Serum Brain-Derived Neurotrophic Factor Levels Are Associated with Skeletal Muscle Function but Not with Muscle Mass in Patients with Heart Failure

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Summary

Heart failure (HF) is associated with aberrant skeletal muscle impairments, which are closely linked to the severity of HF. A low level of brain-derived neurotrophic factor (BDNF), a myokine produced in the skeletal muscle, is known to be involved in reduced exercise capacity and poor prognosis in HF. However, little is known about the factors or conditions of skeletal muscle associated with BDNF levels. We investigated the association between serum BDNF levels and the skeletal muscle mass and function in HF patients ($n = 60$, 63 ± 13 years) and age-matched controls ($n = 29$, 61 ± 16 years). The serum BDNF level was significantly lower in the HF patients compared to the controls (24.9 ± 0.9 versus 28.6 ± 1.3 , $P = 0.021$). In a univariate analysis, BDNF was significantly correlated with the peak oxygen uptake, estimated glomerular filtration rate, 10-m gait speed, and muscle strength, but not with the body mass index or lean mass in the HF group. A multiple linear regression analysis revealed that BDNF was independently associated with muscle strength (β -coefficient = 2.80, 95%CI: 1.89–11.8, $P = 0.008$). Serum BDNF levels were associated with exercise capacity and skeletal muscle function, but not with muscle mass. These novel findings may suggest that BDNF production is controlled by muscle function and activity and consequently regulates exercise capacity, highlighting the importance of adequate training regarding skeletal muscle in HF patients.

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Key words: Muscle strength, Exercise capacity

Heart failure (HF) has become highly prevalent, characterized by reduced activities of daily living and repeated hospitalizations in addition to high mortality rates, comprising a major public health problem.¹⁾ Aberrant skeletal muscle alterations are involved in HF;^{2,3)} these alterations include muscle atrophy, a reduction in muscular strength, a switch in myofibers from slow type I to fast type II, and impaired energy metabolism due to a reduction in mitochondrial density and oxidative function.^{4–6)} These changes have been considered important factors that lead to skeletal muscle impairments, related not only to worse symptoms but also to the severity of HF and the prognosis of patients with HF.^{3,7,8)}

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is abundant in the hippocampus. BDNF has been reported to play a key role in various neurotrophic functions including neuroregeneration, neuroprotection, and synaptic plasticity.^{9,10)} BDNF is also

present in the skeletal muscle and has been shown to be produced in response to muscle contraction.¹¹⁾ Exercise training can increase the serum level of BDNF,¹²⁾ and BDNF is involved in mitochondrial biogenesis and fatty acid metabolism in skeletal muscle.¹³⁾ The skeletal muscles were recently reported to secrete various cytokines and growth factors (collectively called myokines) to regulate their mass and/or function,¹⁴⁾ and BDNF is thus considered one of the myokines.

We reported that the serum BDNF level was decreased in HF patients compared to healthy subjects, and that the serum BDNF level was positively correlated with their peak oxygen uptake (VO_2).¹⁵⁾ We and other groups also observed that decreased serum BDNF was related to all-cause cardiac death and readmission in HF patients, suggesting that the serum BDNF level could be both a less invasive biomarker that reflects the severity of HF and a predictor of prognosis in HF patients.^{16–18)} Using a

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murine model of HF, we recently demonstrated that the administration of human recombinant BDNF improved the exercise capacity of the mice via an improvement in the mitochondrial function of their skeletal muscle.¹⁹⁾ The results of these clinical and basic studies indicate that BDNF is a myokine produced by the contraction of skeletal muscle, and its decrement is associated with a reduction in exercise capacity and skeletal muscle abnormalities in HF patients.

Given that skeletal muscle, i.e., muscle mass, strength and mitochondrial function is an important factor that determines exercise capacity in HF patients,^{2,20)} we hypothesized that the measurement of serum BDNF may show a close relationship between muscle mass and/or strength. The association between BDNF and skeletal muscle function in patients with HF has not been established; we therefore aimed to examine the relationship between serum BDNF and skeletal muscle mass as well as muscle strength in HF patients.

