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Branched-chain amino acids ameliorate heart failure with cardiac cachexia in rats

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ABSTRACT

Aims: Heart failure (HF) is associated with changes in energy metabolism of the heart, as well as in extra-cardiac organs such as the skeletal muscles. Cardiac cachexia is a common complication and is associated with poor prognosis. Branched-chain amino acids (BCAAs) reportedly improve sarcopenia and cancer cachexia. We tested the hypothesis that BCAA ameliorates HF with cardiac cachexia.

Main methods: We used Dahl salt-sensitive (DS) rats fed a high-salt diet as a model of HF. DS rats fed a low-salt diet were used as a control. BCAA were administered in drinking water from 11 weeks of age, when cardiac hypertrophy was established but the cardiac function was preserved. Survival and the cardiac function were monitored, and animals were sacrificed at 21 weeks of age and analyzed.

Key findings: In HF rats, BCAA treatment decreased the heart rate, preserved the cardiac function, and prolonged survival. BCAA also prevented body weight loss, associated with preservation of the skeletal muscle weight. Moreover, gene expression related to mitochondrial biogenesis and function was increased with BCAA in skeletal muscles.

Significance: BCAA preserved the body weight and cardiac function and prolonged survival in HF rats. The expression of genes involved in mitochondrial biogenesis and function in skeletal muscles was increased by BCAA.

1. Introduction

Although the prognosis of patients with heart failure (HF) has been improved, the mortality rate of such patients remains at almost 50% within 5 years of the diagnosis [1]. HF is associated with a significant change in energy metabolism of the heart. This change has been hypothesized to be important in the progression of HF [2].

HF is also associated with a change in systemic energy metabolism, such as insulin resistance [3] and cachexia [4]. Cardiac cachexia is a common complication in HF, and patients with cardiac cachexia show poor prognosis and disability. Several lines of evidence suggest that immune and neurohormonal abnormalities play a critical role in the wasting process and that the abnormal metabolic balance between catabolism and anabolism is associated with the development of cachexia [5]. Skeletal muscle is thought to be important in the development of cachexia [6]. A negative energy balance in skeletal muscle has been shown in an experimental model of HF [7,8]. In cardiac cachexia, wasting and weakness of skeletal muscle are observed and these changes are distinctly different from those of muscle atrophy due to reduced activity [9].

Recent studies showed that branched-chain amino acids (BCAAs) extend the chronological lifespan of *Saccharomyces cerevisiae* [10] and wild-type mice [11]. BCAAs consist of leucine, isoleucine, and valine. These amino acids are known to play a number of roles, such as those in muscle protein synthesis, insulin secretion, and energy production through their catabolism [12]. BCAA improves fiber atrophy of the skeletal muscle due to age-induced sarcopenia in rats [13], and prevent the loss of skeletal muscle weight associated with cancer cachexia in mice [14].

BCAAs improve the cardiac function in global ischemia of isolated rat hearts [15]. Patients with coronary artery disease have a negative protein balance and BCAAs exhibit an anabolic effect on myocardial protein metabolism [16]. Leucine attenuates myocardial infarction in mice [17]. These results show that BCAAs protect the heart from myocardial ischemic injury.

However, there has been no study evaluating the effect of BCAA on HF with cardiac cachexia. The Dahl rat model of HF shows progressive deterioration of the cardiac function [18]. We previously reported that there is a distinct change in the metabolic profile of the heart during the development of HF [19]. In addition, this model shows body weight loss associated with an increase in the level of proinflammatory cytokines, and can be deemed a model of cardiac cachexia [20]. Therefore,

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we tested the hypothesis that BCAAs ameliorate HF with cardiac cachexia using the Dahl rat model of HF.

2. Materials and methods

2.1. Animals

Inbred male Dahl salt-sensitive (DS) rats (Japan SLC, Hamamatsu, Shizuoka, Japan) were fed a 0.3% NaCl (low salt: LS) diet until the age of 6 weeks, after which they were fed an 8% NaCl diet (high salt: HS) [18]. Animal care and experiments were approved by the Institutional Animal Care and Use Committee of Kyoto University and conducted following the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health.

2.2. Protocols

2.2.1. Experiment 1

At 11 weeks of age, the rats fed the LS or HS diet were randomly sorted into four groups to receive tap water or a branched-chain amino acid (BCAA) mixture at a dose of 1.5 mg/g body weight/day in drinking water. The rats were divided into four groups: the rats fed the LS diet and tap water (LS-C, n = 6), LS diet and BCAA (LS-BCAA, n = 6), HS diet and tap water (HS-C, n = 10), and HS diet and BCAA (HS-BCAA, n = 10). Serial measurements of the heart rate and blood pressure were performed before, 24 h after, and 48 h after BCAA supplementation. The heart rate and blood pressure are determined by the tail-cuff method using a noninvasive automated blood pressure apparatus (Softron BP-98A, Softron Co. Ltd. Tokyo, Japan) without anesthesia.

