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Changes in Serum Myostatin Levels in Alcoholic Hepatitis Correlate with Improvement in MELD

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Abstract

Background: Alcoholic hepatitis (AH) is a serious clinical syndrome often associated with muscle wasting. Myostatin, a member of the transforming growth factor β superfamily, has been studied in diseases with muscle wasting, however, the role of myostatin in AH is unknown.

Aims: To investigate the association between myostatin, clinical variables and outcomes in AH.

Methods: We analyzed data for cases of AH and controls of heavy drinkers (HD) in TREAT001 (NCT02172898) with serum myostatin levels (AH: n=131, HD: n=124). We compared characteristics between the two groups at baseline, 30-, and 90-days and explored correlations between myostatin and clinical variables. We then modeled the relationship of myostatin to other variables, including mortality.

Results: Baseline median myostatin was lower in AH compared to HD (males: 1.58 vs 3.06ng/ml, $p<0.001$; females: 0.84 vs 2.01ng/ml, $p<0.001$). In multivariable linear regression, bilirubin, WBC and platelet count remained negatively correlated with myostatin in AH. AH females who died at 90-days had significantly lower myostatin but in a multivariable logistic model with MELD and myostatin, only MELD remained significantly associated with 90-day mortality. During 1-year follow-up, AH cases (n=30) demonstrated an increase in myostatin (mean, 1.73ng/ml) which correlated with decreasing MELD scores ($\rho=-0.42$, $p=0.01$).

Conclusions: Myostatin levels are significantly lower in AH compared to HD and are negatively correlated with total bilirubin, WBC, and platelet count. Myostatin increased as patients experienced decreases in MELD. Overall, myostatin demonstrated a dynamic relationship with AH outcomes and future studies are needed to understand the prognostic role of myostatin in AH.

Keywords

Alcoholism; Biomarker; muscle wasting; TREAT001

Introduction

The prevalence of alcoholic liver disease (ALD) in the United States is increasing due to increasing trends of alcohol consumption [1,2]. This increased prevalence is accompanied by an increase in mortality in ALD patients [3]. ALD encompasses a spectrum of liver disorders ranging from hepatic steatosis to alcoholic hepatitis (AH), and alcoholic cirrhosis [4]. AH is a serious clinical syndrome that results from chronic alcohol abuse and is characterized by specific symptoms such as jaundice, liver failure and non-specific symptoms such as muscle wasting [4,5]. Prognostication and treatment of AH have been challenging as there are many prognostic models with varying degrees of success, and few (if any) effective treatments [6-9]. Due to these shortcomings, there is a consensus that biomarkers can aid in our understanding of AH [10].

Myostatin could be a biomarker of value in AH. Myostatin, a member of the transforming growth factor-beta (TGF-β) superfamily, plays a key role in the homeostasis of muscle mass. Myostatin is produced largely by skeletal muscle and is a potent negative regulator of muscle mass [11]. Elevated serum myostatin has been shown to induce muscle wasting [12]. In fact, myostatin has been studied in a number of different settings in which muscle wasting plays a critical role such as aging, heart failure, and chronic obstructive pulmonary disease [13-16]. In liver disease, there is a growing recognition of the impact of muscle wasting on prognosis, therefore myostatin has been the subject of a few studies [17-19]. Higher serum myostatin in patients presenting for transplant evaluation were observed compared to controls [20] and were associated with mortality over a 7 year follow-up [21]. However, a more recent study showed that men with chronic liver disease and low myostatin levels had higher mortality [22]. In addition to these contradictory results on myostatin and liver disease, there are no such studies in AH.

Given the prominence of muscle wasting in ALD compared to other liver diseases [23,24], recent evidence of the negative impact of sarcopenia on outcomes in AH [25], and animal data on alcohol-induced muscle wasting through a myostatin mediated mechanism [26], the profile of myostatin in AH warrants investigation as better understanding of myostatin may present opportunities for more accurate prognostication or potential targets for therapy. To address this gap in the literature, we conducted an analysis on serum samples from TREAT001 (NCT021728978) which is a prospective observational case-control study with AH cases and heavy drinking (HD) controls. We hypothesized that myostatin levels are altered in those with AH and are associated with key clinical features of AH.

Methods:

Study Cohort

The analyses were performed post-hoc on serum samples obtained from subjects who were recruited as part of the TREAT001 study, funded by NIH 3U01AA021840-07S1. TREAT001 is a prospective registry of AH cases and HD controls that were enrolled and followed up over the course of 3 visits from May 2013 to May 2016. TREAT001 enrolled participants after obtaining written informed consent, was conducted in accord with the declaration of Helsinki, and was approved by the Indiana University IRB. Of the 271 AH cases and 196 HD controls in the registry, 131 and 124 had available myostatin levels and were included in the study (Figure 1). The registry contains detailed data pertaining to participant demographics, alcohol use (reported in daily drinks with each drink containing 17 grams of alcohol), alcohol abstinence (self-reported over past 30-days, quality of life measures, anthropometric measures, laboratory values, and outcomes [27]. The diagnosis of alcoholic hepatitis was based on the following criteria: (1) average daily ethanol consumption of more than 40 g/day for women and more than 60 g/day for men for a minimum of 6 months and within the 6 weeks before study enrolment and (2) total bilirubin concentration greater than 2 mg/dL and aspartate aminotransferase concentration greater than 50 U/L [27]. Heavy drinking controls were those with: (1) an average daily alcohol consumption as well as a duration of drinking similar to that of AH cases but (2) with a normal hepatic panel, (3) no prior history of alcoholic liver disease and (4) an absence of hepatosplenomegaly or stigmata of chronic liver diseases. AH cases were recruited from Indiana University Hospital, Sidney & Lois Eskenazi Hospital (Indianapolis, IN), Mayo Clinic (Rochester, MN), and Virginia Commonwealth University (VCU) (Richmond, VA). HD controls were recruited from the Fairbanks Drug and Alcohol Treatment Center (Indianapolis, IN). They were at least 21 years old without underlying medical illnesses, such as chronic obstructive pulmonary disease, congestive heart failure, diabetes, cancer, or chronic renal failure [28].