Methods

Patients and controls: Sixty Japanese patients with chronic HF (35 men and 25 women, 63 ± 12 years, New York Heart Association [NYHA] functional class I-III) and 29 age-matched healthy Japanese individuals as controls (19 men and 10 women, 61 ± 14 years) were studied. HF was diagnosed on the basis of the Framingham criteria provided in the American College of Cardiology Foundation/American Heart Association Task Force on Practice guidelines.²¹⁾ Eligible subjects were: ambulatory patients at Hokkaido University Hospital; and patients with chronic HF with NYHA class I-III with a history of one or more hospitalizations due to worsening HF and ≥ 1 month post-discharge without worsening events. All patients were on standard medical therapies for HF, and on an optimal diuretic dose.

The exclusion criteria were: patients with NYHA class IV; patients with lower limb dysfunction such as intermittent claudication and orthopedic disease; and those with significant pulmonary disease, neuromuscular disease, or disorders other than cardiac disease that limit exercise performance; patients with severe renal failure (on dialysis), severe liver failure, or infectious disease. The control group was subjects who had no history of HF and had a left ventricular ejection fraction (LVEF) $> 50\%$. The exclusion criteria were the same as those for the HF patients. The protocol was approved by the Medical Ethics Committee of Hokkaido University Hospital in accordance with the ethical principles described in the Declaration of Helsinki (2013 revised version), and written informed consent was obtained from all participating subjects.

Serum BDNF levels: Peripheral venous blood samples were collected in serum tubes from all subjects before cardiopulmonary exercise testing or muscle strength testing. All samples were allowed to clot and centrifuged at $1,000\text{ g}$ for 15 minutes, and then stored at -80°C until analysis. The serum BDNF levels were measured by an enzyme immunoassay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol as described.²²⁾ To ensure accurate measurements, all of the

samples were analyzed in duplicate by investigators blinded to the clinical information.

Assessment of muscle mass and strength: We measured the subjects' body composition by using a Discovery DXA system (Discovery-A, Hologic, Marlborough, MA). The values of total and segmental mass, lean mass, fat mass, and bone masses were recorded. The lean mass was used to estimate the muscle mass.²³⁾ The muscle strength of the quadriceps femoris muscle was evaluated by an isokinetic dynamometer (Biodex System 4, Biodex Medical Systems, Shirley, NY) as described.²⁴⁾ To obtain the isokinetic peak torque, the subject performed 10 dynamic repetitions of concentric knee extensions/flexions at $180^\circ/\text{second}$ each with the right and left legs separately at maximal effort. The highest value of the torque (Nm) recorded during the unilateral knee extension was defined as the muscle strength.

Cardiopulmonary exercise testing: Cardiopulmonary exercise testing was performed using an upright electromechanical bicycle ergometer (Aerobike 75XLII, Combi Wellness, Tokyo) with a ramp protocol as described.²⁵⁾ {Yokota, 2011 #1368; Yokota, 2011 #112} Briefly, after 3 minutes of unloaded cycling, the exercise load was increased continually in 10-15 W/minute increments in the HF patients and 20-25 W/minute increments in the control subjects. The subjects stopped the exercise when they had dyspnea and/or severe leg fatigue. The VO_2 was obtained by a breath-by-breath method throughout the examination by an expired gas analyzer (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). Peak VO_2 was defined as the VO_2 attained at maximal exercise, and the anaerobic threshold (AT) was determined by the V-slope method.

Other clinical variables: Body weight and height were measured, and the body mass index (BMI) ($\text{body weight}/[\text{height}]^2$, kg/m^2) was calculated. The etiology of HF and the medication(s) used by each patient were determined based on the medical records. The 10-m gait speed was measured based on the duration of the time required to walk the middle 10 m during a maximum 14 m-walk.