2.2.2. Experiment 2

At 11 weeks of age, the rats fed the LS or HS diet were randomly sorted into four groups to receive tap water or a branched-chain amino acid (BCAA) mixture at a dose of 1.5 mg/g body weight/day in drinking water. The survival of animals was compared among the rats fed the LS diet and tap water (LS-C, n = 8), LS diet and BCAA (LS-BCAA, n = 8), HS diet and tap water (HS-C, n = 30), and HS diet and BCAA (HS-BCAA, n = 30). Serial measurements of food intake, water intake, body weight, heart rate, and blood pressure were performed every 2 weeks from the age of 11 weeks until they were sacrificed at 21 weeks of age. The heart rate and blood pressure were determined by the tail-cuff method using a noninvasive automated blood pressure apparatus (Softron BP-98A, Softron Co. Ltd. Tokyo, Japan) without anesthesia.

The amino acid contents of the LS and HS diets are listed in Supplementary Table 1, and the content of BCAA mixture in drinking water was determined in a previous report [11] and is listed in Supplementary Table 2.

2.3. Cardiac echocardiography

Transthoracic echocardiography was performed as previously reported [18]. Rats were anesthetized briefly with inhaled diethyl ether (Wako Pure Chemical Industries, Osaka, Japan), and transthoracic echocardiography was performed using a Sonos-5500 echocardiograph (Agilent Technologies, Santa Clara, CA, USA) with a 15-MHz linear transducer every 2 weeks from the age of 11 weeks until being sacrificed. The heart rate (HR), intraventricular septal thickness (IVSd), left ventricular dimension in the diastolic phase (LVDd), and left ventricular dimension in the systolic phase (LVDs) were measured with M-mode echocardiography, and fractional shortening (FS) and the ejection fraction (EF) were calculated with Teichholz formula.

2.4. Tissue sampling

The 21-week-old LS-C group (n = 8), 21-week-old LS-BCAA group (n = 8), 21-week-old HS-C group (n = 11), and 21-week-old HS-BCAA group (n = 17) were sacrificed by decapitation without fasting. The heart, left gastrocnemius muscle, both kidneys, and both lungs were rapidly removed, and their weights were measured. The heart and gastrocnemius muscle were snap frozen in liquid nitrogen and stored at −80 °C, or fixed with 4% paraformaldehyde (PFA).

2.5. Quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from the heart tissue in each group by the acid guanidinium thiocyanate–phenol–chloroform method. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed as in previous reports [21]. The oligonucleotide primers are listed in Supplementary Table 3. The mRNA level of each gene was standardized with the expression level of 18S ribosomal RNA as a control and calculated with the 2-DeltaCt method.

2.6. Fibrosis of myocardium

Hearts were fixed in 4% PFA, embedded in paraffin, and sectioned for histological evaluation. The fibrotic area was quantified in tissue sections with Sirius Red staining, as previously described [22].

2.7. Apoptosis of myocardium

Apoptosis was assessed with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method (Tanaka Bio Inc., Shiga, Japan), as previously reported [19].

2.8. Western blotting

The lysate of heart tissue and gastrocnemius muscle was extracted by homogenization in an ice-cold buffer (10% glycerol, 137 mM NaCl, 20 mM Tris–HCl pH 7.4, 4 g/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 4 g/mL pepstatin, 20 mM NaF, 1 mM sodium pyrophosphate, and 1 mM orthovanadate). The lysate was put on ice for 15 min and centrifuged at 15,000 *g* for 15 min at 4 °C. Then, 80 μg of the lysate was electrophoresed in the gel (Hybrid Gel, Kishida, Osaka, Japan) by the Laemmli method. The primary antibodies used for Western blotting were as follows: mTOR (1:1000, Cell Signaling, Danvers, MA, USA), phospho-mTOR (1:1000, Cell Signaling), p70S6K (1:200, Santa Cruz, Dallas, TX, USA), phospho-p70S6K (1:1000, Cell Signaling), and GAPDH (1:1000, Cell Signaling).

2.9. Measuring thiobarbituric acid reactive substances (TBARS)

TBARS levels in left ventricular tissue were measured according to the manufacturer's instructions (Alexis Biochemicals, Lausen, Switzerland).

2.10. Statistical analysis

Values are expressed as mean ± SEM. The survival of animals was analyzed using the Kaplan–Meier method with a Wilcoxon test. ANOVA was used for comparisons between multiple groups. In all tests, a value of $p < 0.05$ was considered significant.

3. Results

3.1. Short-term effect of BCAA on hemodynamic parameters in DS rats

In experiment 1, after 48 h of BCAA supplementation, the HS-BCAA group showed a significantly lower heart rate compared to that of the HS-C group (Fig. 1). No significant change in the heart rate or systolic blood pressure was noted between the LS-C and LS-BCAA groups (Fig. 1).