Measuring myostatin levels

Serum samples were collected and stored in a freezer at −80°F. Sample mature myostatin (GDF-8) levels were measured in duplicate according to the manufacturer's instructions using the Quantikine ELISA GDF-8/Myostatin Immunoassay (Cat. #DGDF80) from R&D Systems, Inc. (Minneapolis, MN). Briefly, 100 µl serum samples were incubated with 50 µl 1 N HCl for 10 minutes at room temperature, before neutralizing with 1.2 N NaOH/0.5 M HEPES (sample final dilution 1:2). Standards were prepared to generate a standard curve from 0-2000 pg/ml. Samples and standards were plated in duplicate with an equal amount of assay diluent and incubated at room temperature for two hours while shaking at 500 RPM. Wells were washed four times before treating with GDF-8 conjugate and incubating another two hours at room temperature while shaking at 500 RPM. After washing four times, freshly mixed color reagents were applied to the wells and a 30-minute room temperature incubation was performed in the dark. Stop solution was next added and plates were read at 450 and 540 nm on a Molecular Devices VersaMax tunable microplate reader using SoftMax Pro software (ver. 4.7.1) (VWR, Radnor, PA). Samples were run in groups of 40 over the course of one year. Data collected from recombinant human myostatin standards and samples were analysed by 4-parameter logistic regression using the online program at www.elisaanalysis.com (elisakit.com, Melbourne, Australia). Serum values were calculated using the standard curve and the dilution factor. The assay has a sensitivity of 2.25 pg/ml and no interference was detected from human Activin RIIA, Activin RIIB, Decorin, GASP-2, GDF-11, or GDF-15 at 50 ng/ml. However, recombinant human Follistatin and GASP-1 have been shown to interfere at levels >10 ng/ml. Additionally, the assay has an intra-assay variation of < 6% and an inter-assay variation of < 7%.

Statistical Analysis

Continuous variables were described using means with standard deviations or medians with interquartile ranges and were compared with t-test or Wilcoxon rank-sum when variables were normally and non-normally distributed, respectively. Categorical variables were described with counts and percentages and compared with chi-square test or Fisher's exact test. Spearman's correlation was used to test correlation between myostatin and other variables. Myostatin levels were transformed logarithmically to be incorporated into linear regression models. Collinearity was accounted for when appropriate. Percent change in serum myostatin per unit change of model variables was calculated as follows: percent change = $100 \times (e^{\beta} - 1)$) where β is the coefficient for the respective variables. For participants who completed the final study visit and had at least 1 previous study visit, we calculated delta myostatin as the difference between myostatin level at the end of the study and the earliest recorded myostatin level. The same was done for MELD score. For predicting mortality, a backward-forward step-wise logistic regression was used to select appropriate variables based on Akaike information criterion (AIC). A simple logistic regression model was then created based on lowest AIC. All statistical tests were two-sided with a predetermined p-value < 0.05 for statistical significance. All analyses were performed using RStudio (Version 1.2.5033) [29]. Graphs displaying the variation of myostatin and other variables over the course of the study were plotted using ggplot2 v.3.2.0 [30].

Results

Myostatin in Males and Females

Myostatin levels were consistently higher in males compared to females in both AH and control participants (Figure 2). In AH males, median serum myostatin was 1.58 ng/ml (IQR 0.73, 3.17) whereas it was 0.84 ng/ml (IQR 0.56, 1.76) in females (p = 0.003). HD males had median myostatin of 3.06 ng/ml (IQR 2.25, 4.08) whereas, in HD females, it was 2.01 ng/ml (IQR 1.66, 3.07, p = 0.001). Based on these findings and the literature which supports that Myostatin behaves differently in males and females [13,16,21], we elected to complete our analysis segregated by gender.

Cohort Characteristics in AH Cases and HD controls in Males

AH and HD male cohort characteristics are displayed in Table 1. The average age was 47 and 45 years for AH and HD, respectively (p = 0.5). There were more whites in the AH group compared to HD (92% vs. 79%, p = 0.047). At study entry, the number of average daily drinks was lower for AH compared to HD (10 vs. 14, p = 0.011). Baseline average BMI was similar between the two groups while average fasting glucose was higher in the AH group (30 vs. 28 kg/m^2, p = 0.1 and 111 vs. 93 mg/dL, p = 0.012). As expected, baseline ALT, AST, total bilirubin, ALP, INR, WBC, Model for End-Stage Liver Disease (MELD) and Discriminate Function (DF) score were all significantly higher in AH compared to HD while hemoglobin and platelets were both significantly lower (Table 1). Fifteen, 53, and 33% of AH males were in Child Pugh class A, B, and C, respectively. Forearm circumference was also lower in AH compared to HD (25 vs. 26 cm, p = 0.028). Baseline median serum myostatin was lower in AH compared to HD (1.58 vs 3.06 ng/ml, p < 0.001). During follow up, 36% of AH males received corticosteroids. At 30 days, 6 (8%) AH males died. This increased to 10 (13%) by 90 days. No HD males died during the 90 day follow up.

Cohort Characteristics in AH Cases and HD controls in Females

AH and HD female cohort characteristics are displayed in Table 1. Average age was 45 and 42 years for AH and HD respectively (p = 0.14). There were fewer whites in the AH group compared to HD (79% vs 90%, p = 0.35). At study entry, the number of average daily drinks was lower for AH compared to HD (7 vs 15 drinks, p < 0.001). Baseline BMI and fasting glucose were both similar in AH and HD (29 vs 28 kg/m^2, p = 0.5 and 110 vs 97 mg/dL, p = 0.6, respectively). ALT, AST, total bilirubin, ALP, INR, WBC, MELD and DF score were all significantly higher in AH compared to HD (p < 0.001). Hemoglobin and platelets were both significantly lower in AH compared to HD (Table 1). Forearm circumference was also lower in AH compared to HD (20 vs 26 cm, p = 0.028). Four, 62, and 1834% of AH females were in CP class A, B, and, C respectively. Median serum myostatin was lower in AH compared to HD (0.84 vs 2.01 ng/ml, $p < 0.001$). During follow-up, 39% of AH females received corticosteroids. At 30 days 6 (11%) AH females died. This increased to 9 (17%) by 90 days. No HD females died during the 90 day follow up.