Left ventricular (LV) end-diastolic dimension (EDD) and end-systolic dimension (ESD) were measured in the parasternal long-axis view by transthoracic echocardiography. The LV ejection fraction (LVEF) was calculated by the modified Simpson's method from the apical 4- and 2-chamber views. All subjects underwent measurements of hemoglobin, platelets, estimate glomerular filtration rate (eGFR), hemoglobin A1c (HbA1c), and plasma brain natriuretic peptide (BNP). The eGFR was calculated from the serum creatinine values and age, using the Japanese equation.

Statistical analyses: The necessary sample sizes of patients were calculated based on the study by Fukushima, *et al.*¹⁵⁾ To detect the effect compared with the threshold change of 0 under the conditions of $\alpha = 0.05$, $\beta = 0.2$ and the allocation ratio = 2 (HF/controls), the necessary sample sizes calculated were $n = 51$ for the HF group and $n = 25$ for the controls. The results are expressed as the mean $\pm$ SD for continuous variables and as numbers and percentages for categorical variables. We used the unpaired Student *t*-test or the Mann-Whitney *U*-test to compare

Table I. Characteristics of the Control Subjects and Patients with HF

	Control (<i>n</i> = 29)	HF (<i>n</i> = 60)	<i>P</i> -value
Demographic and clinical factors			
Age, years	61 ± 16	63 ± 13	0.632
Male, <i>n</i> (%)	19 (66)	35 (58)	0.514
BMI, kg/m ²	24.9 ± 3.2	23.3 ± 3.7	0.049
Hypertension, <i>n</i> (%)	13 (45)	18 (30)	0.169
Diabetes mellitus, <i>n</i> (%)	7 (24)	15 (25)	0.930
Dyslipidemia, <i>n</i> (%)	16 (55)	38 (63)	0.460
NYHA: I / II / III		8 / 44 / 8	
HFpEF, <i>n</i> (%)		13 (22)	
Medication, <i>n</i> (%)			
ACE-Is or ARBs	8 (28)	58 (97)	< 0.001
Aldosterone antagonists	3 (10)	37 (62)	< 0.001
β-blockers	4 (14)	55 (92)	< 0.001
Diuretics	1 (3)	39 (65)	< 0.001
Statins	14 (48)	38 (63)	0.177
Etiology of HF, <i>n</i> (%)			
Ischemic heart disease	-	18 (30)	
Non-ischemic heart disease	-	42 (70)	
Laboratory test results			
Hemoglobin, g/dL	14.3 ± 1.4	13.3 ± 1.4	0.001
Platelets, × 10 ⁴ /μL	215 ± 51	196 ± 51	0.097
eGFR, mL/minute/1.73 m ²	76.2 ± 18.0	52.3 ± 18.9	< 0.001
HbA1c, %	6.0 ± 0.7	6.0 ± 0.7	0.936
Log BNP, pg/mL	1.1 ± 0.1	2.0 ± 0.1	< 0.001
Serum BDNF, ng/mL	28.6 ± 7.0	24.9 ± 6.8	0.021
Echocardiographic parameters			
LVEDD, mm	47.5 ± 3.2	60.5 ± 11.2	< 0.001
LVESD, mm	30.1 ± 2.2	49.9 ± 14.6	< 0.001
LVEF, %	65.3 ± 4.7	38.1 ± 14.2	< 0.001
Cardiopulmonary exercise test			
Peak VO ₂ , mL/kg/minute	24.8 ± 6.6	16.8 ± 4.3	< 0.001
AT, mL/kg/minute	13.9 ± 2.8	10.2 ± 2.2	< 0.001
Peak respiratory exchange ratio	1.23 ± 0.08	1.20 ± 0.09	0.267
Skeletal muscle findings			
Lean mass, kg	49.3 ± 9.8	41.7 ± 7.5	0.004
10-m gait speed, seconds	5.4 ± 1.1	6.1 ± 1.4	0.023
Muscle strength, Nm	101.8 ± 29.9	84.5 ± 22.9	0.004

Data are mean ± SD. ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AT, anaerobic threshold; BDNF, brain-derived neurotrophic factor; BMI, body mass index; BNP, brain natriuretic peptide; EDD, end-diastolic diameter; EF, ejection fraction; ESD, end-systolic diameter; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HF, heart failure; HFpEF, HF with preserved EF; LV, left ventricular; MVC, maximum voluntary contraction; NYHA, New York Heart Association function class; and VO₂, oxygen uptake.

continuous variables, and the chi-square test for categorical variables. A univariate linear regression model was used to determine the correlations between the serum BDNF levels and other clinical variables.

