Reproductive implications of bone mineral density in an armadillo, the pichi (*Zaedyus pichiy*)

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Armadillos (Cingulata: Dasypodidae and Chlamyphoridae) are the only mammals bearing an osseous carapace. Most mammals have rapid growth rates and require high mineral levels. However, armadillos feed mainly on insects, a poor source of calcium and phosphate. This raises the question how lactating females obtain the needed minerals to provide their offspring sufficient quantities for the development and hardening of their carapace. The objective of this study was to evaluate whether there are differences in the mineral density of the carapace and endoskeleton of reproductive (especially lactating) and non-reproductive female armadillos, and those of males. We performed computed tomography of 32 female and 13 male dead pichis (*Zaedyus pichiy*) of different age classes and reproductive status and compared bone mineral density (BMD) of their marginal scutes, compact and spongy bone of the carapace, and femoral bone. Juveniles of both sexes had significantly lower BMD than adults. Average BMD values of the marginal scutes, compact and spongy bone of the carapace, femoral head, spongy bone, and femoral cortical bone of lactating females were similar to those of adult males and significantly higher than values of adult females without signs of gestation or lactation. We conclude that lactating females do not seem to lose bone density, at least from the carapace and femur, during lactation. However, our results suggest that a high BMD may be necessary to support reproduction in female pichis.

Los armadillos (Cingulata: Dasypodidae y Chlamyphoridae) son los únicos mamíferos que poseen un caparazón óseo. La mayoría de los mamíferos tienen altas tasas de crecimiento y requieren elevados niveles de minerales. Sin embargo, los armadillos se alimentan principalmente de insectos, un recurso pobre en calcio y fósforo. Surge entonces la pregunta cómo las hembras lactantes obtienen la cantidad suficiente de minerales para proveer a sus crías para el desarrollo y endurecimiento de su caparazón. El objetivo de este estudio fue evaluar si existen diferencias en la densidad mineral del caparazón y endoesqueleto de armadillos hembras reproductivamente activas (especialmente lactantes) e inactivas, y de machos. Para esto, se realizaron tomografías computarizadas de piches (*Zaedyus pichiy*) muertos, siendo 32 hembras y 13 machos de diferentes clases etarias y estados reproductivos. Se comparó la densidad mineral ósea (DMO) de sus placas marginales, hueso compacto y esponjoso del caparazón, y hueso femoral. Los juveniles, machos y hembras, presentaron valores de DMO significativamente menores que los adultos. La DMO de las placas marginales, tejido compacto y esponjoso del caparazón, y cabeza femoral de las hembras lactantes fueron similares a las de los machos adultos y significativamente mayores que en hembras adultas sin signos de gestación o lactancia. Concluimos que las hembras lactantes no sufren desmineralización, por lo menos del caparazón y fémur, durante la lactancia. Por otro lado, nuestros resultados sugieren que las hembras requerirían una DMO alta para reproducirse.

Key words: armadillos, BMD, calcium, Cingulata, computed tomography, gestation, lactation, Xenarthra

Lactation involves a series of substantial maternal metabolic adjustments, including marked changes in maternal calcium (Ca) homeostasis. In many species, maternal bone tissues play a key role during this process by supplying important quantities of minerals, especially Ca, to the fetus (Kovacs and Kronenberg 1997). Transfer of Ca and phosphorus (P) from mother to offspring increases after birth as the skeleton develops and begins bearing the increasing mass of the growing animal (Brommage 1989). Maternal reserves of Ca and P in bone may be mobilized to meet the demands of reproduction (Kovacs and Kronenberg 1997). However, loss of bone mass or density may be attenuated if proteins and minerals in the tissues are replenished from dietary supplies (Krebs et al. 1997). Limits against the overuse of maternal endogenous mineral allocated to the litter have been described for female laboratory mice (*Mus musculus*— Hood 2012). According to the cost of reproduction hypothesis, parental investment involves an effort that may reduce their chances of survival or future reproductive success (Trivers 1972; Calow 1979).

Armadillos (Cingulata: Dasypodidae and Chlamyphoridae) have a carapace formed by ossified dermal structures called osteoderms or scutes (Gaudin and McDonald 2008). Although ossification of scutes starts during the fetal period (Vickaryous and Hall 2006), the carapace of fetuses at term is soft and flexible to allow passage through the birth canal. Some species of armadillos have a faster infant growth rate than other mammals of comparable size (Superina and Loughry 2012). Offspring require high mineral levels for the full development and fast hardening of their carapace, for which maternal investment must cover the requirements of infants (Superina and Loughry 2012). However, insects, the predominant food item of most armadillo species, are a poor source of Ca and other minerals (Finke and Oonincx 2013).

The pichi (*Zaedyus pichiy*) is a small (body mass approximately 1 kg), semi-fossorial armadillo endemic to arid and semiarid lands of Argentina and Chile. These opportunistic omnivores primarily feed on insects (Superina et al. 2009a). They can be distinguished from other armadillos by the sharply pointed marginal scutes of their carapace (Superina and Abba 2014). Pichis are the only armadillo species known to hibernate during winter and to enter daily torpor in warmer seasons (Superina and Boily 2007). Their reproductive season is limited to a few months; they mate soon after emerging from hibernation in early spring and give birth to 1–2 offspring after a gestation period of approximately 60 days (Superina et al. 2009b). The offspring remain inside their den during the entire lactation period of 37–40 days, during which they increase their body mass at a linear rate of 9 g/day from 50 to 400 g (Superina et al. 2009b; Superina and Loughry 2012). Their carapace develops very quickly. It is yellow and relatively hard by 2 weeks of age (Superina and Loughry 2012).

