Serum Myostatin Levels are Independently Associated with Skeletal Muscle Wasting in Patients with Heart Failure

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Abstract

Background: It has been reported that skeletal muscle mass and strength are decreased in patients with heart failure (HF), and HF is associated with both reduced exercise capacity and adverse clinical outcomes. Myostatin has been known as a negative regulator of muscle growth, follistatin as the myostatin antagonist, maintaining tissue homeostasis. We thus determined serum myostatin levels in HF patients and whether they are associated with skeletal muscle wasting.

Methods and Results: Forty one consecutive HF patients (58 ± 15 years old, New York Heart Association class I-III) and 30 age-matched healthy subjects as controls (53 ± 8 years old) were studied. Serum myostatin levels were significantly lower in HF patients than controls (18.7 ± 7.4 vs. 23.6 ± 5.2 ng/mL, $P < 0.001$). Circumference of the thickest part of right thigh was significantly small (468 ± 72 vs. 559 ± 37 mm, $P = 0.001$) and lower extremity muscular strength was lower in patients with HF (129 ± 55 vs. 219 ± 52 N×m, $P < 0.001$). Fourteen HF patients (34%) had muscle wasting. By univariate analysis, higher age, higher serum follistatin, and lower serum myostatin were significantly associated with the presence of muscle wasting. By multivariate analysis, serum myostatin levels were independently associated with muscle wasting (OR = 0.77, 95%CI [0.58, 0.93], $P = 0.02$).

Conclusion: Serum myostatin levels were significantly decreased in HF patients and associated with lower extremity muscle wasting, suggesting that myostatin may be important factor for maintaining skeletal muscle mass and strength in HF.

Key Words: heart failure, myostatin, muscle wasting

Introduction

Skeletal muscle abnormalities including impaired muscle energy metabolism, transition of fiber type, and muscle atrophy are frequently observed in patients with heart failure (HF), which is not always related to resting cardiac function, and may contribute to symptoms such as fatigue and dyspnea.[1] These muscle abnormalities are associated with both reduced aerobic exercise capacity and adverse clinical outcomes.[2] It has been also reported that muscle strength and muscle mass are an independent predictor of adverse cardiac event in patients with HF.[3, 4] Muscle strength is closely associated with muscle mass. Therefore, to clarify the regulation of muscle mass in HF is an important issue.

Myostatin, a member of the transforming growth factor-β superfamily maintaining tissue homeostasis, has been known as a negative regulator of muscle growth in mammals and an increase in its expression is reported to be involved in a decrease in muscle mass.[5] Indeed, a child whose myostatin gene naturally occurred a loss-of-function mutation had greater quadriceps muscle in the cross-sectional area assessed by echography than age- and sex-matched controls.[6] Myostatin also plays a crucial role in regulating adult muscle growth and size. Mice with conditional postnatal inactivation of the myostatin gene showed that muscular hypertrophy in skeletal muscle was induced by increasing the size of muscle fibers rather than their number.[7]

It was reported that plasma myostatin levels were shown to be increased in HF patients compared to in healthy controls.[8] However, this study has not reported the association between myostatin level and muscle wasting. On the other hand, in cardiac cachexia, characterized by a severe loss of skeletal muscle, weakness, and exercise intolerance, serum myostatin levels were decreased.[9] Therefore, it is highly

controversial whether myostatin levels were increased or decreased in patients with HF. In the present study, we thus determined whether serum myostatin levels were altered in HF patients and were associated with skeletal muscle wasting.

Methods

Patient Subjects

Forty one consecutive patients suffering from HF (31 men, 58 ± 15 years, left ventricular ejection fraction (LVEF) 32.9 ± 10.8%) and 30 age-matched healthy individuals as controls (26 men, 52 ± 8 years, LVEF 61.9 ± 5.9%) were studied in the present study. HF was diagnosed on the basis of the Framingham criteria by 2 or more cardiologists.[10] Informed consent was obtained from all participating subjects and the protocol, conformed to the ethical guidelines of the Declaration of Helsinki, was approved by the medical ethics committee of Hokkaido University Hospital.