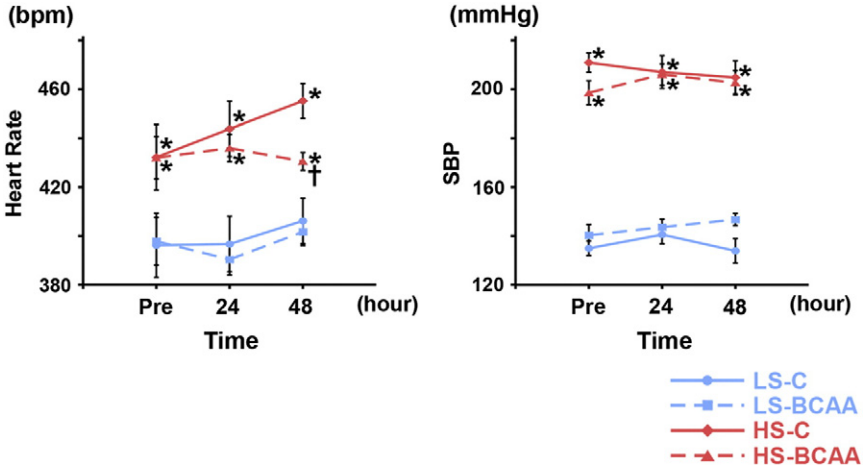

Fig. 1. Hemodynamic parameters in Experiment 1. The heart rate and systolic blood pressure were high in the HS-C group compared with those of the LS-C group. The heart rate was significantly lower at 48 h after BCAA supplementation in the HS-BCAA group compared with that of the HS-C group. There was no difference in the systolic blood pressure between the HS-C and HS-BCAA groups. bpm: beats per minute. SBP: systolic blood pressure. Values are the mean ± SEM. *$p < 0.05$ versus LS-C group. †$p < 0.05$ versus HS-C group.

3.2. BCAA improved survival rate in Dahl salt-sensitive rats

As we previously reported [19], Dahl salt-sensitive (DS) rats fed a high-salt (HS) diet developed hypertension, showed cardiac function degeneration, and died of heart failure. DS rats fed a low-salt (LS) diet did not develop hypertension or heart failure, and were used as controls.

The HS-C group showed a significantly higher heart rate and systolic blood pressure compared with those of the LS-C group. BCAA significantly decreased the heart rate of the HS-BCAA group at 11, 13, 15, and 17 weeks of age compared with the HS-C group (Fig. 2). At 11 weeks of age, we measured hemodynamic parameters 3 days after the start of BCAA administration. No significant change in the heart rate or systolic blood pressure was noted between the LS-C and LS-BCAA groups (Fig. 2).

There was no significant difference in food or water intake between DS rats fed the HS diet and water (HS-C) and DS rats fed the HS diet and water containing BCAA (HS-BCAA), but DS rats fed the LS diet and water containing BCAA (LS-BCAA) showed significantly lower food and water intakes than DS rats fed the LS diet and water (LS-C) (Fig. 3). The survival rate of the HS-C group was lower than that of the LS-C group. BCAA supplementation significantly improved the survival of DS rats fed the HS diet (Fig. 3).

The body weight of the HS-BCAA group at 19 weeks of age was significantly higher than that of the HS-C group. The body weight was significantly lower in the LS-BCAA group than that of the LS-C group (Fig. 3).

3.3. BCAA preserved cardiac systolic function in DS rats

Based on echocardiographic examination, the heart rate (HR) with anesthesia showed no significant difference among all groups during the experimental period (Fig. 4, Supplementary Table 4). The intraventricular septal thickness (IVSd) increased in the HS-C group, indicating the development of left ventricular hypertrophy (LVH). From 17 weeks of age, IVSd decreased, the left ventricular dimension in the diastolic phase (LVDd) increased, and a significant reduction of fractional shortening (FS) was observed in the HS-C group compared to that of the LS-C group (Fig. 4, Supplementary Table 4). The increase of LVDd and the worsening of FS were prevented in the HS-BCAA group. On the other hand, no significant change was observed in IVSd, LVDd, or FS between the LS-C and LS-BCAA groups (Fig. 4, Supplementary Table 4).

3.4. Effect of BCAA on organ weights

All organ and body weights and those corrected by the tibial length are shown in Table 1. At 21 weeks of age, the heart, kidney, and lung weights were significantly increased, while the gastrocnemius muscle weight was significantly decreased in the HS-C group compared with that of the LS-C group (Table 1). BCAA tended to prevent the loss of the gastrocnemius muscle weight in DS rats fed the HS diet (HS-C vs. HS-BCAA, $p = 0.055$). There was no difference in organ weights corrected by the tibial length between the LS-C and LS-BCAA groups (Table 1).