Although pichis eat primarily insects (Superina et al. 2009a), it is possible that females shift their diet to prey with higher mineral content, such as small vertebrates, during reproduction. Some armadillo species also consume prey with greater amounts of Ca and P than insects, such as small vertebrates (Redford and Wetzel 1985; Superina et al. 2009a; Abba and Superina 2016). Seasonal variations in diet composition may occur (Abba et al. 2011). Indeed, Superina et al. (2009a) found lizards and hair in the stomach contents of pichis confiscated during summer. A closely related species, the screaming hairy armadillo (*Chaetophractus vellerosus*), feeds on a considerably higher proportion of mammals during summer than in other seasons (Abba et al. 2011). This change in diet composition may be related to demands of reproduction, as lactation occurs during summer months (Superina and Loughry 2012). Nevertheless, additional studies are needed to confirm this hypothesis.

In this study, we determined whether female pichis use mineral reserves from their own skeleton or carapace during gestation and lactation. Based on the premise that bone mass and density are important determinants of bone strength (Heaney et al. 2000), we hypothesized that demineralization may occur in parts of the carapace that are less important for protection, such as the marginal scutes, but not in its upper part or in the femur that have structural roles important for survival. We analyzed the femoral and carapace bone mineral density (BMD) of lactating female pichis by computed tomography (CT) and compared the values with those of non-lactating females, adult males, and juveniles of both sexes.

Materials and Methods

Animals.—A total of 45 pichis (13 males, 32 females) were analyzed. They had been confiscated dead from poachers by law enforcement authorities of Mendoza Province (Argentina) during the summer and fall (November–May) of 2008, 2009, and 2013. Of these, 41 were from southern Mendoza (36.5°S, 69°W), while the remaining 4 individuals, all females, were from the northern part of the province (32.5°S, 69°W). The armadillos had been eviscerated by poachers and internal reproductive organs were therefore sometimes lacking. The 45 specimens are deposited at the Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA) Mammal Collection, CCT CONICET Mendoza, Argentina (specimens CMI-007525–CMI-007569).

The total number and temporal distribution of confiscated armadillos depends on poaching activity as well as the frequency and success of anti-poaching patrols. Furthermore, poachers are rarely active during the breeding season of wild pichis because of the common belief that eating reproductively active armadillos causes indigestion; hence, confiscations of pregnant females are unusual. It was therefore not possible to obtain similar numbers of samples from all reproductive stages. Nevertheless, this sampling method is an ethically justifiable procedure and often the only legally acceptable means to obtain samples from protected wildlife (Superina 2007). Armadillos are highly susceptible to stress caused by capture and handling, with the consequent risk of abortions (Superina 2007). It would therefore be unethical to perform a longitudinal study using wild or captive-bred pichis.

Pichis were classified as juveniles or adults based on morphological features, such as the appearance and measurements of the carapace (Superina et al. 2009c). The carapaces of juvenile pichis have a smooth, shiny surface, whereas those of adults appear

dull, cracked, and with scars. Carapace length was measured from the anterior edge of the scapular shield to the posterior edge of the pelvic shield, and carapace width on the 4th band. It should be noted that the age of an armadillo cannot be estimated once it reaches adult size (Loughry and McDonough 2013).

To determine the reproductive stage of the females, we inspected the pair of mammary glands, which is located pectorally. Lactating females can be distinguished from non-lactating conspecifics by their turgid mammary glands and enlarged pair of nipples, whereas the latter are considerably shorter in females around or after weaning. Non-lactating females have very small nipples similar to those of males (M. Superina, pers. obs.). Additionally, due to their seasonal breeding pattern, the capture date of pichis is a useful tool to estimate their reproductive stage (Superina et al. 2009b).

Since confiscated pichis were partially or completely eviscerated and at different stages of postmortem decomposition and dehydration, we made the carcasses as uniform as possible for our comparisons. For this, carcasses were defleshed mechanically and using black garden ants (*Lasius niger*; Hymenoptera: Formicidae) to remove soft tissue remains. This procedure did not cause visible damage to the osseous structures. Skeletons and carapaces were sundried and weighed once per week until a constant weight was obtained in 3 consecutive measurements.

Animals were classified into the following 6 categories according to their sex, age class, and reproductive status: females: full lactation (FL), end of lactation (EL), non-lactating (NL), juvenile (JF); males: adult (AM), juvenile (JM).

Computed tomography.—Bone mineral densities were estimated by CT (resolution 0.6 mm; GE Lightspeed 16; General Electric, Milwaukee, Wisconsin) at the Fundación Escuela de Medicina Nuclear (FUESMEN), Mendoza, Argentina. This radiological technique uses X-rays to generate cross-sectional multidimensional images of the body. The viewed image is then reconstructed as a corresponding matrix of picture elements (pixels). Each pixel is assigned a numerical value expressed in Hounsfield Units (HU), which is compared to the attenuation value of distilled water (defined as 0 HU) and displayed on a linear scale (Hounsfield scale) for describing radiodensity, named HU or CT numbers. HU values can be positive or negative. As a negative HU moves away from 0, it means that tissue density is lower; the higher the number, the denser the tissue (Celenk and Celenk 2012). Compact bone will thus have higher HU values than spongy bone (Rho et al. 1995). Hence, HU values are an adequate estimate for assessing BMD (Schreiber et al. 2014).