Demographic, Clinical Characteristics, and Body Composition

Causes of HF were determined based on medical information. Body weight and height were measured, and body mass index (BMI) (body weight/[height]^2, kg/m^2) was calculated. Air displacement plethysmograph, termed BOD POD (Life Measurement Instruments, Concord, CA, USA), was used to evaluate body composition. The BOD POD measures total lean body weight and total fat weight, which is highly reliable method in Japanese population and is considered to be accurate as much as Dual Energy X-ray Absorptiometry (DEXA).[11, 12] The appendicular lean body mass (aLBM) was estimated from height and total fat weight as follows:[13]

aLBM (kg) for men= ‐22.48 + 24.14 × height (m) + 0.21 × total fat mass (kg)

aLBM (kg) for women = ‐13.19 + 14.75 × height (m) + 0.23 × total fat mass (kg)

Assessment of Muscle Strength

The knee extension strength was assessed using an isokinetic dynamometer (Multitrace 2, Lectromed, Jersey, Channel Islands). The maximal strength was measured in both legs in a sitting position with the patient's legs hanging freely, the ankle fixed by a pressure transducer. The best of three measurements was used. Arm strength was analysed using the handgrip dynamometer (Saehan Coporation Korea Hydraulic Hand Dynamometer, model SH5001). Likewise, the best of three measurements was used.

Definition of Muscle Wasting

Muscle wasting was defined according to previously published criteria suggested to diagnose sarcopenia. According to previous study, we defined muscle wasting as both an aLBM and the knee extension strength 2SD below the mean of a healthy young reference group aged 18–40 years.[14]

Serum Myostatin and Follistatin, Cytokines, and Biochemistry

Peripheral venous blood samples were collected in serum tubes from all subjects between 6:00 and 9:00 am. All samples were allowed to clot before centrifuged at 1000*g* for 15 min and were stored at -80℃ until analysis.

Serum myostatin levels were determined by a commercially available enzyme immunoassay kit (R&D System, Inc., Minneapolis, USA) according to the manufacturer's protocol as previously described[15] and its detection limit was 20 pg/mL. Serum follistatin levels were determined by a commercially available enzyme immunoassay kit (R&D System, Inc., Minneapolis, USA) according to the

manufacturer's protocol as previously described[15] and its detection limit was 20 pg/mL.

Serum levels of interleukin (IL) -1β, IL-6, and the tumour necrosis factor-α (TNF-α) were analysed using magnetic cytokine assays purchased from Bio-Rad Laboratories GmbH (Munich, Germany), the lower limits of detection being 0.1, 0.1, and 0.4 pg/mL, respectively.

Hemoglobin, serum albumin, fasting blood glucose, and B-type natriuretic peptide (BNP) were also measured. The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine value and age using the Japanese equation as follows: [16]

$eGFR = 194 \times (\text{serum creatinine in mg/dL})^{-1.094} \times (\text{age in years})^{-0.287} \times (0.739 \text{ if female})$.

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated from the fasting blood glucose (FBG) and fasting serum insulin (FIRI) concentrations by the formula: HOMA-IR=FBG (mg/dL) × FIRI (μU/mL) / 405.

All analyses were performed by investigators blinded to clinical information.

Echocardiography

Left ventricular (LV) end-diastolic dimension (EDD) and LV end-systolic dimension (ESD) were measured in the parasternal long axis view by transthoracic echocardiography. LVEF was measured with biplane Simpson's method via the apical 4- and 2- chamber views.[17]

Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing was performed using an upright electromechanical bicycle ergometer (Aerobike 75XLII, Combi Wellness, Tokyo, Japan) with ramp protocol as described previously.[18] Briefly, after 3 minutes of unloaded cycling, the exercise load was increased in 10-15 watt/minute increments in HF patients and 25 watt/minute increments in control subjects to symptom-limited maximal work. Patients stopped exercise when they had severe leg fatigue and/or dyspnea. Oxygen uptake (VO_2) was measured at rest and throughout the exercise period using a 280E Aero-monitor (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). Anaerobic threshold (AT) was determined by the V-slope method, as described previously.[19] Peak VO_2 was defined as the maximal VO_2 attained during exercise.

