Elevated Myostatin Levels in Patients with Liver Disease: A Potential Contributor to Skeletal Muscle Wasting

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Loss of skeletal muscle mass is a poorly understood complication of end-stage liver disease (ESLD). Based on recent stem cell literature, we hypothesized that the potent negative regulator of muscle mass, myostatin, could play a role in the muscle loss associated with ESLD. In this preliminary investigation, we measured myostatin levels in patients undergoing liver transplant evaluation, using a novel enzyme-linked immunosensitivity assay. Myostatin levels were significantly elevated in patients with ESLD compared with healthy controls. These data suggest that myostatin deserves further investigation as a target for therapies designed to preserve muscle mass in patients with ESLD. (Anesth Analg 2010;111:707–9)

Loss of skeletal muscle mass is a common complication of end-stage liver disease (ESLD) and contributes to weakness in liver transplant patients. Recent discoveries in the complex regulation of muscle mass have not been extended to the study of ESLD patients. In particular, myostatin, a member of the transforming growth factor β superfamily, is an extremely potent negative regulator of muscle mass.[1] In this preliminary study, the first in patients with liver disease, we found that myostatin levels are elevated in patients with ESLD.

METHODS

The study was conducted with Emory IRB approval after informed consent. Nutritional status was evaluated using a widely applied nutritional assessment instrument, the Subjective Global Analysis or SGA.[2] Ten milliliters of venous blood was collected from 36 adult patients presenting for liver transplant evaluation and from 6 healthy volunteer controls. Plasma was frozen at −80°C until analysis.

For enzyme-linked immunosensitivity assay (ELISA), recombinant bovine myostatin (used to prepare the standard curve) and rabbit antimyostatin antibody were provided by Drs. Kambadur and Nicholas, who developed the assay. Assays were done in duplicate in 96-well plates (Maxisorp; Nunc, Rochester, NY). Wells were coated with 2 μg/mL myostatin in 0.05 M bicarbonate buffer, pH 9.8. Samples were diluted 1:10 in PBS-T-PVP (0.01 M phosphate-buffered saline with 0.15 M NaCl, 0.05% Tween-20, and 0.1% polyvinylpyrrolidone [PVP], pH 7.4) in a "mixing" plate (Invitrogen, Carlsbad, CA) with antimyostatin (1:50,000), then incubated overnight (4°C).

After 3 washes, ELISA plates were incubated with PBS-T-PVP for 30 minutes and washed with PBS-T. Solution from the mixing plate was transferred to the ELISA plate and incubated for 2 hours, then washed. Goat anti-rabbit immunoglobulin G secondary conjugated with horseradish peroxidase (1:5000; Dako, Carpinteria, CA) was incubated for 2 hours, followed by PBS-T and PBS washes.

The peroxidase substrate, TMB (3,3′,5,5″-tetramethylbenzidine), was prepared (12.5 mL 0.1 M sodium acetate buffer, pH 5.5 + 125 μL 10 mg/mL TMB in dimethyl sulfoxide), then 125 μL of peroxide (38 μL H_2O_2 in 2.5 mL dH_2O) was added. One hundred microliters of TMB was added to each well, followed by incubation on a shaker for 30 minutes. The reaction was stopped with 50 μL 2 M sulfuric acid/well. Absorbance readings at 450 nm Spectrofluor Plus (Tecan, San Jose, CA) were converted into μg/mL using the standards curve. Because of complex myostatin binding characteristics, these concentrations should be considered estimates but the relative values between groups are reliably captured in the assay.

Data are expressed as mean ± SD. Normally distributed data were compared using Student *t* tests. Because myostatin levels in the study group were not evenly distributed about a mean, the Kolmogorov-Smirnov statistic was used to obtain *P* values. The Kolmogorov-Smirnov statistic is used to compare data without constraining either data set to normal (Gaussian) distribution.

RESULTS

Table 1 shows demographic and laboratory data from the study group. Based on the SGA, 53% of these patients were well nourished, 41% moderately malnourished, and 6% severely malnourished.

The central finding of this study is shown in Figure 1. Mean myostatin levels in patients with ESLD were 4 times those in normals (mean ± SD): 0.53 ± 0.11 vs 0.13 ± 0.03 μg/mL ($P = 0.002$). Myostatin levels of the healthy volunteers were tightly clustered, with minimal overlap of the values from healthy versus ESLD groups.

Because the myostatin assay was colorimetric, it was conceivable that variations in plasma color due to bilirubin

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Accepted for publication May 19, 2010.

Supported by the Department of Anesthesiology, Emory University.

Disclosure: The authors report no conflicts of interest.