We chose the marginal scutes of the 3rd and 6th movable bands of the carapace to study possible sites of demineralization during lactation. Although their function is unknown, one of us (M. Superina, pers. obs.) noted that they acquire a reddish color in lactating females but not in males or non-lactating females. This color shift suggests increased blood circulation and thereby increased metabolic rate. We therefore proposed that marginal scutes may serve as a high-turnover bone to provide the active demand for minerals during lactation. We also selected a region in the upper part of the carapace in which compact and spongy bone are adjacent (Figs. 1A and 1B). Finally, we studied the

femur because we did not expect to find demineralization in a bone subjected to significant mechanical pressures (Fig. 1C). Due to the small size of the bones and the spatial resolution of the equipment, we were able to analyze the femoral head, but could not distinguish the femoral growth plates from the rest of the bone. Thus, measurements were more feasible and with less error in the femoral head. Similarly, the CT equipment did not allow us to analyze changes in trabecular number, thickness, and spacing. We therefore only determined spongy bone density within the medullary cavity and the cortical bone surrounding it.

To obtain the images, the carapace and right femur of each armadillo were scanned together. The CT scanner parameters were set using a voltage of 120 kV, a current of 250 mA, and a slice thickness of 0.625 mm. As a quality control procedure, the GE Lightspeed HU scale was calibrated weekly using a phantom provided by General Electric.

Bone parameters were analyzed with the image-processing program OsiriX, an open-source software for navigating in multidimensional DICOM (Digital Imaging and Communication in Medicine) images such as CT studies (Rosset et al. 2004). The regions of interest (ROIs) were sculpted from the scanned images of each bone structure (Fig. 1) using the "Closed Polygon Tool" available in OsiriX. The contour of a planar closed polygon that defines the ROI was obtained from each slice. The volume of interest (VOI) was defined as the stack of ROIs covering the whole structure of interest. Once the stack of ROIs from a sequence of contiguous slices was established, the values of all pixels contained within the contour boundaries of each VOI were computed, obtaining the average values per volume. Values were measured at 7 sites (Fig. 1): 1) 3rd left marginal scute of the carapace; 2) 6th right marginal scute of the carapace; 3) spongy bone of the carapace, in the ventral and posterior portion of the latter, close to the hip joint; 4) compact bone of the carapace, at the same level as the abovementioned spongy bone; 5) right femoral head; 6) right femoral spongy bone within the medullary cavity, between femoral head and 3rd trochanter; and 7) femoral cortical (compact) bone, at the same level as the abovementioned spongy bone. The number of measured slices at each studied site was established according to its respective bone architecture: sites 1 and 2, 8–12 slices; sites 3 and 4, 8–10 slices; site 5, 4 –5 slices; sites 6 and 7, 6–7 slices.

Statistical analyses.—Results for each category are expressed as the mean of the average VOI ± *SEM*. The statistical significance between categories was evaluated by 1-way analysis of variance (ANOVA) and τ post hoc tests for comparison between females, and with Student's *t*-tests for comparison between males. Homogeneity of the variances was verified with the Bartlett test. Log transformation of the data was performed when variances were not homogeneous. Differences between means were considered significant at $P < 0.05$.

RESULTS

No pregnant females were obtained. Approximately 2/3 of females were lactating, with a greater number at the end of lactation than at full lactation. Carapace length and width for adult females from the different categories were uniform,

suggesting that HU values were not related to size differences (Table 1). Although carapaces of juveniles of both sexes were smaller than those of adults, only the differences between adult and juvenile males were statistically significant (Table 1).

HU values and variations between categories were similar in both marginal scutes analyzed (Figs. 2A and 2B). Lactating females (FL and EL) showed the highest values in both marginal scutes. They were significantly higher ($P < 0.05$) than those of NL and JF (Figs. 2A and 2B) for both scutes. AM had significantly higher values than JM ($P < 0.05$) for the 3rd scute, whereas differences were not significant for the 6th scute (Figs. 2A and 2B). AM had values similar to those of lactating females (Figs. 2A and 2B).

Values for the spongy bone of the carapace showed a pattern similar to that observed in the marginal scutes (Fig. 2C). Values for NL and JF were significantly lower ($P < 0.05$) than those of lactating females (FL and EL; Fig. 2C). JM had significantly lower ($P < 0.05$) values than AM (Fig. 2C). In contrast, HU values of the compact bone of the carapace were similar in all female categories (FL, EL, NL, and JF; Fig. 2D), although JF values tended to be lower. Values were significantly lower ($P < 0.05$) in JM compared with AM (Fig. 2D).

Femoral heads also showed variations similar to the scutes. NL and juveniles of both sexes (JF and JM) had similar values,

Table 1.—Morphometric measurements of the pichi (*Zaedyus pichiy*) carapaces analyzed by computed tomography. Studied pichis (all from Mendoza Province, Argentina) were classified according to their sex, age class, and reproductive status. Measurements are indicated as means ± *SD*. Carapace length and width of adult females from the different categories were uniform, suggesting that bone mineral density values are not related to size differences.

