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# Acute Effects of Branched-Chain Amino Acid Ingestion on Muscle pH during Exercise in Patients with Chronic Obstructive Pulmonary Disease

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## 1. Introduction

The use of $^{31}$P-magnetic resonance (MR) spectroscopy ($^{31}$P-MRS) allows noninvasive measurement of high-energy phosphate compounds such as phosphocreatine (PCr) and adenosine triphosphate (ATP), and the low-energy breakdown product, inorganic phosphate (Pi), in exercising muscle. Ratios of measureable high- and low-energy phosphate metabolites (e.g., PCr/Pi or PCr/(PCr+Pi) have been utilized as indices of the overall bioenergetic state of the cell (Sapega et al., 1987). Extracellular Pi does not exist in sufficient quantities to significantly affect $^{31}$P-MR spectra (Sapega et al., 1987). Intracellular pH (pHi) can also be measured noninvasively based on the pH-dependent chemical shift of cellular Pi that appears as PCr peaks on $^{31}$P-MRS (Sapega et al., 1987; Taylor et al., 1983). A decrease in pHi during exercise suggests lactate accumulation in muscles (Sapega et al., 1987; Taylor et al., 1983).

We have previously found, using $^{31}$P-MRS, that skeletal muscle metabolism in patients with chronic respiratory impairment undergoes specific changes (Kutsuzawa et al., 1992, 1995). Patients with chronic respiratory impairment display significant decreases in PCr and pHi during even mild exercise, suggesting that ATP production is reduced and that lactate levels accumulate rapidly in their muscles, suggesting reduced oxidative capacity. In addition, recent studies have demonstrated that reduced oxidative capacity in skeletal muscles correlates with an accelerated lactate response to exercise in patients with chronic obstructive pulmonary disease (COPD) (Maltais et al., 1996; Saey et al., 2005). Several factors such as inactivity, malnutrition and/or hypoxemia might contribute to altered muscle metabolism (Mannix et al., 1995; Payen et al., 1993; Sala et al., 1999).

Weight loss and muscle wasting are common features in patients with COPD (Bernard et al., 1998) and muscle wasting contributes to muscle weakness and exercise limitations in patients with COPD (Schols et al., 1993). Skeletal muscle is the major protein store that

supplies amino acids to other tissues under specific conditions. Plasma concentrations of free amino acids indicate the balance between exogenous uptake and intercurrent metabolites in protein synthesis and breakdown (Wagenmakers, 1998). The branched-chain amino acids (BCAAs) leucine, isoleucine, and valine account for 35% of the essential amino acids contained in muscle proteins (Harper et al., 1984). Although essential amino acids other than BCAAs are mainly catabolized in the liver, BCAAs can be oxidized in skeletal muscle (Wagenmakers, 1998).

Several investigators have reported that the amino acid profile is altered in the plasma and skeletal muscle of patients with COPD (Engelen et al., 2000b; Hoffored et al., 1990; Pouw et al., 1998; Yoneda et al., 2001). Most of these studies have shown that plasma concentrations of the BCAAs leucine, isoleucine, and valine are reduced (Engelen et al., 2000b; Hoffored et al., 1990; Yoneda et al., 2001). Yoneda et al. (Yoneda et al., 2001) demonstrated that decreased concentrations of BCAAs in COPD are specifically related to weight loss and decreased muscle mass.

Energy expenditure greatly increases in skeletal muscle during exercise, and BCAA oxidization then maximally increases two- to three-fold (Knapik et al., 1991; Wolf et al., 1982). In addition, BCAAs might contribute to energy metabolism during exercise as substrates that expand the pool of tricarboxylic acid (TCA) cycle intermediates (Wagenmakers, 1998). Using $^{31}$P-MRS, we have previously found that plasma concentrations of BCAAs correlate with muscle pH at the completion of exercise in patients with COPD (Kutsuzawa et al., 2009). Those findings were consistent with a role of BCAAs in muscle energy metabolism during exercise in patients with COPD.