Correlation of Serum Myostatin level with Baseline Variables

Correlations of baseline serum myostatin levels with baseline clinical variables are displayed in Table 2. In AH males, serum myostatin correlated negatively and significantly with total bilirubin, ALP, WBC, platelet count, and number of average daily drinks, and positively with fasting serum glucose, INR, and forearm circumference. There were no clinically significant correlations in HD males. In AH females, serum myostatin correlated negatively with total bilirubin, ALP, WBC, platelet count, and AST. In HD females, serum myostatin correlated negatively with AST and positively with WBC.

Because serum myostatin correlated with total bilirubin, ALP, WBC, and platelet count in both males and females with AH, we created a linear regression model for log of serum myostatin as a function of these variables and gender. Total bilirubin, WBC and platelet count remained significantly and negatively correlated with serum myostatin (Supplementary Table 1). ALP was not included in the model due to collinearity with total bilirubin. The interaction between gender and serum myostatin was checked and dropped from the model because it was not statistically significant. Since platelets can be affected by both inflammation and portal hypertension, ascites was added to the model as a surrogate for portal hypertension. Platelets remained significant even after the model was adjusted for ascites. In males with AH, we fit a linear model for log of serum myostatin as a function of fasting glucose, INR, average daily drinks, and forearm circumference; none were significant.

Thirty- and 90-day Follow-up

Differences between those who survived and died at 90 days are displayed in Table 3 and in Supplementary Table 2 for those who died at 30 days. At 90 days, only baseline bilirubin and baseline MELD score were significantly higher in the males with AH that died. In females, those that died at 90 days had higher bilirubin, creatinine and MELD but lower myostatin levels than those who were alive. After bidirectional logistic regression, a simple model with MELD score and serum myostatin was created but only MELD score had p-value < 0.05. At 30 days, there were no statistically significant differences in males with AH between those that died vs. survived including in myostatin levels (1.16 vs. 1.64 ng/ml, $p = 0.183$). In females with AH, those that died at 30 days had higher baseline total bilirubin, creatinine, and MELD score but baseline myostatin levels were lower (0.57 vs. 0.89 ng/ml, $p = 0.020$). Using bidirectional stepwise logistic regression with death at 30 days as the outcome, total bilirubin and serum myostatin were chosen to create a simple model; however, only total bilirubin had a p-value < 0.05.

Trends over a One Year Follow-up

Of the 131 AH cases and the 124 HD controls included in the study, 17 with AH and 24 HD were present for the baseline visit, and the subsequent visits at day 180 and 360.

Trends in their serum myostatin levels by AH vs. HD are displayed in Figure 3. For both genders with AH, myostatin levels increased over time in all except 1 female by an average of 3.5 ng/ml (915%) from baseline, while myostatin levels in HD exhibited no remarkable change. While all initial MELD scores were greater than 15, MELD score decreased in 88% (15/17) AH patients with 59% (10/17) decreasing to a score less than 15 (Supplementary Figure 1). Figure 4a depicts these trends in AH males and females by self-reported alcohol abstinence and increase vs. decrease in MELD. There was a trend of larger increase in median myostatin levels in abstinent AH compared to AH cases who continued to drink (day 0 vs. day 360: 1.7 ng/ml, IQR (−0.5, 4.0) vs. 0.29, IQR (−0.1, 1.2), $p = 0.2$). Figure 4b depicts the changes in myostatin by corticosteroid treatment and increase vs. decrease in MELD. Treated males with AH trended towards having higher levels of serum myostatin compared to untreated but this was not statistically significant (day 0 vs. day 360: 4.10 IQR (3.07, 5.56) vs. 1.73 (0.284, 2.22), $p = 0.08$). Of the 20 females with AH that received treatment at the beginning of the study only one followed up with subsequent thus no meaningful analysis could be conducted. While all initial MELD scores were greater than 15, MELD score decreased in 88% (15/17) AH patients with 59% (10/17) decreasing to a score less than 15 (Supplementary Figure 1 and 2).

Of the 131 AH cases and the 124 HD controls, 42 with AH and 59 HD completed day 360 visit and had at least 1 previous visit over the course of this study. Median delta myostatin was 1.3 ng/ml (IQR −0.14, 3.10) for AH and 0.2 ng/ml (IQR −0.5, 1.305) for HD. There were no statistically significant differences in delta myostatin or delta MELD between those that were abstinent at the end of the study and those that were not for both AH and HD. In AH, delta myostatin correlated negatively with delta MELD ($\rho = -0.42$, $p = 0.01$). This correlation was further tested in abstinent and non-abstinent AH cases (Figure 5). It persisted strongly in non-abstinent AH ($\rho = -0.73$, $p = 0.02$) and showed a similar trend in abstinent AH but was not statistically significant ($\rho = -0.36$, $p = 0.06$).

Discussion

To our knowledge, this is the first study to assess myostatin in AH. This is also the first study to describe myostatin levels over a one-year period in both AH and heavy drinkers without AH. In this prospective case control study comparing AH cases to HD controls, we found that serum myostatin was significantly lower in AH compared to HD in both genders. In addition, we show that serum myostatin levels were correlated with total bilirubin, ALP, WBC, and platelet count in both genders with AH. Our year-long longitudinal follow-up demonstrated an increase in serum myostatin levels in most AH cases in parallel with ceasing alcohol intake and experiencing a decline in MELD. Finally, myostatin levels at baseline were not associated with death 30 or 90 days when controlled for total bilirubin and MELD score, respectively.