Category	n	Carapace	
		Length[a]	Width[b]
Full lactation females (FL)	7	20.33 ± 1.19	21.66 ± 0.86
End of lactation females (EL)	12	20.33 ± 1.42	22.11 ± 1.56
Non-lactating females (NL)	5	19.92 ± 1.91	21.64 ± 0.74
Juvenile females (JF)	8	18.89 ± 1.80	20.65 ± 1.44
Adult males (AM)	8	20.40 ± 1.41	21.76 ± 1.44
Juvenile males (JM)	5	18.23 ± 0.81*	19.53 ± 1.05*

[a]Measured from the anterior edge of the scapular shield to the posterior edge of the pelvic shield.
[b]Measured on the 4th band.
*$P < 0.05$ compared with adult males, Student's *t*-test.
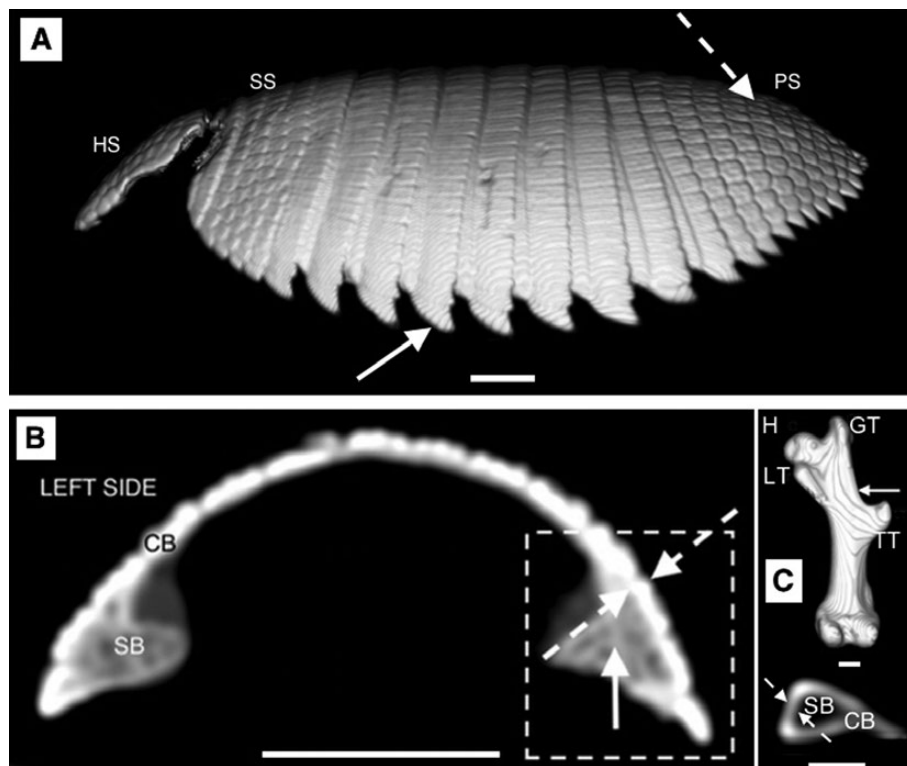

Fig. 1.—Computed tomography images of the carapace and the femur of *Zaedyus pichiy* (CMI-007534). A) 3-dimensional view of the carapace, left side. The white solid arrow points to the 3rd marginal scute. The white dotted arrow marks the transverse plane represented in image B; B) 2-dimensional view of the carapace, transverse plane. The white dotted square shows the analyzed area. The white solid arrow points to the central region of spongy bone. White dotted arrows indicate external and internal limits of compact bone; C) 3-dimensional (upper image, anterior view) and 2-dimensional (bottom image, transverse plane) views of the right femur. The white solid arrow in the upper image points to the site shown in the bottom image. White dotted arrows indicate external and internal limits of cortical bone, respectively. Scale (white bars) represents 20 mm for carapace images and 5 mm for femur images. CB = compact bone; GT = greater trochanter; H = femoral head; HS = head shield; PS = pelvic shield; SB = spongy bone; SS = scapular shield; LT = lesser trochanter; TT = 3rd trochanter.

which were significantly lower ($P < 0.05$) than all other groups (Fig. 3A). FL had significantly lower values ($P < 0.05$) than EL (Fig. 3A), whereas those of AM were comparable to the values of lactating females (Fig. 3A). Interestingly, values for femoral spongy bone within the medullary cavity were similar in all groups (Fig. 3B). Values of the cortical bone of the femur were similar to those observed for the marginal scutes, femoral head, and spongy bone of the carapace (Fig. 3C), with HU values of FL and EL being significantly higher than those of NL and JF, and AM values also significantly higher than JM.

DISCUSSION

The BMD of pichis, as estimated by HU values, varied with age and with reproductive status. Differences were observed in the spongy bone of the carapace, the marginal scutes, and the femurs. These bone structures have varying functions and are subject to different degrees of mechanical loads, suggesting that differences also may occur in other structures of the skeleton. Adult females without signs of having undergone pregnancy or lactation, captured during the same season and of roughly the same size, had significantly lower BMD values than lactating females, which was opposite to our expectation based on the demands of pregnancy and lactation for minerals. Thus, in accordance with the cost of reproduction hypothesis (Calow 1979), a high BMD may be necessary for female pichis to reproduce.