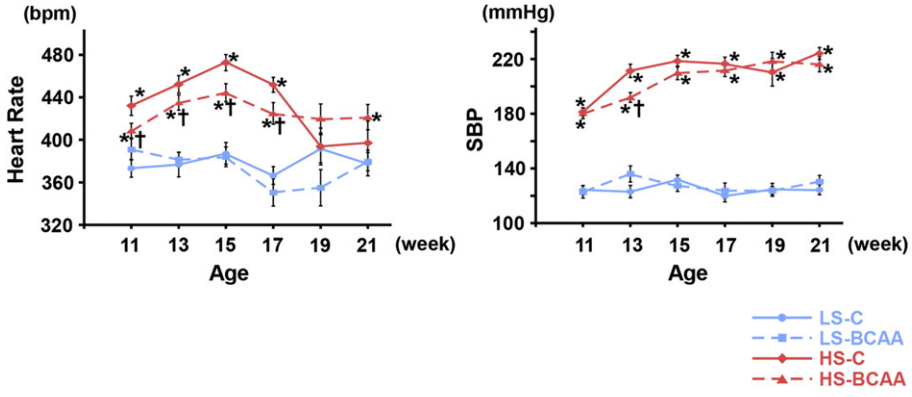

Fig. 2. Hemodynamic parameters in Experiment 2. The heart rate and systolic blood pressure were high in the HS-C group compared with those of the LS-C group. The heart rate was significantly lower at 11, 13, 15, and 17 weeks of age in the HS-BCAA group compared with that in the HS-C group. There was no difference in the systolic blood pressure between the HS-C and HS-BCAA groups. bpm: beats per minute. SBP: systolic blood pressure. Values are the mean ± SEM. *$p < 0.05$ versus LS-C group. †$p < 0.05$ versus HS-C group.

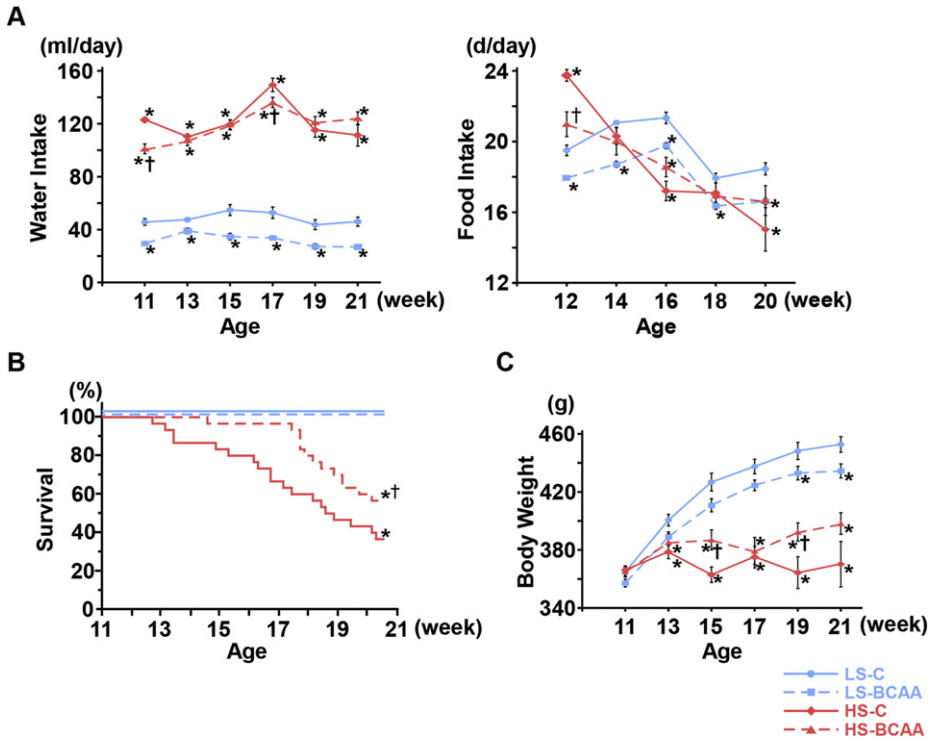

Fig. 3. Water and food intake, survival, and body weight. (A) Water and food intake decreased in the LS-BCAA group compared with that in the LS-C group. There was no difference in water or food intake between the HS-C and HS-BCAA groups. (B) BCAA significantly improved survival in DS rats fed the HS diet. Survival curves were compared between the LS-C (n = 8), LS-BCAA (n = 8), HS-C (n = 30), and HS-BCAA (n = 30) groups. (C) The body weight significantly decreased in the LS-BCAA group compared with that in the LS-C group. The body weight significantly decreased in the HS-C group compared with that in the the LS-C group. The body weight was preserved in the HS-BCAA group compared with that in the HS-C group at 19 weeks of age. LS-C: low-salt diet and water; LS-BCAA: low-salt diet and water containing BCAA; HS-C: high-salt diet and water; HS-BCAA: high-salt diet and water containing BCAA. Values are the mean ± SEM. *$p < 0.05$ versus LS-C group. †$p < 0.05$ versus HS-C group.

3.5. BCAA modified gene expression related to mitochondrial function in skeletal muscles

HF is associated with the abnormal expression of genes related to the mitochondrial function in both heart and skeletal muscles [7,8,19]. A decreased mitochondrial function in oxidative respiration is also observed in the skeletal muscle in the presence of cancer cachexia [23,24]. Hence, we examined gene expression related to the mitochondrial function at 21 weeks of age.

Proliferator-activated receptor-γ coactivator1-α (PGC1-α) is a master regulator of mitochondrial function and biogenesis [25]. In the heart tissue, the expression of *PGC1-α* was reduced in the HS-C group compared with that of the LS-C group. There was no difference in the amount of *PGC1-α* mRNA between the HS-C and HS-BCAA groups. However, in skeletal muscles, the gene expression of *PGC1-α* tended to decrease in the HS-C group compared with that of the LS-C group (LS-C vs. HS-C, $p = 0.056$), and BCAA significantly increased the expression of *PGC1-α* in DS rats fed the HS diet (Fig. 5).