Statistical Analysis

The effect size was calculated to be 1.355 based on the comparison of the serum myostatin levels between normal subjects and patients with chronic obstructive pulmonary disease by Ju et al.[20] To detect the effect compared with the threshold change of 0 under the conditions of α=0.05, β=0.1 and allocation ratio=1.5 (HF/control), sample sizes of the study patients needed were calculated to be 24 for HF and 16 for control. Data are expressed as means ± SD for continuous variables and as numbers and percentages for categorical variables. Myostatin data was normally distributed as proven by the Shapiro-Wilk test. Student's *t* test was used to compare continuous variables. When data were not distributed normally, the Mann-Whitney *U* test was used. Chi-square test was used to compare categorical variables. Univariate linear regression model was used to determine the correlation between variables and serum myostatin levels. Multivariate linear regression analysis including variables with a p value<0.05 in

the univariate model or clinical parameters was performed to identify the independent variables associated with serum myostatin. All analyses were performed using JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). The differences were considered statistically significant when p values were less than 0.05.

Results

Baseline Characteristics in Controls and in Patients with HF

The baseline characteristics of the study subjects are summarized in **Table 1**. Two groups were matched for age, male to female ratio, and BMI. There were 2 patients with NYHA functional class I, 28 patients with class II, and 11 patients with class III. The etiology of HF was ischemic cardiomyopathy in 11 patients, non-ischemic cardiomyopathy in 30 patients, including idiopathic dilated cardiomyopathy, valvular heart disease and hypertensive heart disease. At the time of the study, patients were treated with angiotensin-converting enzyme inhibitors/angiotensin II type I receptor antagonists (ARB) (89%), β-blockers (93%), and diuretics (78%). Only four control subjects had hypertension and received ARBs (13%). Echocardiographic examination revealed that patients with HF had significantly larger LVEDD and ESD with a significantly reduced LVEF of 32.9 ± 10.8%. Peak VO_2 and anaerobic threshold were also lower in patients with HF.

Serum Levels of Neurohormones, Muscle Mass and Strength

Serum levels of myostatin and follistatin have been shown in **Fig 1**. Serum myostatin levels were significantly decreased in HF patients compared to control subjects (18.7 ± 7.4 vs. 23.6 ± 5.2 ng/mL, P = 0.0027) **(Fig. 1A)**. On the contrary, serum follistatin levels were increased (2.7 ± 1.2 vs. 1.9 ± 0.6 ng/mL, P = 0.0015) **(Fig. 1B)**.

The body composition, muscle strength, and blood biomarkers of the study subjects are summarized in **Table 2**. There were no significant differences in body weight and body fat weight. In contrast, lean body weight was lower in HF patients than

in control subjects. Circumference of the thickest part of right thigh was significantly small (468 ± 72 vs. 559 ± 37 mm, P = 0.001) and grip strength (45 ± 9 vs. 33 ± 11 kg, P < 0.001) and lower extremity muscular strength (129 ± 54 vs. 219 ± 51 N×m, P < 0.001) were significantly decreased in patients with HF compared to control.

Hemoglobin, serum albumin, and eGFR were decreased in HF patients compared to control subjects. There were no significant differences in serum IL-6, TNF-α levels and renin activity between groups. In contrast, IL-1β was decreased in HF patients compared to control subjects. Plasma Ang II and BNP were increased in HF patients compared to control subjects.

Univariate and Multivariate Linear Model of Muscle Wasting in Patients with HF

The relationships between serum myostatin and parameters of muscle strength in lower extremity muscle were investigated. There was a significant positive correlation between serum myostatin levels and lower extremity muscular strength (r = 0.580, P = 0.0003) **(Fig. 2A)**, and circumference of thigh (r = 0.481, P = 0.0022) among all study subjects **(Fig. 2B)**.

By univariate analysis, higher age (OR = 1.06, 95%CI [1.01, 1.14], P = 0.04), higher serum follistatin (OR = 2.28, 95%CI [1.20, 5.80], P = 0.04), and lower serum myostatin (OR = 0.76, 95%CI [0.62, 0.88], P = 0.001) were significantly associated with the presence of muscle wasting.