Supplementation with amino acids, particularly BCAAs, should increase exercise capacity in COPD patients, who might be affected by altered muscle and plasma amino acid profiles. Several studies in healthy subjects have examined the effects of BCAA ingestion on lactate metabolism during long-term, exhaustive exercise (De Palo et al., 2001; MacLean et al., 1996; Vukovich et al., 1992). MacLean et al. (MacLean et al., 1996) examined the effects of a large oral dose (308 mg/kg) of BCAAs on muscle amino acid metabolism during 90 min of exercise. They found that lactate release and arterial lactate values were lower in the group given BCAAs compared to a control group. We have studied the effects of BCAA ingestion on muscle pH during repeated bouts of short-term (3-min) exercise in healthy subjects and have found that BCAA supplementation before exercise can cause the attenuation of acidosis in exercising muscle, probably due to a decrease in lactate production (Kutsuzawa et al., 2011). Another study (Doi et al., 2004) of muscle energy metabolism demonstrated that an infusion of glucose and BCAAs before exercise improved acidic pHi during exercise in patients with liver cirrhosis accompanied by a severe amino acid imbalance.

The effects of BCAA ingestion on muscle pH have been investigated in healthy young participants (Kutsuzawa et al., 2011), but whether such supplementation will benefit exercise capacity in patients with COPD remains unclear. We thus used $^{31}$P-MRS to investigate the effects of BCAA ingestion on muscle pH during repeated bouts of short-term (3-min) exercise. Our hypothesis was that BCAA ingestion before a second bout of exercise could prevent metabolic acidosis in exercising muscle.

## 2. Methods

### 2.1 Subjects

Subjects comprised 10 ambulatory male outpatients with stable COPD (mean age, 70.4 ± 8.8 years) diagnosed according to spirometric findings from moderate to very severe airflow limitation (forced expiratory volume in 1 s ($FEV_1$)/forced vital capacity (FVC)<70% and $FEV_1$ <80% of the predicted value) (Global Initiative for Chronic Obstructive Lung Disease, 2010). None of the patients had ever received systemic corticosteroid therapy and two of them had been treated with oxygen inhalation at home. None of them had participated in pulmonary rehabilitation. Exclusion criteria were malignancy, cardiac failure, renal failure, liver cirrhosis, diabetes mellitus, and infection. The ethics committee at our institution approved the study protocol, and both the control individuals and patients provided written informed consent to participate. Tables 1 and 2 show the physical characteristics of the participants.

| | | Patients (n=10) | | |
|---|---|---|---|---|
| Age | years | 70.4 | ± | 8.8 |
| Height | cm | 163.9 | ± | 3.8 |
| Weight | kg | 55.6 | ± | 10.0 |
| BMI | kg/m² | 20.6 | ± | 3.1 |
| Forearm circumference | cm | 22.9 | ± | 1.7 |
| TSF | cm | 5.6 | ± | 3.0 |
| Grip power (left) | kg | 36.7 | ± | 5.5 |

Table 1. Anthropometric data from the patients. Values are given as mean ± SD. BMI, body mass index; TSF, triceps skinfold thickness.

| | | Patients (n=10) | | |
|---|---|---|---|---|
| VC | L | 2.75 | ± | 0.77 |
| VC (%of predicted) | % | 84.1 | ± | 21.8 |
| $FEV_1$ | L | 1.02 | ± | 0.40 |
| $FEV_1$/FVC | % | 40.3 | ± | 9.4 |
| $FEV_1$ (% of predicted) | % | 41.5 | ± | 15.4 |
| pH | | 7.412 | ± | 0.031 |
| $PaCO_2$ | Torr | 41.3 | ± | 4.8 |
| $PaO_2$ | Torr | 79.8 | ± | 7.6 |

Table 2. Spirometric and blood gas analysis data from the patients. Values are given as mean ± SD. VC, vital capacity; $FEV_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; $PaCO_2$, partial pressure of $CO_2$; $PaO_2$, partial pressure of $O_2$.

### 2.2 Study design

All patients fasted overnight, then anthropometric parameters and grip strength were measured at our outpatient clinic. Maximal voluntary grip strength of the non-dominant arm was measured using a dynamometer (DM-100N; Yagami, Nagoya, Japan). Fasting

venous blood was obtained from an antecubital vein. Arterial blood was taken from the brachial artery for blood gas analysis while breathing room air.

