones and the vertebrae (Rasmussen 1977; Zeni et al. 1999); comparable losses are also likely for the mice in this investigation. Losses of bone mineral from other regions have also been described during lactation, including the ribs, skull, mandible, hyoid, and pelvis (Benzie et al. 1955). Cumulative changes in whole-body mineral content have been described during periods of high demand (Widdowson and Dickerson 1964; Hood et al. 2006).

The total-body fat content of females declined with increasing litter size up to the average litter size for this strain of mouse, corresponding to the changes in bone, but that of females assigned the largest litters was similar to that of females with average-sized litters. Allocation to the litter reflected this pattern, with body mass and nutrient accretion increasing to a litter size of 13. Because the nutrient content of cannibalized pups could not be quantified, nutrient allocation by females bearing the largest litters could be characterized only as a range and not as an exact value. Nevertheless, it is probable that allocation remained similar for litter sizes greater than 13. In contrast, allocation to individual pups declined linearly with increasing litter size, as has been described in several other mammals (Mattingly and McClure 1982; König et al. 1988; Sikes 1995; Kunkele 2000; but see Kenagy et al. 1990). It is clear that the food-intake limit alone does not limit allocation to the litter. Females compensate for limited intake by increasing body fat and mineral mobilization from the femur between litter sizes of 8 and 13, but the limit to fat mobilization beyond the average litter size suggests that females may not mobilize their own resources beyond the point at which they will likely jeopardize their own survival (Clutton-Brock 1991).

Allocation of minerals to the full litter followed a pattern similar to that for body mass, but mineral allocation to individual young was comparable across litter sizes rather than
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Figure 6. Relationship between the ash content of females’ femurs at weaning and the number of pups that each female cannibalized (A), the cumulative skeletal size of pups consumed, calculated by the sum of their tail lengths at the time of death (B), and the nursing effort of the female, based on the number of pups suckled for all 21 d of lactation (C). Lines show significant regressions at \( p < 0.05 \).

Declining as litter size increased. It is possible that total energy transfer to the litter was more limiting to offspring development than the minerals necessary to build bone. In general, change in body mass during growth is more sensitive than the skeleton to variation in resource availability (Hoying and Kunz 1998).

Nutrient Recycling, Nutritive Gain by Young, and Savings in Females

There are three possible explanations for higher levels of bone mineral in females suckling the largest litters than in those with average-sized litters in this study: (1) reduced maternal mineral allocation to the litter, (2) mineral recycling, and/or (3) increased mineral availability. Offspring cannibalism was considered as a method of mineral recycling. The number of young cannibalized was not significantly correlated with femoral ash content or bone-breaking load for females assigned 18 young. However, there was a significant relationship between femoral ash content and nursing effort for these females; this relationship explained 44% of the variation in femoral mineral content at weaning. These results suggest that reduced bone mineral loss by females assigned the largest litters was largely associated with a reduction in maternal mineral allocation. Owing to the small sample size, the possibility that mineral recovered from consumed young contributes to maternal bone mineralization should not be discounted. Knight et al. (1986) described the relationship between litter size and milk yield in mice. In early lactation, females suckling 18 young produced significantly more milk than females with 10 young. At peak lactation, however, these differences diminished. Because milk composition is not expected to vary for females suckling large litters (Fiorotto et al. 1991), it can be implied that nutrient transfer at peak lactation was likely similar among females assigned 13 and 18 offspring regardless of the incidence of cannibalism. Thus, cannibalistic females most likely incurred the greatest mineral saving but reduced their litter size during early lactation. Increased mineral absorption by the intestine and decreased mineral excretion by the kidney are common adaptations to the increased mineral demand of reproduction (Kovacs and Kronenberg 1997). These variables were not examined here, but the effect of litter size on these variables is worthy of investigation.

Bone Mobilization and Reproductive Performance

The question of what limits the reproductive performance of an individual has puzzled biologists for decades. Many ecological and evolutionary physiologists have approached this problem by looking for intrinsic constraints on the total amount of energy that females transfer to their young. For example, Hammond and Diamond (1992) and others (Drent and Daan 1980; Scaletbury et al. 2000) have considered the assimilation capacity as a possible constraint on energy absorption (central-limitation hypothesis), Brown et al. (2004) suggested that the architecture of the circulatory system responsible for nutrient delivery to the tissues could limit energy expenditure (the metabolic theory of ecology), and Hammond and colleagues (Hammond and Diamond 1994; Hammond et al. 1994, 1996) and Speakman and colleagues (Johnson et al. 2001; Król and Speakman 2003) have also considered that the synthetic capacity of tissues (peripheral-limitations hypothesis) could also limit energy transfer during lactation. More recently, Król et al. (2007) have proposed that an animal’s ability to dissipate heat may be an important constraint on maximum performance during


in improved skeletal condition for females at weaning. The possibility that cannibalism could have been a response to the→ Christian D.P., T.E. Manning, and C.J. Harth. 1993. Sodium and potassium balance of captive meadow voles (Microtus pennsylvanicus) fed laboratory chow and vegetation diets. Comp Biochem Physiol A 106:571–579.

increase in demand placed on females associated with the experimental manipulation could not be ruled out, nor is it possible→ Clutton-Brock T.H. 1984. Reproductive effort and terminal investment in iteroparous animals. Am Nat 123:212–229.


theless, the changes in maternal condition in cannibalistic females remain intriguing, particularly given that cannibalism is→ ———. 1988. The effect of porosity and mineral content on the Young’s modulus of elasticity of compact bone. J Biomech 21:131–139.


condition were lacking before this investigation (Hrdy 1979; Labov et al. 1985). Here I showed that cannibalistic females improved the mineral content of their femurs by reducing the overall demand of suckling. After weaning, bone goes through a period of rapid anabolism (Bowman and Miller 1999), and thus cannibalism likely improves a female’s immediate probability of survival during lactation rather than having a sustained effect between reproductive events.

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Literature Cited


