Progeny Status May be a Useful Surrogate if Neuter Status is not Known in Canine Osteoporosis Studies: An Analysis of Dogs in "The Georgie Project"

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Disclosures: The authors have nothing to disclose.

INTRODUCTION: Canine have been studied as a possible translational model for estrogen-deficient osteoporosis induced via gonadectomy, but results of many of these studies show that the canine does not undergo similar bone modeling/remodeling patterns observed in post-menopausal human females [1, 2]. Canines are di-estrus whereas human females are poly-estrus, and canines have significantly lower circulating estrogen levels during estrus cycles than human females [1]. However, some studies show that after gonadectomy canines show significant reduction in bone volume for a short time following gonadectomy [3-6]. A major issue with studies of the hormone-related effects on bone remodeling in canines is that the time period between the surgery and final follow-up is relatively short (< 24months), which seems to be an inadequate to evaluate the long-term effects of decreased endogenous sex hormone levels on bone mass. Additionally, the changes in bone mass/density in post-menopausal human females are gradual [7]. This suggests that observing changes in the canine femur, which is thought to be less dependent on endogenous hormones, within 24 months is an inadequate duration when compared to humans. Unfortunately in our sample of Portuguese Water Dogs (PWDs), the originators of "The Georgie Project" did not have information from the owners if the dog had been castrated or had their gonads intact [10,11]. In this study we addressed this major shortcoming and some of its potential consequences by estimating the neuter status as whether or not the dog had progeny, which can be determined because American Kennel Club (AKC) contracts between breeder and acquirer require that pet-quality dogs be spayed or neutered. The progeny status of our specimens was obtained using each of our PWD's AKC identification number. We sought to elucidate if the estimated gonadectomy/progeny status had a significant influence on various measurements of the femur that are correlated with osteoporosis such as cortical thickness/index and cross-sectional cortical bone area [8, 9].

MATERIALS AND METHODS: PWD carcasses were obtained from "The Georgie Project" [10-11]. With IACUC approval we examined the right femora (total sample= 415, males= 145, females= 207, unknown sex= 63) from the remaining skeletally mature animals that had been donated for these studies by the animal owners. Age range: 2-16 years old. Cortical thicknesses (CT) were measured at the medial, lateral, anterior, and posterior aspects of each bone diaphysis at 50% and 70% of bone length from the condyles. These cross-sections were then scanned and digitally analyzed using ImageJ to find the medullary and cortical areas. Statistical analyses were conducted using the statistical software NCSS (2020). To control for the confounding effects of specimen body size and mass we conducted multiple regression analyses to elucidate the effect of age, sex, and progeny status on the parameters reported. We measured and calculated the medial-lateral cortical index (CI) at the diaphyseal medullary isthmus, which is calculated as the outer extra-cortical diameter (OD) minus the inner endosteal diameter (ID) divided by the OD. The mean cortical thickness (CT) at the level measured was calculated as the average of the medial-lateral CTs and the anterior-posterior CTs.

RESULTS: The multivariate analyses (**Table 1**) indicate that progeny status did not have significant effects on cortical thickness and diameter in either the medial-lateral or anterior-posterior planes, except for the medial-lateral mean cortical thickness at the isthmus (Dist.CT= distal CT) ($p < 0.05$). Most of the variation of the parameters measured was explained by the total length of the femur (Ltot), sex, and/or age. The CI at the isthmus did not show any significant relationship with progeny status, but was most significantly related to sex. Females had on average a significantly lower CI at the isthmus than males when we controlled for size variations of the specimen.

DISCUSSION: Despite the oversight of not knowing the neutering status of the PWDs in The Georgie Project, our results are encouraging because they suggest that neuter-related hormone status likely has negligible effect on the morphological aspects of our osteoporosis-related research on the femora of this model. The results of our study mirror those of previous investigators that have suggested that the canine is a poor model for estrogen and androgynous hormone depleted osteoporosis [1, 5, 12]. This study was conducted to explore the possible effects of neuter status in our sample because we will ultimately study the genetic influences on bone histology in our sample, and hormones can effect gene expression. The statistical significant reduction in the mean Dist.CT showed that on average those specimens that did not give offspring had a lower mean Dist.CT of -0.09mm, which is not a clinically or biomechanically significant reduction. Additionally the model explained little about the variation in the mean Dist.CT, therefore conclusions regarding the model are statistically weak. In other words, our results suggest that there is no clinically or biomechanically significant effect of neutering on the parameters reported in this study, with a positive neuter status being estimated as a specimen that did not have progeny. The large majority of the variation in the measured parameters is explained by specimen body size, age, and/or sex. It would be expected in female PWD underwent similar bone modeling/remodeling patterns observed in post-menopausal women then there would be a significant interaction between sex and progeny status. This was not the case in our sample, and the significant effects of sex are not conditional on whether or not the specimen had progeny. In the near future our lab will obtain bone tissue mineralization and cortical porosity data in hopes to further test the effects of progeny status on bulk and tissue mineralization across the broad age range of our sample.

RELEVANCE/CLINICAL SIGNIFICANCE: The results of this study are important in estimating the hormonal environment of our sample for our future studies into genetic effects on bone histology.

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Table 1

	Adj. R^2	Ltot	Body Mass	Age	Sex	Progeny
70% Bone Length						
Cort.Ar	0.34	**0.3*** **	**0.2*** **	-0.3	**-5.2*** **	-0.9
MD.Ar	0.11	**0.5*** **	0.2	**1.2*** **	0.3	2.1
50% Bone Length						
Cort.Ar	0.32	**0.2*** **	**0.2** **	**-0.6** **	**-7.0*** **	-1.4
MD.Ar	0.13	**0.5*** **	**0.2***	**1.2*** **	1.3	1.4
Medio-Lateral						
Mean Prox.CT	0.03	0.003	<0.001	-0.002	-0.08*	-0.04
Mean Dist.CT	0.16	<0.001	0.004*	**-0.1***	**-0.2*** **	**-0.09***
Anterior-Posterior						
Mean Prox.CT	0.12	0.002	0.005	**-0.05*** **	**-0.3*** **	-0.2
Mean Dist.CT	0.15	0.001	0.005	**-0.04*** **	**-0.4*** **	-0.1