Our results provide evidence of a correlation between clinical makers of liver disease severity inflammation and low myostatin in AH. In both males and females with AH, myostatin was negatively correlated with markers of AH severity activity and inflammation such as total bilirubin, white blood cell count, and platelet count in multivariable regression. This correlation was not seen in our HD controls due to lack hepatic inflammation in this group. Furthermore, platelet count remained significantly and negatively correlated with myostatin even after adjusting for ascites. Our results are in line with a prior study in hospitalized patients with cirrhosis where serum myostatin was found to correlate negatively with both WBC and C-reactive protein [22]. Such a contextual role of myostatin is plausible because it is a member of the TGF-β superfamily, important modulators of immunity and inflammation [31].

In mice, alcohol intake increases myostatin levels in both muscle and serum [26,32]. Our data do not replicate the mouse data; in our HD males and females as well as AH females, there was no correlation between number of drinks and myostatin levels. In addition, while serum myostatin level was negatively correlated with number of daily drinks in AH males, this the correlation did not persist when adjusted for other variables such as INR, fasting glucose level, and forearm circumference. It is possible we are not able to replicate the mouse data due to lack of non-drinking controls.

Our one year follow up trends showed that myostatin levels increased in subjects with AH as they achieved sobriety and as MELD scores decreased. No such increase was observed in the HD group, most of whom continued to consume alcohol. Corticosteroids did not significantly affect these trends. Though all but one patient with AH had an increase myostatin, some increases were more dramatic than others. It is difficult to definitively determine the reasons behind these trends due to small sample size but our negative correlation between delta myostatin and delta MELD show that patients with greater decreases in MELD score had greater increases in myostatin. This correlation persisted in non-abstinent AH cases with a trend towards a similar correlation in abstinent AH. We did not observe correlations between renal function (measured by serum creatinine) and serum myostatin or 90-day clinical outcomes. In kidney disease, negative correlations between eGFR and myostatin levels have been reported [33] and future studies looking at other makers of renal function in alcoholic hepatitis and their relationship to myostatin and sarcopenia are needed. Given our study design, we cannot determine the true relationship between myostatin levels and AH prognosis, however, our data do support the hypothesis that increasing myostatin levels may herald improvement in AH.

When looking at serum myostatin levels at baseline and 30- and 90-day outcomes, our results showed that low baseline myostatin levels were associated with mortality in females with AH. Low baseline myostatin taken during hospitalization in a cirrhosis cohort was shown to be associated with mortality in males over a median follow-up period of more than 250 days [22]. Conversely, high baseline myostatin levels have been shown to be associated with decreased survival in both men and women with cirrhosis over a 10 year follow up period [21]. This discrepancy between study results most likely stems from the discrepancy in disease etiology in different studies. Our result is in accord with Skladany et al, whose cohort consisted mostly of patients with ALD. That of Nishikawa et al. on the other hand was mostly cirrhosis due to viral infection, with ALD being < 10%. Furthermore, as we have shown correlations between inflammatory makers and myostatin levels, the discrepancy between studies may be due to the acute inflammatory nature of AH in our study participants vs. the chronic liver disease cohorts included in prior studies.

Taken together, it remains unclear if myostatin is playing a direct role in the disease pathogenesis and subsequent mortality in AH, or if myostatin levels in the serum are simply associated with other features of AH such as synthetic function or abstinence from alcohol. Our 1 year follow up data was limited due to small sample size which did not allow us to draw more tangible conclusions on long term myostatin trends. Another limitation of our study is that it does not have a quantitative measure of skeletal muscle mass or body composition data. This prevents us from drawing conclusions regarding the relationship between myostatin, muscle volume and sarcopenia, which can be a prominent feature of AH despite its acute nature. We did show that myostatin only weakly correlated with forearm circumference in males with AH. We are also not able to comment on the associations between presence of liver fibrosis and myostatin levels as the AH diagnosis was made based on widely-accepted clinical parameters [4,34] and not all participants underwent liver biopsy. Underlying advanced fibrosis and cirrhosis are frequently seen in those with AH and it is possible the myostatin levels differ by level of fibrosis. These associations will need to be explored in future studies.

In addition, the impact of alcohol intake on myostatin is not fully elucidated by our data as We also do not have data on healthy non-drinkers were not enrolled during TREAT001 for comparison because our controls were heavy drinkers. While this can be considered a limitation, the use of HD as a comparison allowed us to evaluate the effect of alcohol consumption on myostatin which is largely unknown in humans. Furthermore, our cohort is very well characterized, and the diagnosis of AH is the only difference between cases and HD controls. This allows us to state with confidence that the difference in myostatin between the two groups is indeed due to AH. Finally, heterogeneity between commercially available myostatin assays prevents us from comparing our AH and HD controls myostatin levels with levels from published literature on cirrhosis, other chronic end-stage diseases, or acute inflammatory states. To put our results into perspective, using the same assay, median myostatin levels were significantly higher in a NAFLD cohort enrolled at our institution [male, 3.70 ng/ml (IQR: 2.82, 5.06); females, 2.71 ng/ml (IQR: 1.96, 3.85)] (Are V, unpublished data).

In conclusion, myostatin levels are altered in AH and display a dynamic relationship with markers of disease activity. While our study cannot establish the utility of myostatin in prognostication, our results indicate that low serum myostatin levels are a maker for AH and increasing myostatin levels may herald improvement in AH. Larger studies are needed to confirm our findings and understand the unique role of myostatin in AH and liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AH	Alcoholic Hepatitis
HD	Heavy Drinking
TREAT001	Translational Research and Evolving Alcoholic Hepatitis Treatment 001 study
INR	International Normalized Ratio
WBC	White blood cells
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline Phosphatase
MELD	Model for End-stage Liver Disease
DF	Discriminate Factor
TGF-®	Transforming growth factor ®
GDF-8	Growth Differentiation Factor-8
ELISA	Enzyme-linked immunosorbent assay
AIC	Akaike information criterion