Subsequently, we measured the expression of genes in the mitochondrial respiratory chain related to oxidative phosphorylation. In heart tissue, expressions of *alpha-subcomplex 9* (*αS9*), *succinate dehydrogenase b* (*SDHB*), and *cytochrome c oxidase* (*COX*) *4* were significantly decreased in the HS-C group compared with those of the LS-C group. There was no difference in the expressions of those genes between the HS-C and HS-BCAA groups (Fig. 5). In skeletal muscles, the expressions of *αS9* and *COX1* were significantly decreased in the HS-C group compared with those of the LS-C group, and the expressions of *αS9* and *SDHB* were significantly increased in the HS-BCAA group compared with those of the HS-C group (Fig. 5).

Since the generation of reactive oxygen species (ROS) is an important function of mitochondria, we measured a marker of ROS in heart and skeletal muscle tissues. Thiobarbituric acid reactive substances (TBARS) is a marker of lipid peroxidation, the TBARS level was increased in the HS-C group compared to that of the HS-BCAA group, and there was no difference between the HS-C group and the HS-BCAA group both in heart tissue and skeletal muscles (Supplementary Fig. 1).

3.6. Effect of BCAA on gene expression related to inflammation and ubiquitin ligase

Inflammatory mediators, such as interleukin-1 β (*IL1-β*) and interleukin-6 (IL-6), induce cardiomyopathy [26,27] and skeletal muscle atrophy [27]. Muscle RING finger protein 1(MuRF-1) and muscle atrophy f-bx (MAF-bx) are ubiquitin ligases, and those expressions are significantly upregulated during the development of skeletal muscle atrophy [28]. Thus, we measured the expression of genes related to inflammation and wasting. In heart tissue, there was no difference in the expressions of *IL1-β*, *IL-6*, *MuRF1*, and *MAF-bx* among all groups (Supplementary Fig. 2). In skeletal muscles, although the expression of *IL1-β* was significantly decreased in the HS-C group compared with that of the LS-C group, there was no difference in the expression of *IL1-β*, *Il-6*, *MuRF1*, or *MAF-bx* between the HS-C and HS-BCAA groups (Supplementary Fig. 2).

3.7. Effect of BCAA on cardiac fibrosis and apoptosis

At 21 weeks of age, the area of cardiac fibrosis of the left ventricular tissue was significantly increased in the HS-C group compared to that of the LS-C group. There was no difference between the HS-C and HS-BCAA groups (Supplementary Fig. 3A). At 21 weeks of age, the number of apoptotic cells of whole heart tissue was significantly increased in the HS-C group compared to that of the LS-C group. There was no difference between the HS-C and HS-BCAA groups (Supplementary Fig. 3B).