The similar BMD values between full lactation and end of lactation indicate that female pichis do not demineralize their carapace or femur during lactation to provide minerals to the
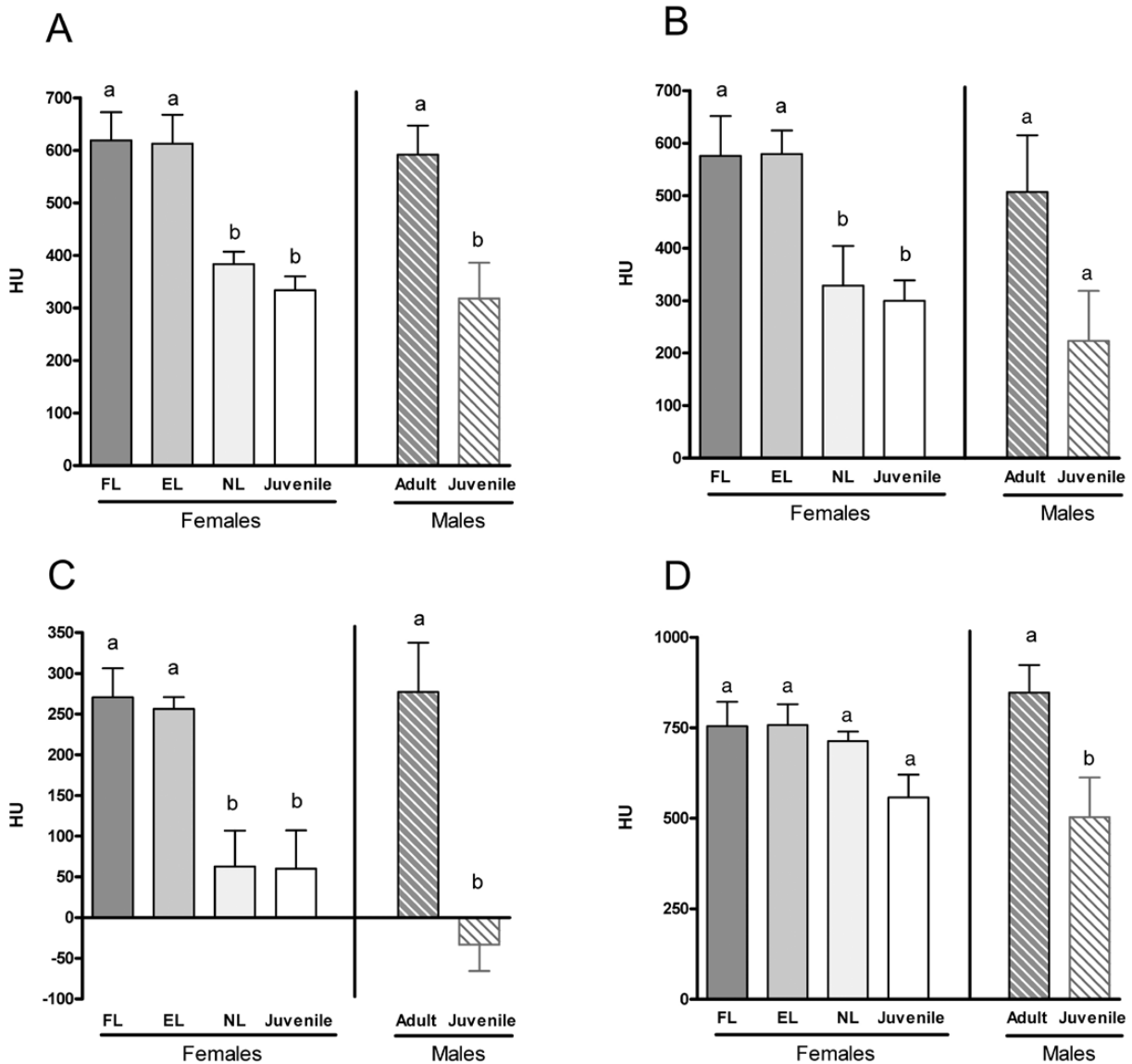

