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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 the 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 an important factor for maintaining skeletal muscle mass and strength in HF.

1. 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

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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.

2. Methods

2.1. 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.

2.2. 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]:

$$\text{aLBM (kg) for men} = -22.48 + 24.14 \times \text{height (m)} + 0.21 \times \text{total fat mass (kg)}$$

$$\text{aLBM (kg) for women} = -13.19 + 14.75 \times \text{height (m)} + 0.23 \times \text{total fat mass (kg)}$$

2.3. 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 analyzed using the handgrip dynamometer (Saehan Corporation Korea Hydraulic Hand Dynamometer, model SH5001). Likewise, the best of three measurements was used.

2.4. 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 2 SD below the mean of a healthy young reference group aged 18–40 years [14].

2.5. 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 °C 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 tumor necrosis factor-α (TNF-α) were analyzed 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]:

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

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.

2.6. 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].

2.7. 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 min of unloaded cycling, the exercise load was increased in 10–15 W/min increments in HF patients and 25 W/min 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.

2.8. 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 $\alpha = 0.05$, $\beta = 0.1$ 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.

3. Results

3.1. 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

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.