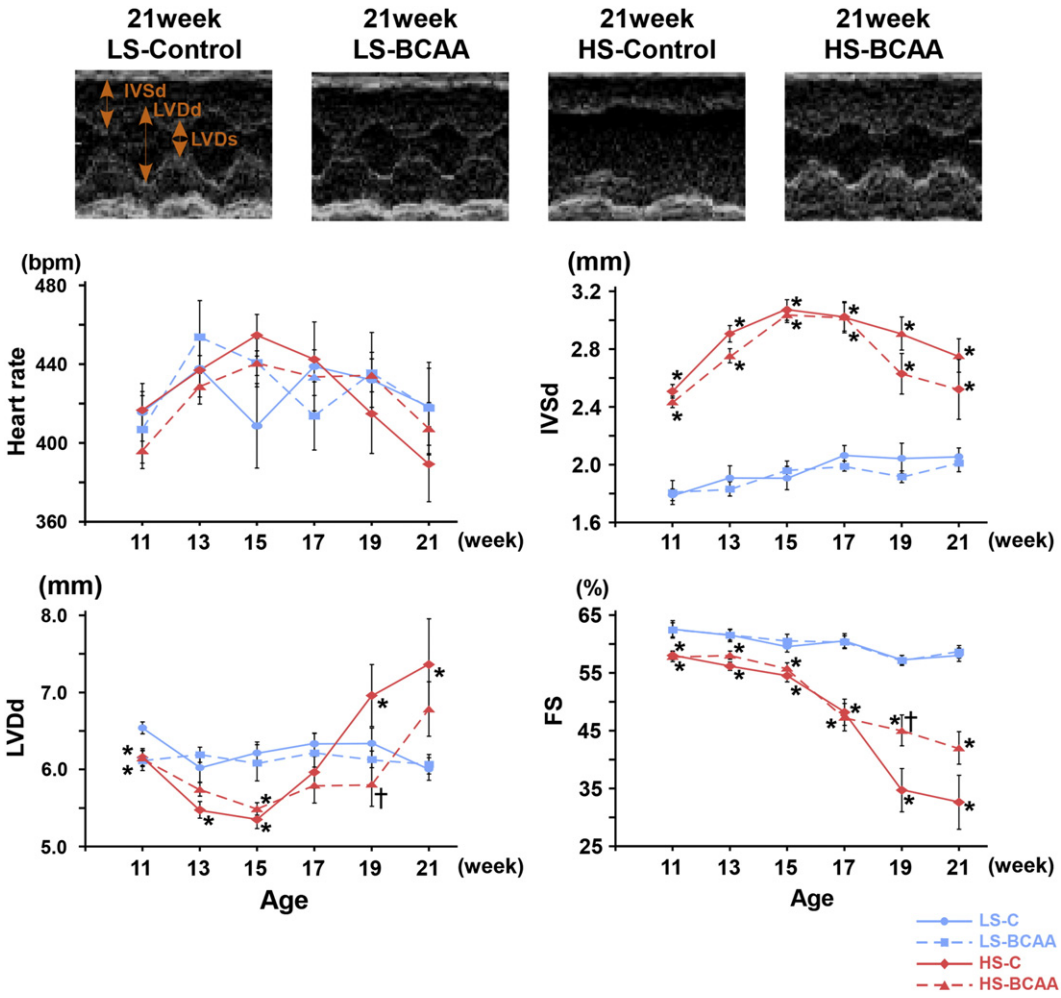

Fig. 4. Echocardiography. A representative M-mode echocardiogram at 21 weeks of age is shown in the upper panel. There was no difference in the heart rate among all groups. IVSd and LVDd increased in the HS-C group and FS decreased in the HS-C group compared with those in the LS-C group. LVDd and FS were preserved in the HS-BCAA group compared with those in the HS-C group. HR: heart rate; IVSd: interventricular septum dimension; LVDd: left ventricular diastolic dimension; LVDs: left ventricular systolic dimension; FS: fractional shortening. Values are the mean ± SEM. *$p < 0.05$ versus LS-C group. †$p < 0.05$ versus HS-C group.

3.8. Effect of BCAA on pathway of mammalian target of rapamycin (mTOR) pathway

In previous reports, cardiac hypertrophy induced by pressure overload causing aortic binding was dependent on the mTOR pathway [29]. Since BCAAs were reported to be positive regulators of the mTOR pathway [30], we examined the effect of BCAA supplementation on the mTOR pathway in heart tissue and the gastrocnemius muscle at 21 weeks of age. In the heart tissue, the phosphorylation of p70S6K was reduced in the HS-C group compared with that of the LS-C group. The amount of phosphorylated p70S6K or mTOR was not different between the HS-C and HS-BCAA groups (Supplementary Fig. 4A). In the skeletal muscle, the phosphorylation of p70S6K and mTOR was reduced in the HS-C group compared with that of the LS-C group. The amount of

Table 1
Body and organ weights.

	LS-C	LS-BCAA	HS-C	HS-BCAA
Numbers of animals	8	8	11	17
Body weight (g)	453 ± 5	435 ± 5*	370 ± 16*	398 ± 7*
Heart weight (g)	1.38 ± 0.03	1.34 ± 0.02	1.89 ± 0.06*	1.96 ± 0.06*
Gastrocnemius muscle weight (g)	2.92 ± 0.10	2.86 ± 0.08	2.27 ± 0.16*	2.67 ± 0.07*,†
Kidney weight (g)	2.82 ± 0.04	2.81 ± 0.05	4.01 ± 0.09*	4.20 ± 0.10*
Lung weight (g)	1.66 ± 0.19	1.53 ± 0.03	3.28 ± 0.47*	2.54 ± 0.30*
Tibial length (cm)	4.13 ± 0.01	4.12 ± 0.01	4.00 ± 0.01*	4.04 ± 0.01*,†
Body weight/TL (g/cm)	109.7 ± 1.18	105.4 ± 1.01	92.4 ± 3.70*	98.5 ± 1.70*
Heart weight/TL (g/cm)	0.34 ± 0.01	0.32 ± 0.01	0.47 ± 0.02*	0.49 ± 0.01*
Gastrocnemius muscle weight/TL (g/cm)	0.71 ± 0.02	0.69 ± 0.02	0.57 ± 0.04*	0.66 ± 0.02*,#
Kidney weight/TL (g/cm)	0.68 ± 0.01	0.68 ± 0.01	1.00 ± 0.02*	1.04 ± 0.03*
Lung weight/TL (g/cm)	0.44 ± 0.04	0.37 ± 0.01	0.82 ± 0.11*	0.63 ± 0.08

Values are expressed as the mean ± SEM. LS-C: low-salt control; LS-BCAA: low-salt treated with BCAA; HS-C: high-salt control; HS-BCAA: high-salt treated with BCAA. TL; tibial length.
* $p < 0.05$ versus LS-C group.
† $p < 0.05$ versus HS-C group.
# $p = 0.055$ versus HS-C group.

