INTRODUCTION
Myostatin (GDF-8) suppresses muscle growth, development, and regeneration, and mice lacking myostatin show a significant increase in muscle mass. We have found that the receptor for myostatin, ActRIIB, is expressed in bone marrow derived stem cells, and mice lacking myostatin show increased bone density and strength. It is also known that myostatin is expressed in the fracture callus early (<48 hrs) in the healing process, but the role of myostatin in fracture repair is not clear. Myostatin can circulate in an inactive form, bound to a propeptide from which it must be cleaved to form an active ligand. A recombinant myostatin propeptide effectively inhibits active myostatin in vitro and in vivo. We used a fibula osteotomy model in mice to test the hypothesis that blocking active myostatin with systemic injections of a recombinant myostatin propeptide would improve bone repair.

METHODOLOGY
Adult mice, 4-6 months of age, were included for study. The sample utilized here includes 12 mice (6 male, 6 female) injected i.p. with recombinant propeptide at a dose of 20 mg/kg and 12 control mice (6 male, 6 female) injected i.p. with saline. Fibula osteotomy was performed on the left leg under isofluorane anesthesia. Treatments were administered immediately following osteotomy, 5 days following surgery, and 10 days following surgery. Recovery of musculoskeletal function was assessed 48-72 hours after surgery using rotarod and open field testing. Animals were sacrificed according to IACUC-approved procedures 5 days after the last treatment (15 days after surgery). Mice were weighed, blood collected via cardiac puncture, and the left quadriceps femoris and triceps brachii muscles weighed. The extensor digitorum longus (EDL) and soleus muscles were snap frozen, sectioned using a cryostat, and stained for fast- and slow-twitch muscle fibers. Muscle fiber cross-sectional areas were then measured using image analysis software. Serum osteocalcin was measured using immunonasays. The left hindleg was fixed in buffer for formalin, transferred to 70% ETOH, and then radiographed using a FAXITRON x-ray cabinet. The fracture callus was then imaged using microCT, and bone volume calculated from microCT images 0.5 mm either side of the callus center.

RESULTS
ANOVA s demonstrate that mice treated with the myostatin propeptide were ~7% heavier than saline-treated mice (Fig. 1a), and muscle mass of the propeptide-treated animals was ~18% greater than that of saline-injected mice (P<0.01, Fig. 1b). Analysis of muscle fiber areas showed that there was no change in areas of slow-twitch fibers in the soleus, but size of fast-twitch fibers was increased by ~18% in the EDL of propeptide treated mice. Propeptide- and saline-treated mice did not differ from one another in rotarod performance, free cage activity (measured either as total distance traveled, or ambulatory counts), or serum osteocalcin. Callus bone volume was, however, increased by approximately 20% in the propeptide-treated mice (Fig. 2).

DISCUSSION
The morphological and molecular events involved in the process of fracture healing are relatively well understood. The process can be separated into three general phases: an initial inflammatory phase, a chondrogenic phase, and an osteogenic phase. The role of early GDF8 (myostatin) expression in the callus during the inflammatory phase of fracture healing has been difficult to interpret since this factor is most well known for its effects on myogenesis. As noted earlier, the receptor for myostatin is expressed in bone-marrow derived stromal cells. Myostatin can stimulate adipogenic differentiation in mesenchymal stem cells whereas its absence increases osteogenic differentiation. Myostatin regulates myogenic differentiation in part by suppressing the expression of myogenic factors such as MyoD, but it also inhibits myoblast proliferation by increasing levels of p21. We previously showed that mice lacking myostatin showed an enlarged fracture callus following fibula osteotomy compared to normal mice, suggesting that the early expression of myostatin in the callus is likely to suppress the proliferation and recruitment of progenitor cells at the site of injury. Data presented here using a myostatin propeptide support this hypothesis by showing that fracture healing is enhanced with a myostatin inhibitor. While myostatin is most well known for its effects on muscle development, it is also clear that myostatin plays a significant, direct role in bone formation and regeneration.

REFERENCES

Funding for this research was provided by the Office of Naval Research.