Fig. 2.—Average bone mineral density values, estimated by Hounsfield Units (HU), of regions of the carapace from pichis (*Zaedyus pichiy*) of different reproductive, age, and sex categories. A) 3rd left marginal scute; B) 6th right marginal scute; C) spongy bone of the carapace; D) compact bone of the carapace. Sample sizes: FL = full lactation, $n = 7$; EL = end of lactation, $n = 12$; NL = non-lactating females, $n = 5$; juvenile females, $n = 8$; adult males, $n = 8$; and juvenile males, $n = 5$. Bars represent the group mean ± *SEM*. Distinct letters indicate categories with significantly different BMD values within each sex, at $P < 0.05$. HU values of the 3rd and 6th marginal scutes were log transformed for statistical analyses to homogenize variances.
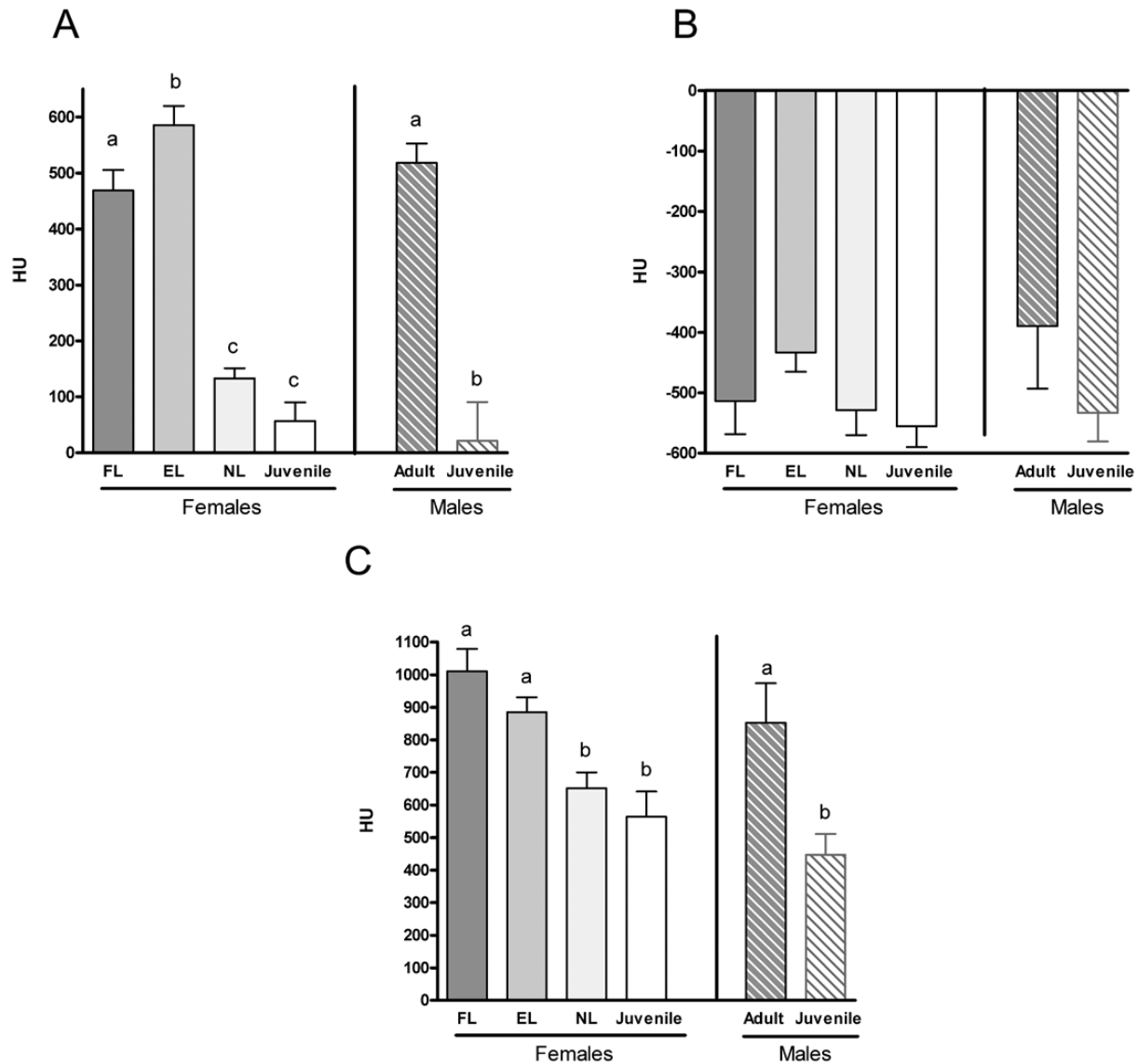
Fig. 3.—Average bone mineral density values, estimated by Hounsfield Units (HU), of regions of the femurs from pichis (*Zaedyus pichiy*) of different reproductive, age, and sex categories. A) femoral head; B) femoral spongy bone within the medullary cavity; C) femoral cortical bone. Sample sizes: FL = full lactation, $n = 7$; EL = end of lactation, $n = 12$; NL = non-lactating females, $n = 5$; juvenile females, $n = 8$; adult males, $n = 8$; and juvenile males, $n = 5$. Bars represent the group mean ± *SEM*. Distinct letters indicate categories with significantly different HU values, within each sex, at $P < 0.05$.

milk. The only significant difference between groups was the slightly higher femoral head BMD at the end of lactation than during full lactation. Our results match the findings of Hood et al. (2006), who suggested that mineral concentration may never reach critically low levels in lactating brown bats (*Eptesicus fuscus*). This may be a consequence of the lower skeletal mass of small mammals compared to larger species (Prange et al. 1979) that limits the quantity of minerals available for transfer to their offspring (Hood et al. 2006) without compromising bone integrity.

Considering that the carapace may have influenced the evolution of other features of extant armadillos, such as ecological, physiological, and reproductive traits (Superina and Loughry 2012), it is possible that preservation of its structural integrity may be a priority. Tsurusaki et al. (2000) proposed

that trabecular (spongy) bone is more responsive to metabolic changes than cortical bone. This hypothesis is supported by the fact that the BMD of the compact bone of the carapace was similar among female groups, whereas values of marginal scutes and spongy bone of the carapace were significantly different between lactating and non-reproductive females.

The higher values in lactating than non-lactating females suggest the possibility of an increase in BMD prior to lactation, which may anticipate the loss of minerals during this period. Based on fecal hormone analyses in pregnant pichis, Superina et al. (2009b) demonstrated a rapid decrease in both progestogen and estrogen concentrations immediately before or after parturition. In humans, progesterone and estrogens promote a trophic effect and reduce resorption in bone tissues, respectively (Prior 1990; Turner et al. 1994). Pregnant pichis

may therefore overcompensate bone mineralization (Ákesson et al. 2004) to achieve sufficient mineral reserves to cope with gestation and lactation. However, the lack of specimens of pregnant pichis precluded us from testing this hypothesis, which may be addressed by additional studies using other methodologies.

Exercise may reduce bone loss in lactating women (Lovelady et al. 2009). The mechanical stress produced by exercise appears to stimulate a redistribution of bone tissue in pregnant or lactating laboratory mice (Hood and Hobensack 2015). Pichis have solitary habits and males do not participate in the rearing of offspring (Superina 2007). Lactating females must therefore engage in foraging activities sufficient to successfully rear their litter on their own. Lactating females may experience an increased loading on their femurs, associated with digging and foraging movements, that may limit mineral loss.