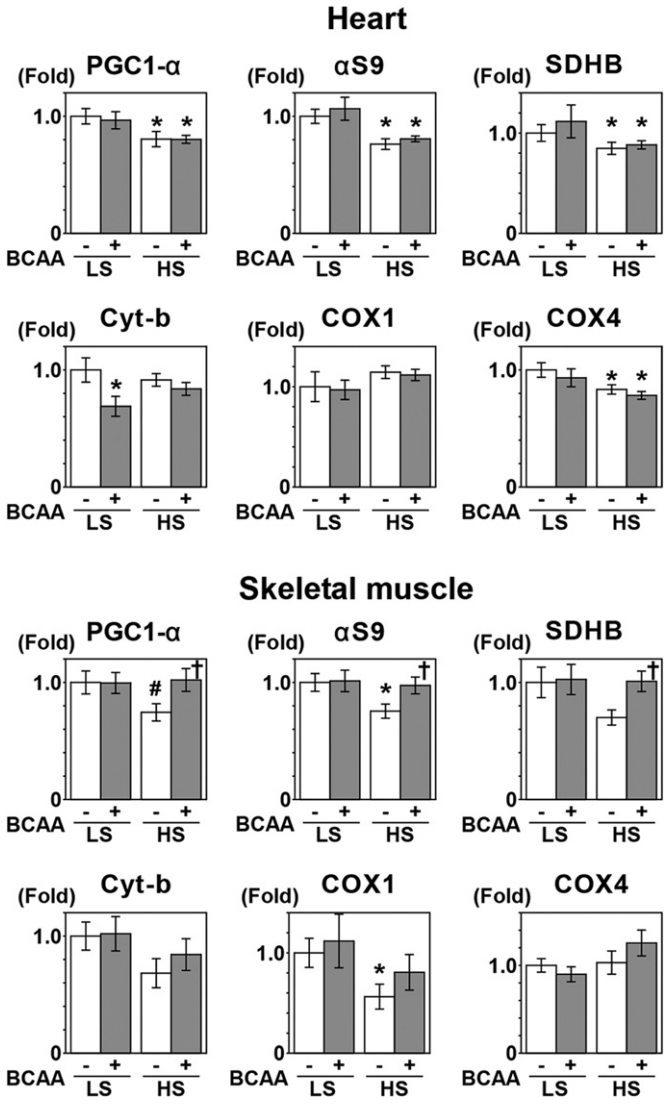

Fig. 5. Expressions of the *PGC1-α* gene and genes related to mitochondrial oxidative phosphorylation in the heart and skeletal muscle. The expression of genes in the heart is presented in the upper figure and that in the skeletal muscle is shown in the lower figure at 21 weeks of age. Expression of the *PGC-1α* gene decreased in the HS-C group compared with that in the LS-C group both in the heart and skeletal muscles. The expression of *PGC-1α* increased in the HS-BCAA group compared with that in the HS-C group in skeletal muscle. In the heart, the expressions of *αS9*, *SDHB*, and *COX4* decreased in the HS-C group compared with those in the LS-C group. There was no difference in the expression of genes between the HS-C and HS-BCAA groups. In skeletal muscle, the expressions of *αS9* and *COX1* decreased in the HS-C group compared with those in the LS-C group. The expressions of *αS9* and *SDHB* increased in the HS-BCAA group compared with those in the HS-C group. LS-C, n = 8; LS-BCAA, n = 8; HS-C, n = 11; HS-BCAA, n = 17. Values are the mean ± SEM. *$p < 0.05$ versus LS-C group. †$p < 0.05$ versus HS-C group. #$p = 0.056$ versus LS-C group.

phosphorylated mTOR and p70S6K was not different between the HS-C and HS-BCAA groups (Supplementary Fig. 4B).

4. Discussion

In the present study, BCAA decreased the heart rate, preserved the body weight, delayed the deterioration of the cardiac function, and prolonged the survival of HF rats with cardiac cachexia. BCAA also prevented the decrease in the mass of skeletal muscles and modified the expression of genes related to mitochondrial function in these muscles.

Although the mechanism by which BCAA ameliorated the body weight loss and cardiac dysfunction and improved the survival of DS rats fed the HS diet is not clear, there were clues to speculate on the mechanism. Firstly, HR decreased in the early phase of the experiment by BCAA administration. Secondly, BCAA preserved the mass of skeletal muscles and the gene expression related to the mitochondrial function in the muscles at 21 weeks of age.

It is possible that the decrease of HR maintained with BCAA for several weeks had a beneficial effect on the cardiac function. Although the mechanism by which BCAA modulated HR is not clear, it was previously reported that leucine decreased the atrial contraction rate in isolated rat hearts [31]. Persistent tachycardia leads to left ventricular dysfunction [32], and pharmacologic reduction of HR preserves the cardiac function in a rat model of myocardial infarction [33]. In addition, HR reduction improves the survival of patients with HF [34,35]. It is possible that the decrease in HR in the early phase of the experiment delayed the deterioration of the cardiac function, and ultimately improved survival.

Another possible mechanism for BCAA improving survival may involve their effect on extra-cardiac organs. BCAA preserved the mass of the gastrocnemius muscle at 21 weeks of age in DS rats fed the HS diet. This was associated with preservation of the body weight at 21 weeks of age. Skeletal muscle has been suggested to be one of the target organs on which BCAAs act [12]. Several studies have suggested that BCAAs are effective in treating muscle atrophy. Amino acid supplements which contained BCAA improved fiber atrophy due to age-induced sarcopenia in rats [13], and BCAA also prevented dexamethasone-induced soleus muscle atrophy in rats [36]. In mice bearing the cachexia-induced tumor, leucine and valine prevented skeletal muscle weight loss [14].

