MUSCLE FUNCTION LOCALLY MEDIATES BONE HOMEOSTASIS

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INTRODUCTION

Muscle contractions serve as one of the two major sources of mechanical loading on bones and thereby directly effect bone homeostasis [1]. However, the means by which muscle function serves to maintain bone health is poorly understood. Recently, we observed that acute muscle paralysis induced by Botulinum neurotoxin (Botox) injection rapidly precipitates substantial muscle and bone degradation in mice [2]. As well, preliminary data from the model indicated that individual mice demonstrating the greatest loss of muscle also experienced the greatest bone degradation [3]. Here, we examine the hypothesis that local muscle function is required to maintain local bone homeostasis.

METHODS

Twenty female C57B6 mice (16 wk) were randomized into three groups: 1) saline (S; n = 5), 2) quadriceps and calf Botox injections (QC; n = 5), and 3) quadriceps Botox injection (Q; n = 10). At day zero, each IM injection consisted of either 2.0 unit/100 g body weight of Botox or equal volume of saline. This dose of Botox is within the range approved for human use. All mice were allowed free cage activity and food and water ad libitum for 3 weeks. At sacrifice, the quadriceps and calf wet mass were determined. The right femurs and tibiae were imaged via micro-CT at a 10.5 μm voxel resolution (Scanco Viva CT). Bone volume/total volume (BV/TV, %) are reported for the distal femur epiphysis and proximal tibia metaphysis. At the tibia mid-shaft, a 0.1 mm thick transverse cross section was assessed for cortical volume (Ct.V; mm^3) and endocortical volume (Ec.V; mm^3). Treatment effects were evaluated using ANOVA ($p = 0.05$) while linear regression was used to explore relations between muscle mass and site-specific bone alterations.

RESULTS AND DISCUSSION

Intramuscular injection of Botox induces temporary muscle paralysis by blocking the release of acetylcholine into the neuromuscular junction [4]. In the murine Botox model, lameness is maximal by 3 days post-Botox injection, with gradual restoration of weight bearing occurring within 2 wk. Weight bearing remains compromised compared to control mice for 4 to 6 wk. Muscle mass was significantly diminished at each Botox injection site when compared to S (range: -52% to –60%). In contrast, calf muscle mass in the Q group was diminished –29% (Table 1). Both Botox groups demonstrated similar degradation of femur and tibia BV/TV when compared to S (QC: -52 and -70%; Q: -46 and -51% respectively, $p < 0.05$; Table 1). However, while QC mice demonstrated a 15% loss of cortical volume due to endocortical expansion, the tibae of Q mice were not altered compared to S mice (Figure 1). Quadriceps wet mass was strongly predictive of femoral BV/TV for all Botox mice (QC: $r = 0.995$, $p < 0.001$ and Q: $r = 0.957$, $p = 0.011$), but calf wet mass was related to tibia Ct.V only in the presence of calf paralysis (QC: $r = 0.751$, $p < 0.001$ and Q: $r = 0.334$, $p > 0.05$).

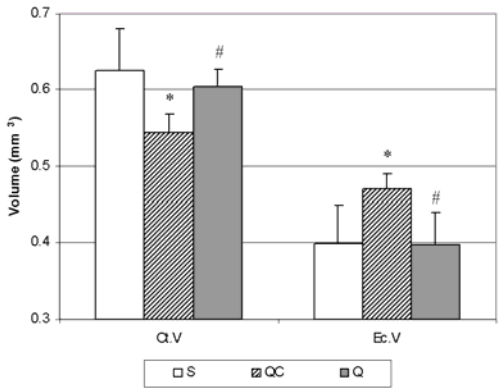

Figure 1: Tibia cortical bone volume and endocortical volume. *QC different from S, #Q different from QC.

The only observed functional difference between the Botox injected groups was that the Q mice continued to perform calf contractions during attempted ambulation, while the QC mice were unable to contract the calf muscles. As such, it is likely that the Q mice maintained a physiologic level of small magnitude muscle contractions in the face of minimal large contractions (due to limited weight bearing). Thus, the absence of tibial cortical bone loss in the Q mice supports our initial hypothesis while implicating small muscle contractions as one mechanism by which muscle function may locally mediate bone homeostasis.

REFERENCES

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ACKNOWLEDGEMENTS

This work was supported, in part, by NIH AR45665 and the Sigvard T. Hansen, Jr. Endowed Chair.

Table 1: Muscle wet mass and bone parameters *QC different from S, ^Q different from S, #Q different from QC, $p<0.05$.

	Muscle Wet Mass (mg)		Femur Epiphysis	Tibia Metaphysis
	R Quadriceps	R Calf	BV/TV (%)	BV/TV (%)
Saline	201.8 ± 7.4	142.6 ± 4.7	27.6 ± 1.5	7.7 ± 1.9
Botox QC	96.0 ± 16.0*	57.4 ± 6.1*	13.3 ± 3.3*	2.3 ± 0.8*
Botox Q	97.4 ± 3.9^	101.1 ± 2.3^#	15.0 ± 2.1^	3.8 ± 0.5^