Multivariate analysis showed that serum myostatin levels were independently associated with muscle wasting (OR = 0.77, 95%CI [0.58, 0.93], P = 0.024) **(Table 3)**.

Discussion

The major finding of the present study was that serum myostatin levels were significantly decreased and serum follistatin levels were increased in HF patients, and serum myostatin levels were independently associated with lower extremity muscle wasting.

Previous papers reported that myostatin expression was upregulated in the sheep's hearts after myocardial infarction and the rat HF model of chronic pressure overload.[21, 22] In patients with HF, some reports have also shown an increase in serum myostatin levels.[8, 23] However, in the present study, serum myostatin levels were significantly lower in HF patients than control subjects. Serum follistatin, known as the myostatin antagonist, levels were increased. Our results have been supported by previous reports that serum myostatin levels were decreased in HF patients with compensatory status, cachexia, or with treatment of exercise training.[9, 24, 25] Previous animal study reported that myostatin levels were increased in muscle atrophy due to denervation (a model of disuse), and stretching and electrical stimulation to muscle, which mimic exercise training, decreased myostatin levels.[26] Therefore, serum myostatin levels depend on the various conditions including severity of HF and treatment including exercise therapy. In the present study, all patients with HF were already compensated and about 70% of HF patients performed exercise training when they tested.

Lower extremity muscular strength and circumference of thigh were significantly lower in HF patients than control subjects, and thus patients with HF in our cohort showed skeletal muscle wasting. By univariate and multivariate analysis, serum

myostatin levels were significantly and independently associated with muscle wasting. Myostatin has been known as a negative regulator of muscle mass, and mainly expressed in and secreted from the skeletal muscle. In general, its increase leads to muscle atrophy, and its decrease leads to muscle hypertrophy. Therefore, muscle wasting in patients with HF might be caused by the increase in myostatin. However, our results suggest that lower serum myostatin and higher serum follistatin could be compensatory mechanisms of skeletal muscle for muscle wasting induced by HF.

The mechanisms for muscle wasting in patients with HF has never been know. Some animal studies have reported its molecular signal.[27] We previously reported that infusion of high-dose of angiotensin II into mice caused skeletal muscle atrophy via the activation of ubiquitin-proteasome pathway.[28] Other reports showed that inflammatory cytokines were increased in patients with HF. Therefore, we investigated the association between serum inflammatory cytokines, renin activity, or angiotensin II levels and muscle wasting. However, we could not find significant association. This may suggest that several factors but not single factor are associated with muscle wasting. Other possible explanation for discrepancy between our results and animal studies is that we did not measure these in local level of the skeletal muscle. Further studies are needed to clarify the mechanisms for skeletal muscle wasting in patients with HF.

Study limitations

There are several limitations in our observational study, including its cross-sectional design, the relatively small sample size, and the lack of measurements of muscle mass by computed tomography, which is thought to be standard method of measurement of lower extremity muscle mass, to fully explain the complex underlying

pathophysiology. However, our observations are unique, because the relationship between myostatin and skeletal muscle wasting in patient with HF has not been previously reported. Future studies are necessary to better understand the exact pathophysiology underlying the mechanism of how myostatin work in the body and whether the main source of myostatin is skeletal muscle.

Clinical implication

During the acute phase, bed in rest causes the progressive decline in skeletal muscle in patients with HF, especially elderly, which leads to the impairment in activities of daily living. This is one of clinical problem to be resolved. The increase in myostatin level could be associated with the development in this process. At the chronic phase, minimum stimulation to the muscle (i.e. standing, walking, and exercise training) inhibits an excess myostatin expression in or secretion from muscle, which maintains muscle mass. In stable patients with HF, serum myostatin is secreted from skeletal muscle and their levels reflect muscle mass, which could be a novel marker for muscle mass.

Conclusions

Serum myostatin levels were significantly decreased in HF patients and associated with lower extremity muscle wasting, suggesting that myostatin may be important factor for maintaining skeletal muscle mass and strength in this disease state.

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Disclosures

The authors have no conflicts of interest to disclose.