Moreover, in the present study, BCAA promoted expressions of the *PGC1-α* gene in skeletal muscles of DS rats fed the HS diet. PGC1-α is considered to be a regulator of energy metabolism, and its overexpression sustained mitochondrial biogenesis and prevented skeletal muscle wasting in murine models of Duchenne muscular dystrophy and amyotrophic lateral sclerosis (ALS) [37,38]. In cardiac cachexia, body weight loss is an independent risk factor of a poor prognosis [4]. In an animal experiment, skeletal muscle-specific Akt ameliorated the cardiac dysfunction in animal models of heart failure [39]. In addition, in patients with HF, exercise training [40] and pharmacological intervention [41] were considered for skeletal muscle as a therapeutic target, and they ameliorated the cardiac function. These findings suggest that BCAA preserved the mass and function of skeletal muscles, which may improve survival in DS rats fed the HS diet. The cause–effect relation between the effects of BCAAs on skeletal muscle and heart should be determined in future studies.

Though we did not have evidence that BCAA directly protected the heart, both detrimental and beneficial effects of BCAAs on the heart were reported. BCAAs were metabolized to branched-chain α-keto acids (BCKAs) in non-hepatic tissues, such as neuron, kidney, and cardiac and skeletal muscles [42]. BCKAs are then metabolized by the branched-chain α-keto acid-dehydrogenase complex (BCKD), a rate-limiting enzyme of BCAA catabolism, in the liver as well as in other tissues. One of the key regulators in branched-chain amino acid catabolism is a mitochondrial targeted 2C-type serine/threonine protein phosphatase (PP2Cm). PP2Cm positively regulates the activity of BCKD [43]. The gene expression of PP2Cm was decreased in hypertrophied hearts and was further reduced in failing hearts [43]. The systemic knockdown of PP2Cm led to the increase of the plasma levels of BCAAs [44] and accelerated heart failure after mechanical overload induced by transaortic constriction in mice [45]. Suppression of PP2Cm expression induced cell death of cultured cardiomyocytes [43]. The proposed mechanisms for the detrimental effects of decreased PP2Cm on the heart were dysregulation of mitochondrial permeability transition pore, the increase of reactive oxygen species, and the accumulation of tissue BCAAs [43,44]. Furthermore, leucine induced insulin resistance, which potentially perturbed normal glucose homeostasis [46]. Metabolomic analysis of pressure-overload mice also showed the insulin resistance in the failing hearts associated with increased BCAA levels

in cardiac tissue [47]. These results indicated that chronically elevated BCAA/BCKA in cardiac tissue can potentially block the normal bioenergenic homeostasis and induce a series of pathologic remodeling and dysfunction of the heart. On the other hand, there are also several reports suggesting that BCAAs have beneficial effects on the heart. Oral BCAA supplementation promoted mitochondrial biogenesis of the heart and skeletal muscles through eNOS-derived nitric oxide, decreased ROS in cardiac and skeletal muscles, and prolonged the survival of mice [11]. A leucine diet attenuated myocardial infarction in mice accompanied by a significant increase in cardiac ATP content [17], and leucine and isoleucine improved cardiac systolic function in septic isolated rat heart [48].

Of interest, BCAA significantly decreased food and water intakes in DS rats fed the LS diet but did not change food or water intake in DS rats fed the HS diet. Leucine administration decreases food intake via its effect on the hypothalamus [49]. This may explain the loss of appetite and body weight in DS rats fed the LS diet receiving BCAA. Although in a clinical situation, the oral administration of BCAA reportedly increased the appetite of cancer patients [50] and malnourished uremic patients undergoing hemodialysis [51]. In the present study, BCAA did not change the food intake in HF rats.

In diabetic rats, several amino acids are used for treatment, and dietary supplementation of taurine, not other amino acids, attenuates diabetes-induced changes in cardiac function [52]. In septic rats, several components of amino acids are used, and they show different effects on muscle weakness [53]. In addition, in cancer-related wasting in human patients, different amino acid components show different effects on muscle atrophy [54]. Although we used a single composition of amino acids, these data suggest that other compositions of amino acids would generate results different from those shown in the current report.

The present study was a preliminary experimental report that BCAA might be useful for the treatment of patients with HF associated with cachexia. However, several limitations should be considered. Firstly, all animals were sacrificed and analyzed at 21 weeks of age, which was the terminal phase of HF. Serial analysis at different time points of the experiment would clarify the potential role of BCAA in mitochondria, ROS, inflammation, ubiquitin ligases, fibrosis, apoptosis, or mTOR pathway, although these analyses would require a large number of animals. Secondly, since only gene expression related to mitochondrial function was examined and few mitochondrial functions were measured, the role of mitochondria remains to be elucidated in future studies. Thirdly, we did not measure plasma BCAA levels nor determined the uptake and catabolism of BCAA which would be elucidated in another experiment with isotope-labeled amino acids.

5. Conclusions

BCAA preserved the body weight and cardiac function and prolonged survival in HF rats with cardiac cachexia.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2015.06.021.

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