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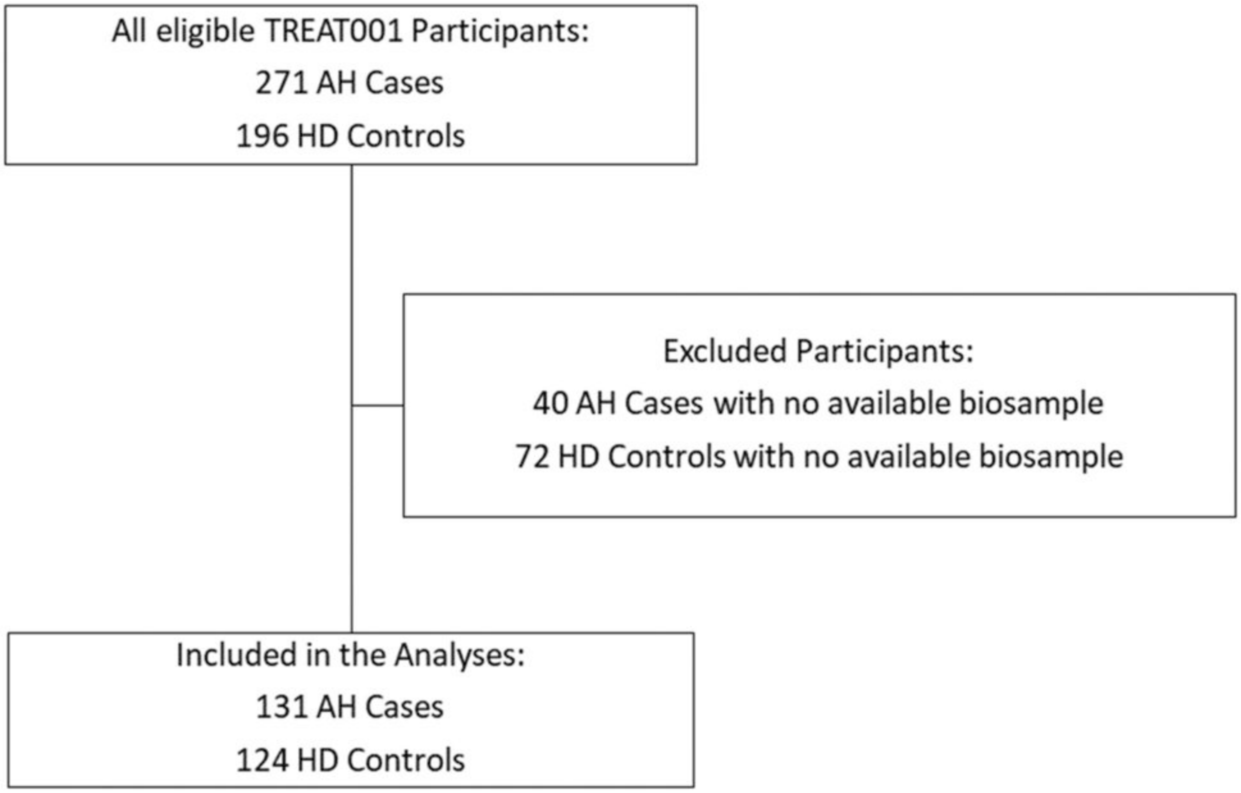

Figure 1. Inclusions and Exclusion Flow Diagram.
Flow diagram showing participants in TREAT001 who were included in the study analyses

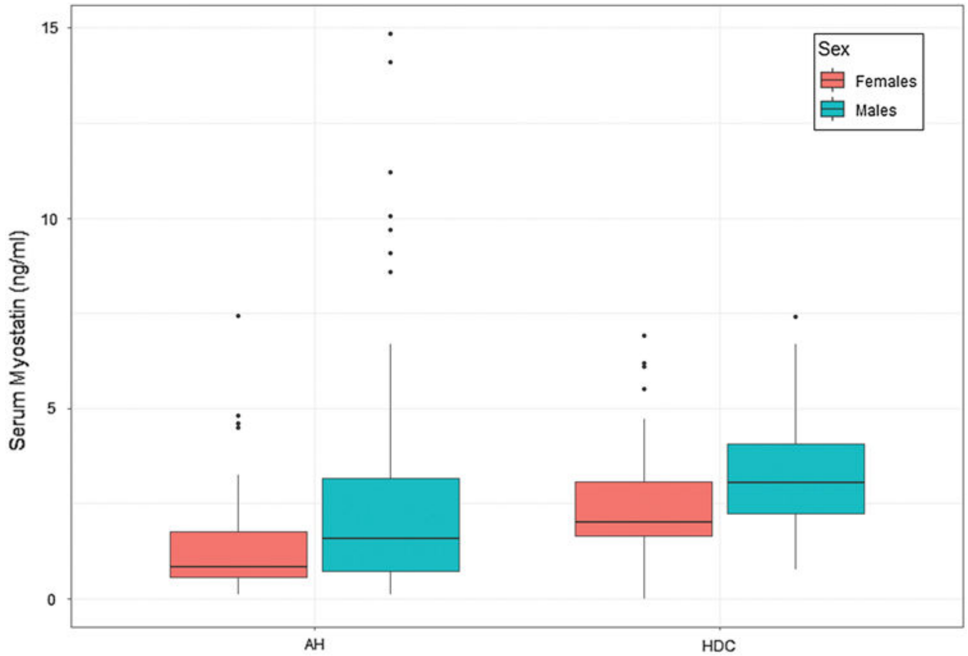

Figure 2. Myostatin Levels in Alcoholic Hepatitis (AH) and Heavy Drinking (HD) Controls at Baseline in Males and Females.