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Table 1. Clinical, echocardiographic, and cardiopulmonary exercise parameters in control subjects and in patients with HF

	Controls (n = 30)	HF (n = 41)	P value
Baseline characteristics			
Age, years (mean±SD)	52 ± 8	58 ± 15	0.069
Male, n (%)	26 (87)	31 (76)	0.247
BMI, kg/m^2	23.8 ± 3.2	23.1 ± 4.1	0.462
NYHA (I /II /III)	-	2/28/11	
Cause of HF, n (%)			
Ischemic	-	11 (27)	
Non ischemic	-	30 (73)	
Medical history, n (%)			
Hypertension	4 (13)	12 (29)	0.112
Diabetes mellitus	-	14 (33)	
Medication Use, n (%)			
ACEI	-	24 (60)	
ARB	4 (13)	12 (29)	0.112
β-blocker	-	38 (93)	
Diuretics	-	32 (78)	
Echocardiographic parameters			
LV EDD, mm	46.6 ± 3.2	63.9 ± 11.0	<0.001
LV ESD, mm	30.2 ± 3.8	54.8 ± 12.6	<0.001

LVEF, %	61.9	±	5.9	32.9	±	10.8	<0.001
Cardiopulmonary exercise variables							
Peak VO_2, ml/kg/min	29.5	±	6.7	13.6	±	3.2	<0.001
AT, ml/kg/min	15.5	±	4.1	8.8	±	2.1	<0.001

Values are means ± SD; HF indicates heart failure; BMI, body mass index; NYHA, New York Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; LV, left ventricle; EDD, end-diastolic diameter; ESD, end-systolic diameter; EF, ejection fraction; VO_2, oxygen uptake; AT, anaerobic threshold.

Table 2. Body composition, muscle strength, and blood biomarkers in control subject and in patients with HF

	Controls (n = 30)			HF (n = 41)			P value
Body weight, kg	68	±	9	63	±	15	0.087
Body fat weight, kg	16	±	6	16	±	9	0.989
Lean body weight, kg	52	±	6	46	±	10	0.006
Circumference of thigh, cm	56	±	4	47	±	7	<0.001
Grip strength, kg	45	±	9	33	±	11	<0.001
Lower extremity muscular strength, N×m	219	±	51	129	±	54	<0.001
Hemoglobin, g/dL	14.8	±	0.9	13.2	±	1.8	<0.001
Serum albumin, g/dL	4.6	±	0.3	4.1	±	0.4	<0.001
eGFR, $ml \times min^{-1} \times 1.73m^{-2}$	75.7	±	13.2	60.2	±	23.9	0.001
HOMA-IR	1.85	±	1.91	2.31	±	2.30	0.375
Interleukin-6, ng/mL	1.1	±	3.0	1.0	±	1.8	0.827
Tumor necrosis factor-α, pg/mL	0.095	±	0.23	0.064	±	0.17	0.516
Interleukin-1β, pg/mL	2.6	±	2.4	0.50	±	1.4	<0.001
Renin activity, ng/mL/hr	2.5	±	6.0	10.9	±	11.3	0.220
Ang II, pg/mL	10.2	±	13.0	22.5	±	28.7	0.039
BNP, pg/mL	13.8	±	9.04	403.8	±	473.1	<0.001

Values are means ± SD; HF indicates heart failure; eGFR, estimated glomerular filtration rate; HOMA-IR, Homeostasis model assessment-insulin resistance; Ang, angitotensin; BNP, B-type natriuretic peptide.