Not surprisingly, juveniles of both sexes had significantly lower bone densities in most bone structures studied, reflecting their immaturity. BMD values also were similar across all studied bone tissues of lactating females and adult males, suggesting that these values may be normal for reproductively active females. There is no consensus concerning differences in BMD between the sexes in other species (Martin et al. 1981; Fürst et al. 2008; Vulcano et al. 2008), and discrepant results exist even within a single species (Kelly et al. 1990; Looker et al. 2001).

Reproductive events are linked to ecological and physiological costs (Speakman 2008). These reproductive costs are expected to be higher when animals have limited time to recover from the negative effect caused by resource depletion (Humphries and Boutin 2000). In pichis, this recovery period overlaps with the time during which they must accumulate substantial fat reserves to enter hibernation (Superina and Boily 2007), which in turn immediately precedes the next reproductive season. Because new bone formation does not occur during hibernation (Kwiecinski et al. 1987), the ability of pichis to recover and replenish their mineral reserves shortly after lactation also may affect their reproductive success in the next breeding season. Due to their predominantly insectivorous diet (Superina et al. 2009a), pichis may ingest sufficient protein and energy (Finke and Oonincx 2013) but it may be difficult for them to achieve an adequate intake of minerals between 2 consecutive reproductive seasons. As a consequence, females may not be able to produce a litter every year. Alternatively, female pichis might modify their diet during the reproductive season toward food items richer in minerals, such as small vertebrates or eggs from ground-nesting birds, as has been described for other armadillo species (Abba et al. 2011; Loughry and McDonough 2013).

It is possible that bone loss to support minerals withdrawn during lactation occurs from areas not analyzed here. For example, Baksi and Newbrey (1989) indicated that to support the development of antlers, bone loss in reindeer (*Rangifer tarandus*) appears to occur primarily in the ribs. On the other hand, the function of the characteristic marginal scutes of pichis is unknown. The high BMD and the reddish color observed in the marginal scutes of lactating females suggest that these bone structures could serve as a high-turnover bone to provide the active demand for minerals during lactation. However, further studies are necessary to test this hypothesis. The relatively small litter size of pichis also may be an adaptation to limit bone loss in lactating females.

In summary, pichis do not demineralize regions of the carapace or femur over the lactation period. BMD values are higher in females that reproduced than in those that did not successfully breed. These results suggest that a minimum BMD level may be necessary for female pichis to reproduce. Pregnant and lactating pichis may obtain the necessary minerals using other strategies, such as a change in diet or a period of bone density overcompensation before giving birth.

Acknowledgments

Literature Cited

Abba, A. M., G. H. Cassini, M. H. Cassini, and S. F. Vizcaíno. 2011. Historia natural del piche llorón *Chaetophractus vellerosus* (Mammalia: Xenarthra: Dasypodidae). Revista Chilena de Historia Natural 84:51–64.

Abba, A. M., and M. Superina. 2016. *Dasypus hybridus* (Cingulata: Dasypodidae). Mammalian Species 48:10–20.

Ákesson, A., M. Vahter, M. Berglund, T. Eklöf, K. Bremme, and P. Bjellerup. 2004. Bone turnover from early pregnancy to postweaning. Acta Obstetricia et Gynecologica Scandinavica 83:1049–1055.

Baksi, S. N., and J. W. Newbrey. 1989. Bone metabolism during antler growth in female reindeer. Calcified Tissue International 45:314–317.

Brommage, R. 1989. Measurement of calcium and phosphorus fluxes during lactation in the rat. The Journal of Nutrition 119:428–438.

Calow, P. 1979. The cost of reproduction – a physiological approach. Biological Reviews 54:23–40.

Celenk, C., and P. Celenk. 2012. Bone density measurement using computed tomography. Pp. 123–136 in Computed tomography – clinical applications (L. Saba, ed.). InTech, Rijeka and Shanghai.

Finke, M. D., and D. G. A. B. Oonincx. 2013. Insects as food for insectivores. Pp. 19–49 in Mass production of beneficial organisms: invertebrates and entomopathogens (J. A. Morales-Ramos, M. G. Rojas, and D. I. Shapiro-Ilan, eds.). Academic Press, London, United Kingdom.

Fürst, A., D. Meier, S. Michel, A. Schmidlin, L. Held, and A. Laib. 2008. Effect of age on bone mineral density and micro architecture in the radius and tibia of horses: an extreme computed tomography study. BMC Veterinary Research 4:3.

GAUDIN, T. J., AND H. G. MCDONALD. 2008. Morphology-based investigations of the phylogenetic relationships among extant and fossil xenarthrans. Pp. 24–36 in The biology of the Xenarthra (S. F. Vizcaíno and W. J. Loughry, eds.). University Press of Florida, Gainesville.

HEANEY, R. P., ET AL. 2000. Peak bone mass. Osteoporosis International 11:985–1009.

HOOD, W. R. 2012. A test of bone mobilization relative to reproductive demand: skeletal quality is improved in cannibalistic females with large litters. Physiological and Biochemical Zoology 85:385–396.

HOOD, W. R., AND M. HOBENSACK. 2015. The effect of locomotion on the mobilization of minerals from the maternal skeleton. PLoS One 10:e0122702.

HOOD, W. R., O. T. OFTEDAL, AND T. H. KUNZ. 2006. Variation in body composition of female big brown bats (Eptesicus fuscus) during lactation. Journal of Comparative Physiology B 176:807–819.

HUMPHRIES, M. M., AND S. BOUTIN. 2000. The determinants of optimal litter size in free-ranging red squirrels. Ecology 81:2867–2877.

KELLY, P. J., L. TWOMEY, P. N. SAMBROOK, AND J. A. EISMAN. 1990. Sex differences in peak adult bone mineral density. Journal of Bone and Mineral Research 5:1169–1175.