Males had higher myostatin levels than females and HD had higher myostatin levels than AH. *HDC: heavy drinking controls

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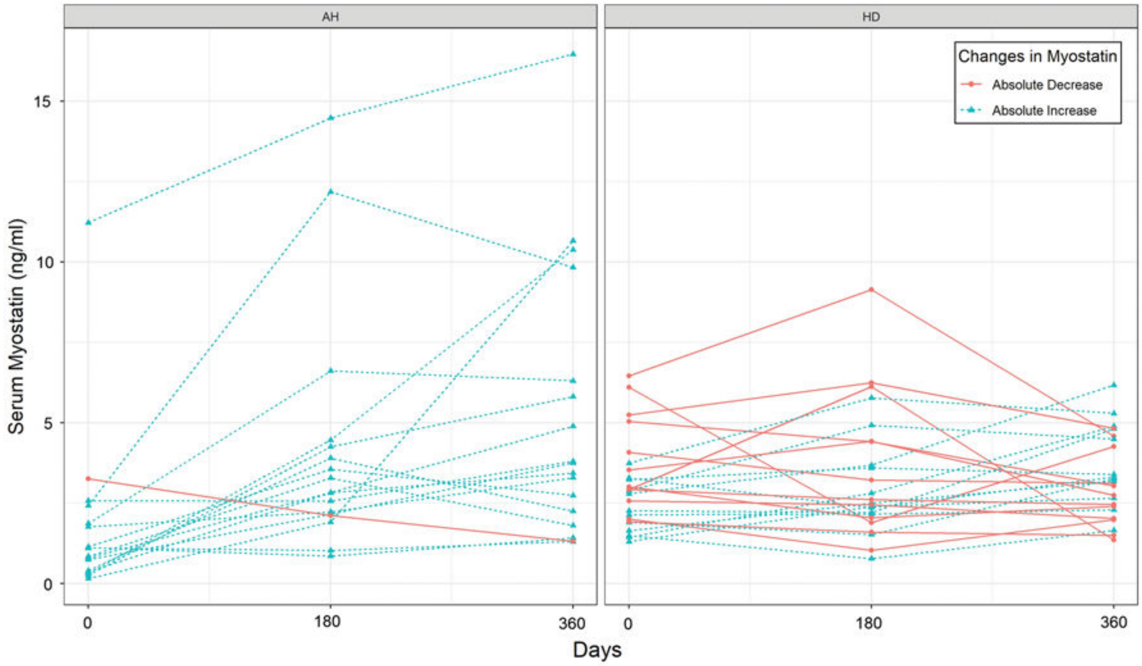

Figure 3. Trends in Myostatin over a One Year Follow up in Alcoholic Hepatitis (AH) and Heavy Drinkers (HD).

Trends in myostatin levels over the one year follow up period showing that AH displayed a trend of increasing myostatin levels while HD did not display a trend.

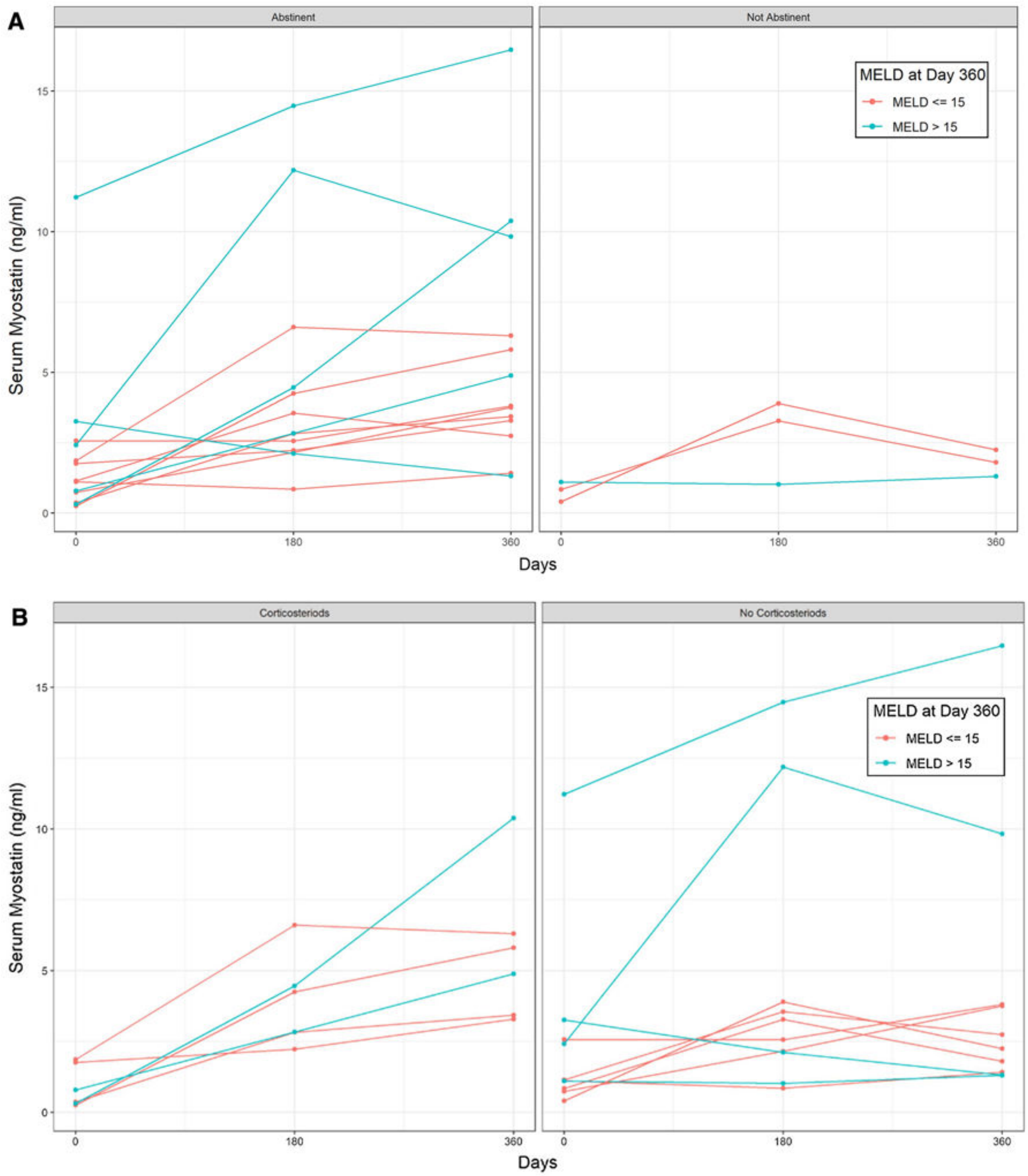

Figure 4. Trends in Myostatin over a One Year Follow up in Females and Males with Alcoholic Hepatitis, Stratified by Abstinence (panel A) or Corticosteroid use (panel B) and Change in MELD.

