INTRODUCTION
We have previously demonstrated that transient paralysis of the murine calf muscles causes profound degradation of trabecular and cortical bone in the adjacent tibia within 3 weeks, primarily a result of osteoclastic bone resorption [1]. In more recent studies, we have begun to identify the temporal and spatial nature of this bone loss through acute time course micro-CT (µCT) imaging of trabecular bone resorption (proximal tibia metaphysis) and endocortical bone resorption (tibia diaphysis, [2]). The timing and magnitude of bone resorption differs greatly between the two compartments. Within the metaphyseal region, significant trabecular bone resorption was identified by d.3 (-25.46% reduction in bone volume/total volume: BV/TV) and occurs at locations of high bone turnover nearest the growth plate, suggesting an enhancement of basal osteoclast activity is the initial cellular mechanism underlying trabecular bone loss following muscle paralysis [3]. Bone resorption in the tibia diaphysis, however, occurs much more slowly, with endocortical bone volume (E.Vol) expansion (indicating cortical bone resorption) first identified at d.12 [2].

Given the differences in timing and magnitude of acute bone resorption in the tibia metaphysis and diaphysis and the spatial distance between these compartments, we hypothesized that bone loss in the proximal tibia metaphysis and tibia diaphysis occur through distinct osteoclastogenic events.

METHODS
To assess our hypothesis, we performed two in vivo experiments. In the first study, six female C57B6 mice (16 wk) received IM injections of Botox (2.0 units/100 g) in the right calf immediately following a µCT scan. Additional in vivo µCT scans were obtained on d.6 and d.13, with a terminal scan obtained on d.20. The scan length was 6.3 mm beginning at the distal end of the proximal growth plate and continuing through the midshaft of the tibia. The scanning location and time points enabled determination of whether osteoclast activity temporally and spatially migrated from the metaphysis to the diaphysis or occurred in two distinct events as hypothesized. As the scan volume used encompassed both the highly trabecularized metaphyseal compartment and the diaphyseal compartment lacking trabecular bone, endocortical expansion was the primary outcome measure used to contrast osteoclast activity across these compartments. The scan volume was discretized into six discrete bins (each 1.05 mm) starting at the growth plate and E.Vol calculated within bins (Fig 1A). Alterations in E.Vol occurring between each µCT scan were integrated within each bin to assess temporal osteoclast activity.

In the second experiment we determined if the magnitude of bone loss initiation within the diaphysis was independent of the magnitude of bone loss initiation in the metaphysis following transient muscle paralysis. Previously, we reported that young mice (6 wk) demonstrated a rate of trabecular bone loss following calf paralysis that greatly exceeded that of adult mice [4]. Here, we examined the magnitude of endocortical bone resorption in the same specimens. Specifically, ex vivo scans of the tibia midshaft were obtained 14 days following transient muscle paralysis for experimental and contralateral tibias and alterations in the endocortical volume were calculated versus contralateral controls (previously shown to have no change in endocortical volume in contralateral limb following muscle paralysis, [1]).

In the first experiment, statistical significance in the metaphyseal and diaphyseal regions were analyzed separately (p<0.05). Specifically two-way ANOVAs, with bin location and scanning time periods being the main effects, were calculated for each compartment with TUKEY post-hoc analysis. In the second experiment, significant differences were determined using unpaired T-tests (p<0.05).
RESULTS AND DISCUSSION
Consistent with previous data in adult mice, osteoclast activity within 6 days of muscle paralysis was profound and occurred predominantly in the proximal tibia metaphysis nearest the growth plate (Fig 1B-black bars). Osteoclastic activity within this bone compartment was significantly reduced at later timepoints. In contrast, osteoclast activity in the diaphyseal region occurred almost entirely within the d.6 to d.13 time period, with significantly decreased bone resorption in the preceding and trailing time periods (p<0.01). Within this time period bone loss was not significantly different between bins.

In young growing mice, the magnitude of bone resorption 3 days following muscle paralysis was significantly enhanced versus adult mice (significant decrease in BV/TV, Fig 1C). However, there was no significant difference in the magnitude of endocortical diaphysis expansion between young and adult mice (Fig 1C).

Taken together, these data suggest that two distinct osteoclastogenic events mediate bone loss in the metaphyseal and diaphyseal compartments. In contrast with metaphyseal bone loss, there was no effect of spatial location on bone loss magnitude in the diaphysis, suggesting osteoclast migration between the compartments did not occur. The young animal data in which increased metaphyseal bone loss did not correlate with increased diaphyseal resorption also supports this thesis. The timing and spatial homogeneity of bone resorption within the diaphyseal region suggests a distinct osteoclastogenic event from that driving acute trabecular resorption. Given that the timing of bone loss initiation in this compartment (d.6 at the earliest) correlates well with the amount of time required to form osteoclasts from monocyte/macrophage precursors in vitro and in vivo [5,6], one possible explanation for these data is that profound osteoclastogenesis within the marrow cavity drives a secondary wave of bone resorption.

CONCLUSIONS
Initial bone resorption following paralysis is achieved through an increase in basal osteoclast activity within the proximal tibia metaphysis, while profound de novo osteoclastogenesis within the bone marrow is the predominant mechanism driving resorption elsewhere. This data suggests the presence of two distinct osteoclastogenic pathways that may be targeted to prevent bone resorption in models of severe muscle dysfunction such as spinal cord injury or sarcopenia.

REFERENCES

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