INTRODUCTION
Enhanced skeletal fragility associated with aging occurs in parallel with diminished muscle function (1). However, it is unknown whether acutely diminished muscle function in the elderly precipitates an equivalent loss of bone mass as occurs in young skeletons. Given that muscle strength has already substantially declined in the elderly, it is reasonable to speculate that further diminishment of muscle function by acute paralysis would be less influential in contributing to decreased bone mass than paralysis in a young adult skeleton. We have recently demonstrated that acute hindlimb muscle paralysis induced by Botox provokes a precipitous loss of bone in the young adult mouse (2). As older mice, like humans, demonstrate reduced bone morphology and diminished muscle function compared to young adult mice, we hypothesized that the aged skeleton would be less sensitive to bone loss associated with acute muscle paralysis.

METHODS
Female C57Bl/6J mice of two ages (16 wk vs 2 yr) were contrasted in this study. Mice of both ages were randomly assigned to either saline (16 wk: n=10; 2 yr: n=6) or Botox (16 wk n=10, 2 yr: n=6) groups. At day zero all mice received IM injections of saline (10 µl) or Botox (10 µl 0.2 unit/100 g) in both the quadriceps and calf of the right leg. Following sacrifice, quadriceps and calf wet weights were determined (the tibia of one 2 yr saline mouse fractured during dissection and was not included in further analyses). The right tibia of each mouse was imaged via micro-CT at a 10.5 µm voxel nominal resolution. Analyses were performed at the proximal tibial metaphysis of each tibia to determine tissue volume (mm³), bone volume (mm³), bone volume/tissue volume (BV/TV, %), trabecular number (#/mm), thickness (mm), and spacing (mm). At the tibia mid-diaphysis, a cortical bone site, periosteal volume (mm³), cortical volume (mm³), endocortical volume (mm³), cortical thickness (mm), and cortical thickness (mm) were each quantified. Due to non-equal group size, comparisons were performed using non-parametric Mann-Whitney tests (p=0.05).

RESULTS
At the tibia metaphysis, the mean (± S.D.) BV, TV, BV/TV, trabecular number, thickness and spacing did not differ statistically in the saline-treated limbs of 16 wk and 2 yr mice. Trabecular number was increased 25.5% in 2 yr old mice (4.23 ± 1.31 vs 3.37 ± 0.23 #/mm, p=0.13), while cortical bone volume was therefore achieved primarily via endocortical expansion (16 wk: 16.3%, p<0.001; 2 yr old: 9.9%, p<0.02). Cortical thickness was similarly diminished by Botox treatment in 16 wk (-19.5%, p<0.001) and 2 yr old mice (-15.9%, p<0.001). According to our hypothesis, however, acute hindlimb muscle paralysis induced equivalent losses in both trabecular and cortical bone in 2 yr old mice relative to respective saline mice. Startlingly, the mean cortical thickness of the 2 yr old mice following Botox treatment was only 2.7 fold greater than the mean trabecular thickness found in a 16 wk old mouse. In both the 16 wk and 2 yr old mice, micro-CT data suggested that bone alterations were primarily achieved via substantial osteoclastic activation, but this observation remains to be confirmed via histomorphometry. These data do suggest that whatever role muscle function plays in maintaining bone homeostasis, age induced musculo-skeletal alterations do not mitigate this effect. In fact, given that the aged skeleton already demonstrates reduced structural capacity, the superimposition of further degradation may extract a non-linear structural cost. Given that the aged are more likely to undergo acute bouts of diminished muscle function due to illness or surgery, such events may serve to incrementally elevate fracture risk, similar to the structural consequences of menopause.

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References

Figure 1. The mean (S.D.) ratio of tibia metaphysis bone volume to tissue volume (BV/TV, %, a) and mid-diaphysis cortical volume (Ct.Vol, mm³, b) in the right tibia of 16 wk and 2 yr old saline and Botox treated mice. Ct.Vol, but not BV/TV, was significantly diminished in older vs younger saline mice (*, p<0.001). Acute hindlimb muscle paralysis significantly diminished both BV/TV and Ct.Vol in both 16 wk and 2 yr old mice (±, all p < 0.01).