Multiple linear regression analysis was conducted to identify the independent variables associated with serum BDNF levels. Clinical parameters or variables with a *P*-value < 0.2 in the univariate model were considered for inclusion. All analyses were performed using JMP 14.0.0 software (SAS, Cary, NC). The differences were considered significant when the *P*-values were < 0.05.

Results

Patient characteristics: The age, gender, and past history of the HF and control groups were comparable, whereas

the BMI was significantly lower in the HF group (*P* = 0.049) (Table I). Regarding the NYHA functional class, 8 patients were in class I, 44 class II, and 8 class III. The etiologies of HF were ischemic heart disease in 18 patients and non-ischemic heart disease in the other 42 patients. Thirteen patients (22%) had HF with preserved EF (HFpEF), and the other 48 patients had HF with reduced EF (HFrEF). Angiotensin-converting enzyme inhibitors or angiotensin II type I receptor antagonists were used in 97% of the HF patients; β-blockers in 92%, aldosterone antagonists in 62%, and diuretics in 63%.

In the echocardiography, the HF patients had significantly greater LVEDD and significantly lower LVEF values (both *P* < 0.001) compared to the control group. The peak VO₂ and AT values were significantly lower in the HF patients (both *P* < 0.001). The plasma BNP values

Figure 1. Serum BDNF levels in the control subjects (open circles, $n = 29$) and patients with HF (closed circles, $n = 60$).

were significantly higher and the hemoglobin and eGFR values were significantly lower in the HF patients compared to the control subjects (both $P < 0.001$). The values of lean mass, 10-m gait speed, and muscle strength were significantly lower in the HF group.

Serum BDNF levels: The serum BDNF levels were significantly lower in the HF patients compared to the control subjects (24.9 ± 0.9 versus 28.6 ± 1.3 ng/mL, $P = 0.021$) (Figure 1). Among 60 HF patients, the BDNF levels did not differ between the males and females ($P = 0.939$) or between other clinical variables, including diabetic and non-diabetic patients ($P = 0.882$). In addition, the BDNF levels did not differ between the HFpEF and HFrEF patients ($P = 0.152$) (data not shown).

Correlation between serum BDNF and clinical variables in the HF patients: In the univariate analysis, the serum BDNF levels of the HF patients were significantly correlated with their values of peak VO_2 ($r = 0.372$, $P = 0.007$), AT ($r = 0.372$, $P = 0.006$), hemoglobin ($r = 0.412$, $P = 0.001$), eGFR ($r = 0.305$, $P = 0.018$), 10-m gait speed ($r = -0.368$, $P = 0.005$), and muscle strength ($r = 0.342$, $P = 0.009$), but not with their age, BMI, lean mass, LVEDD, LVEF, HbA1c, or the plasma log BNP level (Table II, Figures 2, 3).

In the multiple analysis, muscle strength (β -coefficient = 2.80, 95%CI: 1.89-11.8, $P = 0.008$) was identified as an independent determinant of the serum BDNF level, conditional upon the other predictors (Table III).

Discussion

The main findings of the present study are that (1) the serum BDNF level was lower in HF patients compared to the control subjects, and it was correlated with exercise capacity, and (2) the serum BDNF level in the

Table II. Univariate Linear Model of BDNF in the HF Group

Variable	Univariate correlation coefficient	P-value
Age, years	0.018	0.891
BMI, kg/m ²	-0.001	0.994
LVEDD, mm	-0.103	0.434
LVEF, %	0.174	0.185
Peak VO_2 , mL/kg/minute	0.321	0.019
AT, mL/kg/minute	0.372	0.006
Hemoglobin, g/dL	0.412	0.001
eGFR, mL/minute/1.73 m ²	0.305	0.018
Log BNP, pg/mL	-0.176	0.186
HbA1c, %	-0.143	0.278
Lean mass, kg	0.018	0.907
10-m gait speed, sec	-0.368	0.005
Muscle strength, Nm	0.342	0.009

Abbreviations are explained in the Table I footnote and the text.