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DOI: 10.1213/ANE.0b013e3181eac1c9

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Table 1. Basic Information on Liver Transplant Candidates (Study Group)

Demographic/laboratory data	Mean ± SD
Age (y)	53 ± 8
Male:female ratio	25:11
Body mass index (kg/m^2)	29.8 ± 6.6
Ideal body weight (%)	132 ± 30
Albumin (g/dL)	2.8 ± 0.5
Total bilirubin (mg/dL)	3.2 ± 2.9
Creatinine (mg/dL)	1.2 ± 1.3
INR	1.6 ± 0.5
MELD	16 ± 6

INR = international normalized ratio of the prothrombin time; MELD = Model for End-stage Liver Disease score.

Data are from 36 liver transplant candidates. Ideal body weight calculation for men was 50 ± 2.3 kg/in. over 5 ft., and for women, 45.5 ± 2.3 kg/in. over 5 ft.

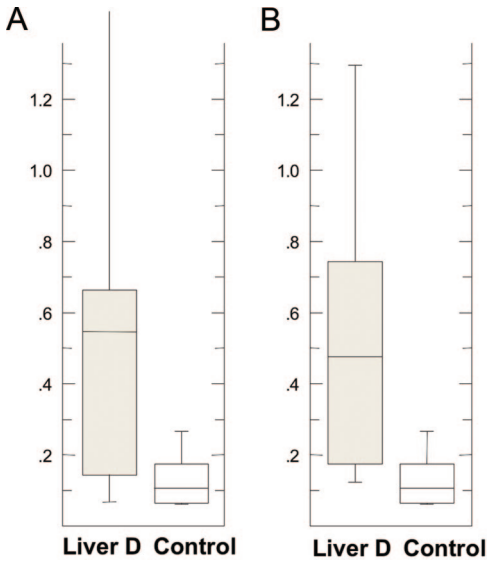

Figure 1. Myostatin levels in patients with liver disease (D) versus healthy controls. A, Myostatin levels determined by enzyme-linked immunosensitivity assay in patients with end-stage liver disease (ESLD) (left bar) are significantly elevated compared with levels in a group of healthy controls (right bar). B, As a technical control, myostatin levels were measured in ESLD patients with a total bilirubin <1.4 mg/dL (left bar). Mean myostatin levels and distribution were similar to levels in the entire ESLD group. Error bars depict the range.

could have caused an overestimation of myostatin levels. Therefore, we compared myostatin levels between the control group and a subset of the ESLD group with normal total bilirubin (<1.4 mg/dL). That assay (Fig. 1B) yielded similar results to those obtained from the whole study group, confirming that bilirubin did not significantly affect myostatin measurements.

We tried to determine whether particular diagnoses, laboratory findings, or other factors were significantly associated with high myostatin levels. Men and women had similar levels. Myostatin levels were also not significantly different as a function of diagnosis (comparing hepatitis C/B versus ethanol versus nonalcoholic steatohepatitis versus cryptogenic cirrhosis), but a larger study may be required to dissect such differences. Comparison of laboratory values (Table 1) and physical examination findings (body mass index; triceps skin fold, calf circumference, and SGA data) with myostatin levels did not identify a correlation with a particular subgroup of patients based on nutritional status.

DISCUSSION

Our data suggest that myostatin levels are elevated in patients with ESLD. This finding is likely significant, because in all preclinical settings studied to date, increased myostatin levels contribute to muscle loss although the mechanism of action is not fully understood. Some studies emphasize the antiproliferative effect of myostatin on muscle stem cells.[3] Others suggest the mechanism of action is inhibition of protein synthesis independent of stem cells,[4] in part through Akt regulation,[5] ultimately leading to proteasome-mediated breakdown. In a rat portocaval shunt model, muscle loss was associated with increased myostatin levels, decreased insulin-like growth factor-1 (IGF-1 is a positive regulator of muscle mass), and impaired stem cell function.[6]

Muscle loss secondary to liver disease is the result of increased muscle protein breakdown and decreased liver and muscle protein synthesis.[7] Liver disease is characterized by global hormonal dysregulation because most hormones are metabolized in liver; muscle loss in this setting is attributed to hormonal disruption (particularly insulin resistance) and to cytokines including adipokines. The complex underlying mechanism is acknowledged to be poorly understood, and myostatin is likely only one of many mediators yet to be linked to ESLD-associated muscle wasting.

Increased myostatin levels in patients with ESLD may have implications beyond skeletal muscle. Because myostatin knockout animals have both increased muscle and bone mass,[8] myostatin may also be a clinically relevant negative regulator of bone mass. Osteopenia and frank osteoporosis are well-known complications of ESLD.[9] Because of the potency of myostatin in causing muscle loss, several therapeutic candidates are in development to block the effects of myostatin in various chronic diseases.[10,11] Given the benefit of antimyostatin strategies in preclinical models, our study suggests that antimyostatin therapies deserve further exploration in the setting of ESLD.

AUTHOR CONTRIBUTIONS

PG conducted assays and statistical analyses and helped write the manuscript; AC conducted patient interviews and collected samples; RK and GN provided reagents and expertise for the ELISA; and MC designed and supervised the study, and wrote the IRB protocol and manuscript. All authors contributed to the final manuscript preparation and agree with its contents.

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