Table 3. Independent predictors of muscle wasting in patients with HF

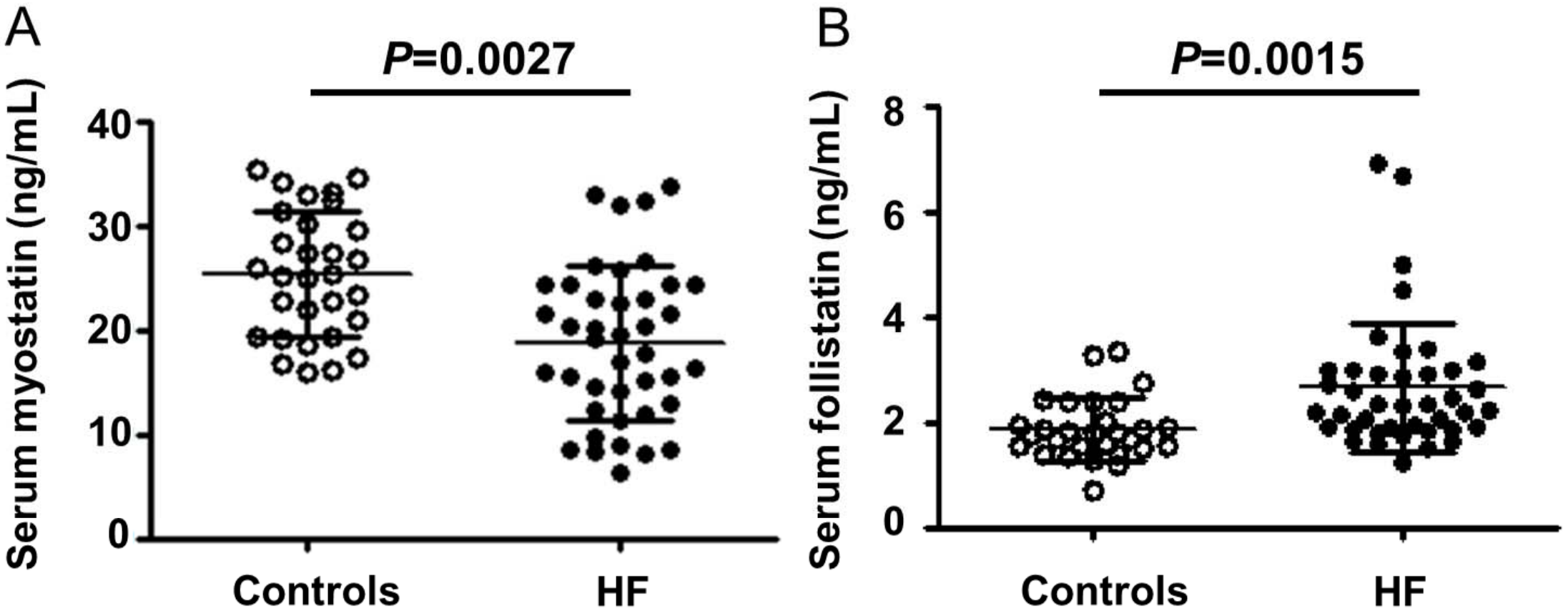
Variable	Univariate analysis		Multivariate analysis		
	OR	P-value	OR	95%CI	P-value
Age, years	1.06	0.038	1.03	0.95 - 1.16	0.411
Gender	0.41	0.224			
BMI, kg/m^2	0.61	0.005	0.66	0.37-0.95	0.080
LVEF, %	1.03	0.240	1.15	1.02-1.36	0.011
Peak VO_2, mL/kg/min	0.87	0.238			
Serum myostatin, ng/mL	0.76	0.001	0.77	0.58-0.93	0.024
Serum follistatin, ng/mL	2.28	0.036	1.89	0.59-8.44	0.374
Interleukin-6, ng/mL	0.68	0.291			
Tumor necrosis factor-α, pg/mL	0.29	0.571			
Interleukin-1β, pg/mL	0.49	0.314			
Plasma renin activity, ng/mL/hr	0.95	0.156			
Plasma Ang II, pg/mL	0.98	0.195			
Plasma BNP, pg/mL	1.00	0.256			

Male and female were assigned values of 0 and 1, respectively. HF indicates heart failure; OR, odds ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricular ejection fraction; VO_2, oxygen uptake; Ang, angiotensin; BNP, B-type natriuretic peptide.

Figure Legends

Figure 1 Serum myostatin levels in control subjects (**open circles**, n = 30) and in patients with heart failure (**closed circled**, n = 41) (**A**), and Serum follistatin levels in control subjects (**open circles**, n = 30) and in patients with heart failure (**closed circles**, n = 41) (**B**). HF indicates heart failure.