KOVACS, C. S., AND H. M. KRONENBERG. 1997. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocrine Reviews 18:832–872.

KREBS, N. F., C. J. REIDINGER, A. D. ROBERTSON, AND M. BRENNER. 1997. Bone mineral density changes during lactation: maternal, dietary, and biochemical correlates. The American Journal of Clinical Nutrition 65:1738–1746.

KWIECINSKI, G. G., L. KROOK, AND W. A. WIMSATT. 1987. Annual skeletal changes in the little brown bat, Myotis lucifugus lucifugus, with particular reference to pregnancy and lactation. The American Journal of Anatomy 178:410–420.

LOOKER, A. C., T. J. BECK, AND E. S. ORWOLL. 2001. Does body size account for gender differences in femur bone density and geometry? Journal of Bone and Mineral Research 16:1291–1299.

LOUGHRY, W. J., AND C. M. MCDONOUGH. 2013. The nine-banded armadillo: a natural history. University of Oklahoma Press, Norman.

LOVELADY, C. A., M. J. BOPP, H. L. COLLERAN, H. K. MACKIE, AND L. WIDEMAN. 2009. Effect of exercise training on loss of bone mineral density during lactation. Medicine and Science in Sports and Exercise 41:1902–1907.

MARTIN, R. K., J. P. ALBRIGHT, W. S. JEE, G. N. TAYLOR, AND W. R. CLARKE. 1981. Bone loss in the beagle tibia: influence of age, weight, and sex. Calcified Tissue International 33:233–238.

PRANGE, H. D., J. F. ANDERSON, AND H. RAHN. 1979. Scaling of skeletal mass to body mass in birds and mammals. The American Naturalist 113:103–122.

PRIOR, J. C. 1990. Progesterone as a bone-trophic hormone. Endocrine Reviews 11:386–398.

REDFORD, K. H., AND R. M. WETZEL. 1985. Euphractus sexcinctus. Mammalian Species 252:1–4.

RHO, J. Y., M. C. HOBATHO, AND R. B. ASHMAN. 1995. Relations of mechanical properties to density and CT numbers in human bone. Medical Engineering & Physics 17:347–355.

ROSSET, A., L. SPADOLA, AND O. RATIB. 2004. OsiriX: an open-source software for navigating in multidimensional DICOM images. Journal of Digital Imaging 3:205–216.

SCHREIBER, J. J., P. A. ANDERSON, AND W. K. HSU. 2014. Use of computed tomography for assessing bone mineral density. Neurosurgical Focus 37:E4.

SPEAKMAN, J. R. 2008. The physiological costs of reproduction in small mammals. Philosophical Transactions of the Royal Society of London, B. Biological Sciences 363:375–398.

SUPERINA, M. 2007. Natural history of the pichi (Zaedyus pichiy) in Mendoza Province, Argentina. Ph.D. dissertation, University of New Orleans, New Orleans, Louisiana.

SUPERINA, M., AND A. M. ABBA. 2014. Zaedyus pichiy (Cingulata: Dasypodidae). Mammalian Species 46:1–10.

SUPERINA, M., AND P. BOILY. 2007. Hibernation and daily torpor in an armadillo, the pichi (Zaedyus pichiy). Comparative Biochemistry and Physiology A 148:893–898.

SUPERINA, M., F. FERNÁNDEZ CAMPÓN, E. L. STEVANI, AND R. CARRARA. 2009a. Summer diet of the pichi Zaedyus pichiy (Xenarthra: Dasypodidae) in Mendoza Province, Argentina. Journal of Arid Environments 73:683–686.

SUPERINA, M., N. CARREÑO, AND G. A. JAHN. 2009b. Characterization of seasonal reproduction patterns in female pichis Zaedyus pichiy (Xenarthra: Dasypodidae) estimated by fecal sex steroid metabolites and ovarian histology. Animal Reproduction Science 116:358–369.

SUPERINA, M., M. M. GARNER, AND R. F. AGUILAR. 2009c. Health evaluation of free-ranging and captive pichis, Zaedyus pichiy (Mammalia, Dasypodidae) in Mendoza Province, Argentina. Journal of Wildlife Diseases 45:174–183.

SUPERINA, M., AND W. J. LOUGHRY. 2012. Life on the half-shell: consequences of a carapace in the evolution of armadillos (Xenarthra: Cingulata). Journal of Mammalian Evolution 19:217–224.

TRIVERS, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 in Sexual selection and the descent of man (B. Campbell, ed.). Aldine, Chicago, Illinois.

TSURUSAKI, K., M. ITO, AND K. HAYASHI. 2000. Differential effects of menopause and metabolic disease on trabecular and cortical bone assessed by peripheral quantitative computed tomography (PQCT). The British Journal of Radiology 73:14–22.

TURNER, R. T., B. L. RIGGS, AND T. C. SPELSBERG. 1994. Skeletal effects of estrogen. Endocrine Reviews 15:275–300.

VICKARYOUS, M. K., AND B. K. HALL. 2006. Osteoderm morphology and development in the nine-banded armadillo, Dasypus novemcinctus (Mammalia, Xenarthra, Cingulata). Journal of Morphology 267:1273–1283.

VULCANO, L. C., F. A. MARQUES DOS SANTOS, AND C. L. BUCHMANN DE GODOY. 2008. Determinação da densidade mineral óssea da extremidade distal do rádio-ulna em gatos: correlação entre peso, sexo e idade. Ciência Rural 38:124–128.