Trends in myostatin in males and females with AH showing that both genders had a trend to increase myostatin levels while achieving abstinence and decreasing their MELD scores. Trends were not different by corticosteroid use.

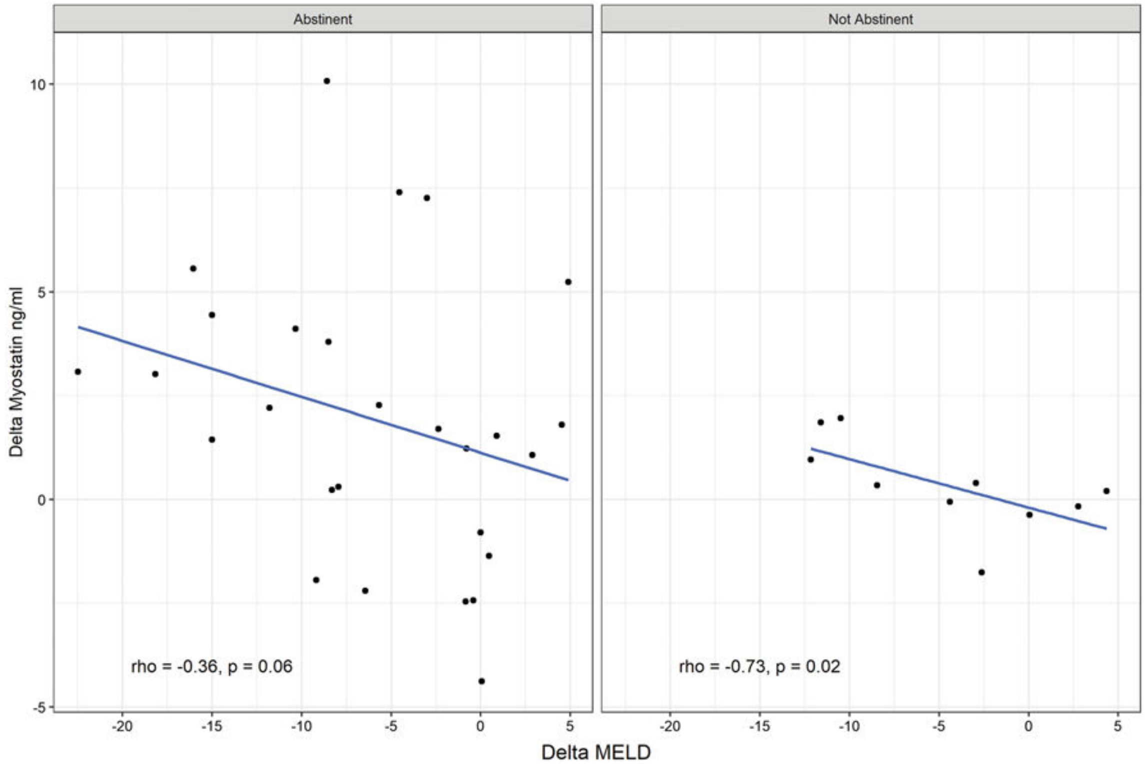

Figure 5. Delta Myostatin as a function of Delta MELD score in Abstinent and Non-abstinent AH Cases.

Scatter plot with a fitted linear approximation showing delta myostatin correlating negatively with delta MELD score in both abstinent and non-abstinent AH cases.

Table 1.

Cohort Characteristics in Cases and Controls Stratified by Gender

	Males (N = 162)			Females (N = 93)		
	AH (N=78)	**HD (N=84)**	**p-value**	**AH (N=53)**	**HD (N=40)**	**p-value**
Age, years	46.7 (12.5)	45.2 (13.5)	0.454	45.2 (9.7)	42.1 (10.7)	0.143
Race			0.047			0.353
White	72 (92%)	66 (79%)		42 (79%)	36 (90%)	
Black or AA	4 (5%)	13 (16%)		7 (13%)	3 (8%)	
Other	2 (3%)	5 (6%)		4 (8%)	1 (3%)	
Daily drinks #	10.3 (8.0)	14.4 (11.7)	0.011	7.1 (5.4)	14.7 (12.0)	< 0.001
BMI, kg/m^2	29.8 (6.5)	28.2 (7.0)	0.130	28.9 (8.0)	28.0 (5.3)	0.526
Fasting Glucose, mg/dL	110.9 (23.6)	92.7 (14.2)	0.012	110.9 (47.8)	97.6 (33.8)	0.599
ALT, IU/L	76.4 (82.8)	29.5 (13.0)	< 0.001	42.3 (20.5)	25.1 (10.2)	< 0.001
AST, IU/L	143.4 (98.5)	30.7 (9.0)	< 0.001	130.2 (71.8)	28.05 (9.4)	< 0.001
Total Bilirubin, mg/dL	13.9 (10.7)	0.5 (0.3)	< 0.001	14.5 (9.3)	0.4 (0.3)	< 0.001
ALP, IU/L	202.1 (170.8)	79.1 (34.9)	< 0.001	196.2 (100.6)	79.3 (25.4)	< 0.001
Hemoglobin, mg/dL	10.5 (2.1)	13.3 (2.4)	< 0.001	9.5 (1.6)	12.1 (1.5)	< 0.001
WBC, x10^9/L	10.1 (5.9)	7.5 (3.0)	< 0.001	12.1 (7.0)	6.4 (2.0)	< 0.001
Platelets, x10^9/L	142.2 (82.6)	241.7 (81.3)	< 0.001	154.9 (81.2)	237.0 (73.0)	< 0.001
Creatinine, mg/dL	0.9 (0.6)	0.9 (0.3)	0.682	0.9 (0.5)	0.8 (0.3)	0.425
INR	1.8 (0.5)	1.1 (0.2)	< 0.001	1.9 (0.4)	1.1 (0.4)	< 0.001
DF Score	51.2 (29.3)	14.1 (10.6)	< 0.001	59.7 (25.8)	17.1 (24.6)	< 0.001
MELD	22.0 (5.5)	7.9 (2.4)	< 0.001	23.5 (4.7)	7.6 (2.6)	< 0.001
Child-Pugh Class			< 0.001			< 0.001
A	11 (15%)	19 (100%)		2 (4%)	9 (100%)	
B	40 (53%)	0 (0%)		33 (62%)	0 (0%)	
C	25 (33%)	0 (0%)		18 (34%)	0 (0%)	
Ascites	40 (51%)	1 (1%)	< 0.001	38 (72%)	1 (3%)	< 0.001
Corticosteroid	28 (36%)	2 (2%)	< 0.001	20 (39%)	0 (0.0%)	< 0.001
Serum Myostatin, ng/ml	1.58 (0.73, 3.17)	3.06 (2.25, 4.08)	< 0.001	0.84 (0.56, 1.76)	2.01 (1.66, 3.07)	< 0.001
Forearm circumference, cm	24.7 (4.3)	26.3 (4.8)	0.028	19.7 (6.2)	24.6 (6.4)	< 0.001
30-day Mortality	6 (8%)	0 (0%)	0.010	6 (11%)	0 (0%)	0.028
90-day Mortality	10 (13%)	0 (0%)	< 0.001	9 (17%)	0 (0%)	0.006