Figure 2 Correlation between serum myostatin levels and lower extremity muscular strength (**A**), and correlation between serum myostatin levels and circumference of thigh (**B**) in patients with heart failure (n = 41).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P=0.0027
Serum myostatin (ng/mL)
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Figure 1

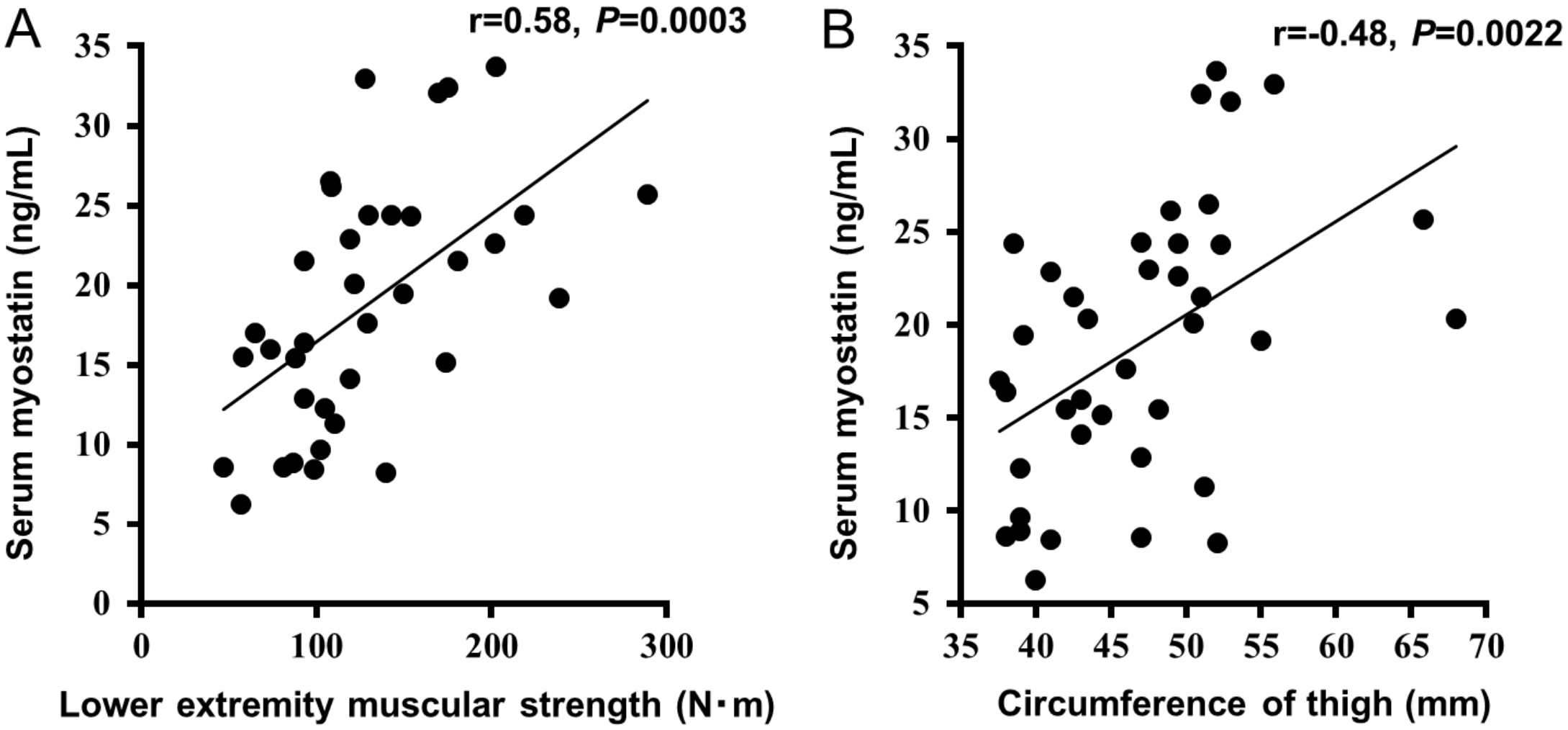
A
r=0.58, P=0.0003
Serum myostatin (ng/mL)
0
5
10
15
20
25
30
35
0
100
200
300
Lower extremity muscular strength (N·m)
B
r=-0.48, P=0.0022
Serum myostatin (ng/mL)
5
10
15
20
25
30
35
35
40
45
50
55
60
65
70
Circumference of thigh (mm)

Figure 2