HF patients was significantly correlated with the muscle function measurements, but not with the BMI or lean mass.

We also observed that the serum BDNF level was correlated with aspects of exercise capacity, i.e., the peak VO_2 and AT. This result is concordant with our previous findings^{15,16} as well as reports from other groups.^{17,26} Interestingly, the serum BDNF levels of the HF patients in the present study were higher compared to those in previous reports. This difference may be due to the severity of HF of the enrolled subjects: 87% of the HF patients in this study were NYHA class I/II and 13% were NYHA class III, with a mean peak VO_2 of 16.8 mL/kg/minute, and our HF group showed a mean BDNF level of 24.9 ng/mL. Our previous study¹⁵ with a mean BDNF level at 16.6 ng/mL examined HF patients with more severe HF and lower exercise intolerance: 76% of that patient series was NYHA class I/II and 24% was NYHA class III, and the mean peak VO_2 was 14.0 mL/kg/minute. Kadowaki, *et al* reported the mean BDNF level of 14.7 ng/mL in HF patients, 53% of whom were NYHA class II and 47% of whom were NYHA III/IV, including patients with decompensated HF.²⁶ Therefore, serum BDNF is present at lower values in patient groups with a higher severity of HF, and together with the finding that lower serum BDNF is associated with exercise intolerance and worse prognosis in HF patients,^{15,16} these results indicate that the serum BDNF level well reflects the severity of HF.

In HF, aberrant skeletal muscle impairments are known to occur both in quantity and quality,^{4,6,27} and these changes have been considered important factors of exercise intolerance in HF patients. We have reported the association between serum BDNF and exercise intolerance; however, the relationship between BDNF and skeletal muscle mass and/or function remained unclear. The present study revealed for the first time that serum BDNF shows correlations with skeletal muscle function such as the 10-m gait speed and muscle strength, but not with the BMI or lean mass. Tsai, *et al* reported similarly that exercise training increased the serum BDNF levels in type 2 diabetic patients, and they noted that the serum BDNF levels were correlated with the changes of muscle

Figure 2. Correlation between the serum BDNF level and peak VO_2 (A), and AT (B) in the HF patients.

Figure 3. Correlation between the serum BDNF level and the BMI (A), lean mass (B), 10-m gait speed (C), and muscle strength (D) in the HF patients.

strength, but not with the BMI.²⁸⁾ Accordingly, these results suggest that muscle function or activity, rather than muscle mass, is important for the increase in BDNF levels.

BDNF seems to play a key role in mediating the benefits of exercise. It has been well documented that ex-

ercise increases the production of BDNF. For example, exercise increases BDNF levels in the hippocampus, contributing to improvements in cognitive function.²⁹⁻³¹⁾ Several other studies revealed that acute aerobic exercise and endurance training also increase serum BDNF.³²⁾ However, the source of the increased circulating BDNF remains to

Table III. Multiple Regression Analysis of BDNF and Other Clinical Variables in the HF Group

Variable	Correlation coefficient	95%CI	P-value
Age, years	3.67	-1.72 - 9.07	0.175
Male (0)	-1.16	-4.97 - 2.65	0.541
LVEF, %	-1.89	-2.66 - 6.43	0.406
Log BNP, pg/mL	-0.31	-4.89 - 4.27	0.892
Lean mass, kg	1.44	-5.68 - 8.56	0.684
Muscle strength, Nm	6.85	1.89 - 11.8	0.008

Male and female were assigned values of 0 and 1, respectively.

be elucidated. Several possibilities are proposed; first, most of the circulating BDNF is stored in platelets, and its activated release from platelets in response to exercise might contribute to the increased level of BDNF.³³⁾ Second, the brain might be another source, since cerebral BDNF is known to pass through the blood-brain barrier,³⁴⁾ and preliminary evidence indicated that 70%-80% of the increase in BDNF due to exercise is derived from the brain.³⁵⁾