mean number of drinks, each drink containing 17 grams of alcohol

Table 2.

Spearman Correlations for Serum Myostatin and other Variables in Cases and HDC Stratified by Gender

Serum MSTN*	AH Males	HD Males	AH Females	HD Females
Age	0.14	−0.21	0.12	−0.19
BMI	0.18	0.12	0.11	−0.13
Fasting Glucose	0.59¶	−0.08	0.20	−0.60
Daily Drinks #	−0.30¶	0.14	−0.20	0.21
ALT	−0.02	0.13	−0.15	−0.29
AST	−0.22	0.00	−0.33¶	−0.42¶
Total Bilirubin	−0.43¶	−0.08	−0.43¶	−0.30
ALP	−0.31¶	−0.12	−0.29¶	−0.07
Hemoglobin	−0.04	−0.02	−0.22	0.23
WBC	−0.35¶	−0.32¶	−0.50¶	0.39¶
Platelets	−0.40¶	0.07	−0.50¶	0.27
Cr	−0.02	0.20	−0.06	0.20
INR	0.24¶	0.02	0.19	−0.05
DF Score	0.03	0.00	0.05	−0.21
MELD	−0.15	−0.04	−0.10	−0.13
Forearm Circumference	0.31¶	0.15	0.06	0.21

*MSTN: Serum Myostatin (ng/ml)

#mean number

¶p-value < 0.05

Table 3.

Characteristics in Cases Stratified by Death at 90 Days for Males and Females

	Males			Females		
	Dead (N=10)	**Alive (N=68)**	**p value**	**Dead (N=9)**	**Alive (N=44)**	**p value**
Age, years	45.5 (15.8)	46.9 (12.1)	0.749	41.7 (8.7)	45.9 (9.9)	0.237
Race			0.620			0.242
White	10 (100.0%)	62 (91%)		9 (100%)	33 (75%)	
Black or AA	0 (0%)	4 (6%)		0 (0%)	7 (16%)	
Other	0 (0%)	2 (3%)		0 (0%)	4 (9%)	
BMI, kg/m^2	29.7 (6.9)	29.8 (6.5)	0.958	29.3 (7.6)	28.8 (8.1)	0.869
Fasting Glucose, mg/dL	140.5 (33.2)	106.3 (19.7)	0.052	86.8 (11.8)	118.3 (52.5)	0.261
Daily drinks	12.6 (10.5)	9.97 (7.6)	0.333	6.2 (3.0)	7.3 (5.7)	0.621
ALT, IU/L	76.3 (74.4)	76.4 (84.46)	0.997	45.7 (20.3)	41.6 (20.7)	0.591
AST, IU/L	159.9 (117.6)	141.0 (96.1)	0.574	137.1 (90.5)	128.8 (68.5)	0.754
Total Bilirubin, mg/dL	20.5 (9.2)	12.9 (10.6)	0.035	24.3 (7.7)	12.5 (8.4)	<0.001
ALP, IU/L	170.8 (90.7)	206.7 (179.6)	0.539	201.3 (117.4)	195.1 (98.3)	0.868
Hemoglobin, mg/dL	10.3 (3.2)	10.5 (2.0)	0.763	9.2 (1.3)	9.5 (1.7)	0.649
WBC, $x10^9/L$	11.3 (5.4)	9.9 (6.0)	0.489	14.6 (5.1)	11.6 (7.2)	0.242
Platelets, $x10^9/L$	137.2 (55.2)	143.0 (86.1)	0.838	174.2 (69.5)	150.9 (83.5)	0.437
Creatinine, mg/dL	1.0 (0.4)	0.9 (0.6)	0.535	1.3 (0.8)	0.9 (0.3)	0.002
INR	2.0 (0.7)	1.7 (0.5)	0.140	1.9 (0.3)	1.9 (0.5)	0.973
DF Score	76.6 (19.5)	48.8 (29.1)	0.118	71.7 (3.2)	57.6 (27.5)	0.397
MELD	25.9 (3.3)	21.3 (5.6)	0.015	27.7 (4.2)	22.8 (4.5)	0.009
Child-Pugh class			0.101			0.658
A	0 (0%)	11 (17%)		0 (0%)	2 (5%)	
B	4 (40%)	36 (55%)		5 (56%)	28 (64%)	
C	6 (60%)	19 (29%)		4 (44%)	14 (32%)	
Ascites	8 (80%)	32 (47%)	0.052	7 (78%)	31 (71%)	0.657
Corticosteroid	4 (40%)	24 (35.3%)	0.772	6 (67%)	14 (33%)	0.063
Serum Myostatin, ng/ml	1.16 (0.84, 1.89)	1.64 (0.70, 3.29)	0.511	0.56 (0.18, 0.58)	0.89 (0.60, 1.87)	0.006
Forearm circumference, cm	24.0 (4.8)	24.8 (4.3)	0.564	18.8 (7.3)	19.9 (6.0)	0.651