All patients then performed two consecutive, 3-min constant work-rate exercises (a control bout and a BCAA bout), using the non-dominant forearm. Differences in MRS variables among individuals might be smaller for the non-dominant arm because routine activities might not vary as much as those performed with the dominant arm. Ten minutes after the first bout (control bout) of exercise, the patients consumed 8.0 g of powdered BCAAs (2 packs of LIVACT®; Ajinomoto, Tokyo, Japan) with 100 ml of water. The second bout (BCAA bout) of exercise started at 60 min after BCAA ingestion. Patients remained seated during the interval between first and second bouts of exercise and were allowed water. Muscle metabolism was measured using $^{31}$P-MRS during 1.5 min of rest, 3 min of exercise, and 4 min of recovery.

The exercise consisted of repetitively gripping a lever attached to a weight via a pulley system at a rate of 20 grips/min for 3 min while supine and breathing room air. The weight was lifted 5 cm by gripping the lever. To normalize the exercise intensity, the weight was adjusted to 7% of the maximum grip strength, which was suitable for this study because all patients could complete the exercise. Moreover, repetitive hand-gripping exercise in which the same weight (7% of maximal hang grip) is lifted 5 cm 20 times per min can differentiate healthy from altered muscle metabolism (as a decrease in pHi) (Kutsuzawa et al., 1992, 1995).

### 2.3 BCAA supplementation

LIVACT® is a BCAA powder that was developed to treat imbalances in amino acids among patients with liver cirrhosis. One pack of LIVACT (4.75 g) contains 4.0 g of BCAA (3808 mg of leucine, 1904 mg isoleucine and 2288 mg of valine). The composition ratio of leucine, isoleucine and valine at 2:1:1.2 effectively balances nitrogen as well as the plasma amino acid profile (Ohashi et al., 1989). The plasma BCAA level at 2 h after ingesting 8 g of BCAA increases 2-fold (Hamada et al., 2005).

### 2.4 Nutritional assessment

Nutritional status was evaluated by biochemical blood testing and anthropometric measurements including height, weight, non-dominant forearm circumference and triceps skin fold thickness (TSF). Body mass index (BMI) was calculated based on height and weight. The circumference of the non-dominant forearm was measured at the proximal third of the forearm, where the MRS surface coil was positioned. TSF of the non-dominant arm was measured using an EIYOKEN-TYPE skinfold caliper (Yagami).

Fasting blood samples from all patients were obtained by venipuncture before the control bout. In six of the 10 patients, second blood samples were obtained before the BCAA bout. Portions of samples were immediately cooled on ice and plasma obtained by centrifugation at 4°C was stored at -80°C for later amino acid analysis. Samples were deproteinized using 5% sulfosalicylic acid, then plasma levels of amino acids were measured by ion-exchange, high-pressure liquid chromatography with fluorometric detection (Model 8500; Hitachi, Tokyo, Japan) (Dyel et al., 1986). Total BCAAs comprised

leucine + isoleucine + valine, and total amino acids included all measured amino acids. Serum albumin and prealbumin, as indices of nutritional status, were determined by routine methods in other portions of the samples.

### 2.5 $^{31}$P-MRS

Unlocalized MR spectra were obtained using a 2.0-T, 31-cm-bore BEM 250/80 superconducting magnet (Otsuka Electronics Co., Osaka, Japan). The spectrometer was operated at 85 MHz for $^{1}$H and at 34.5 MHz for $^{31}$P. A 4-cm surface coil was placed on the proximal third of the non-dominant forearm. We accumulated $^{31}$P-MR spectra every 3 s for 1 min using a single 90° pulse (50 μs) (Kutsuzawa et al., 1992). We then analyzed the $^{31}$P-MR spectrum at rest (1 min before onset of exercise) and at the end of 3 min of exercise.

The signal area for Pi and PCr was determined from each spectrum by Gaussian curve fitting (Kutsuzawa et al., 1992). Relative concentrations of PCr and Pi were evaluated using normalized units of PCr/(PCr+Pi).

Muscle pH (i.e., pHi) was calculated as a difference in the chemical shift between Pi and the PCr peak (Stevens, 1987; Taylor et al., 1983). At physiological pH, Pi exists as $HPO_4^{2-}$ and $H_2PO_4^{-}$ ions. The signal from $HPO_4^{2-}$ is shifted downfield by 2.25 ppm from that of $H_2PO_4^{-}$. However, the two forms are in rapid exchange, so a single Pi peak is formed with a chemical shift reflecting the relative proportions of the two ions. The chemical shift in Pi can thus be used as a pH indicator, as follows:

$$pH = 6.75 + \log (\delta - 3.27)/(5.69 - \delta),$$

where δ is the difference of chemical shift in parts per million between the Pi and PCr signals (Taylor et al., 1983). The pH was determined from the weighted average of both Pi peaks if the peak was split.