BDNF was also shown to be synthesized by skeletal muscle in response to contraction and exercise.^{11,36,37)} Indeed, Matthews, *et al* demonstrated that skeletal muscle cells themselves produce BDNF in response to contraction.¹¹⁾ However, they also observed that the elevation of skeletal-muscle BDNF did not correlate with serum BDNF, suggesting that skeletal muscle BDNF does not transfer into circulation and acts locally, enhancing fatty acid oxidation in the muscle, at least in the acute phase after exercise. In the chronic phase, the role of BDNF in skeletal muscle post-exercise was reported to be more than the regulation of energy metabolism.³⁸⁾

Our present findings indicate that in conditions of chronic skeletal muscle impairments (as in the setting of HF), it is possible that BDNF production from skeletal muscle is involved in the serum BDNF level, and that muscle activity is important for the increase in serum BDNF, since the muscle function was more closely associated with BDNF compared to the muscle mass. However, more evidence is needed to evaluate the source of BDNF in HF patients.

BDNF is known to affect exercise capacity by regulating skeletal muscle mitochondrial function and content, but the cause-and-result relationship between BDNF and muscle function is not clear. BDNF was shown to activate the AMP-activated protein kinase (AMPK) and enhance fatty acid oxidation,¹¹⁾ and it triggers the AMPK/CREB/PGC-1 α pathways to increase cellular respiration by promoting mitochondrial biogenesis in skeletal muscle.¹³⁾ The depletion of BDNF in C2C12 myoblasts decreased the level of phosphorylation of AMPK and PGC-1 α proteins.³⁹⁾ We recently reported that BDNF was reduced in the skeletal muscle of a murine model of HF after myocardial infarction, and that the administration of human recombinant BDNF ameliorated the reduction of the endurance exercise capacity and improved the mitochondrial respiration of the skeletal muscle.¹⁹⁾

Taken together, all of these findings indicate that BDNF regulates mitochondrial function through AMPK/

PGC-1 α and affects the endurance exercise capacity. To date, there is no evidence showing that BDNF directly regulates muscle mass or function. Based on the present results, it is plausible that the intensity of muscle function regulates the production of BDNF, rather than that BDNF controls muscle function. Further elucidation of the underlying mechanisms is needed.

Elderly patients with HF have a high prevalence of sarcopenia, which is a state of muscle impairment in both mass and function.⁵⁾ Sarcopenia is an independent prognostic factor in HF,⁴⁰⁾ but it has been a great concern whether the exercise intolerance is mainly due to muscle dysfunction or the decrease in muscle mass considering the pathophysiology of HF patients. Exercise training is beneficial for HF patients with sarcopenia, but muscle training generally improves muscle strength in advance of an improvement in muscle mass,⁴¹⁾ making it difficult for such patients to gain muscle mass. In light of our present findings, it appears that the improvement in muscle function may increase myokines (including BDNF) and may contribute to the multiple health benefits associated with exercise, providing a significant impact in clinical practice.

The present study revealed for the first time that serum BDNF was associated with skeletal muscle function, but not with muscle mass. We speculate that BDNF is secreted by muscle activity, regulates mitochondrial function in the skeletal muscle, and defines exercise endurance. This novel finding may provide a crucial clue for future therapies, such as medications for HF-related muscle impairments.

There are several limitations of this study that should be acknowledged. First, we cannot make a conclusion regarding the causal relationship between BDNF and muscle function in this cross-sectional observation with a relatively small number of patients, and thus a large portion of the mechanism remains speculative. Further investigations and large-population studies are warranted. Second, our HF group was comprised of both HFpEF and HFrEF patients. Although the BDNF levels did not differ between the groups, we did not compare further group values due to the limited sample size. Finally, the source of the circulating BDNF is not clear at present. Although evidence shows BDNF production in skeletal muscle by contraction, the precise roles of muscle and serum BDNF remain to be elucidated.

Disclosure

Conflicts of interest: The authors state that there are no conflicts of interest to declare.

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