We determined ΔpHi or ΔPCr/(PCr+Pi) as the difference between values just before and at the end of the exercise period.

### 2.6 Statistical analysis

All data are presented as means ± standard deviation (SD). MRS variables between the control and BCAA bouts of exercise and between resting and the end of exercise were compared using Student's paired *t*-test. The ΔpHi and ΔPCr/(PCr+Pi) between control and BCAA bouts were compared to determine the effects of BCAA supplementation using the paired Student's *t*-test. We used linear regression analysis to evaluate correlations between concentration of BCAA and BMI, between BCAA and spirometric data, and between BCAA and the MRS variable (ΔpHi of the first exercise bout) using the least-squares method. Goodness of fit was examined using the $\chi^2$-test. A value of $p < 0.05$ was considered indicative of a significant difference.

## 3. Results

### 3.1 Physical characteristics and nutritional assessment

Table 1 shows the physical characteristics of the 10 patients. Mean body mass index of patients was 20.6 ± 3.1 kg/m$^2$ and 3 of the patients were considered malnourished (BMI <

20). Table 2 shows spirometric data from the patients with COPD. Mean $FEV_1$ was 1.02 ± 0.40 L. Based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (Global Initiative for Chronic Obstructive Lung Disease, 2010), 3 of the 10 patients were categorized as stage II, 4 as stage III and 3 as stage IV, but none showed severe hypoxemia (partial pressure of $O_2$ ($PaO_2$), 79.8 ± 7.6 Torr).

### 3.2 Nutritional assessment

Values of serum albumin did not decrease in the patients. Although the mean level of prealbumin was within normal range (22-40 mg/dl), two of the patients showed a prealbumin level below the lower limit of the normal range (Table 3).

| | | Patients (n=10) | | |
|---|---|---|---|---|
| Albumin | g/dl | 4.5 | ± | 0.4 |
| Prealbumin | mg/dl | 25.9 | ± | 6.5 |

Table 3. Albumin and prealbumin levels in fasting venous blood from patients. Values are given as mean ± SD.

| Amino acid (nmol/L) | Range | Patients (n=10) | | |
|---|---|---|---|---|
| Taurine | 40 - 93 | 56.4 | ± | 14.8 |
| Threonine | 67 - 190 | 101.5 | ± | 21.0 |
| Serine | 72 - 190 | 92.1 | ± | 20.8 |
| Asparagine | 45 - 97 | 43.1 | ± | 8.5 |
| Glutamic acid | 12 - 63 | 38.1 | ± | 12.0 |
| Glutamine | 420 - 700 | 699.5 | ± | 146.1 |
| Proline | 78 - 270 | 132.2 | ± | 35.1 |
| Glycine | 150 - 350 | 167.9 | ± | 40.6 |
| Alanine | 210 - 520 | 339.5 | ± | 55.0 |
| Citrulline | 17 - 43 | 36.5 | ± | 14.7 |
| Aminobutyric acid | 7.9 - 27 | 13.2 | ± | 4.1 |
| Valine | 150 - 310 | 199.9 | ± | 34.6 |
| Cystine | 29 - 49 | 49.4 | ± | 8.2 |
| Methionine | 19 - 40 | 23.7 | ± | 5.2 |
| Isoleucine | 40 - 110 | 59.4 | ± | 12.8 |
| Leucine | 48 - 180 | 103.2 | ± | 21.6 |
| Tyrosine | 40 - 90 | 65.2 | ± | 13.8 |
| Phenylalanine | 43 - 76 | 56.8 | ± | 11.2 |
| Histidine | 59 - 92 | 70.7 | ± | 12.7 |
| Tryptophan | 37 - 75 | 58.6 | ± | 9.3 |
| Ornithine | 30 - 100 | 53.1 | ± | 16.3 |
| Lysine | 110 - 240 | 191.5 | ± | 47.8 |
| Arginine | 54 - 130 | 100.1 | ± | 28.7 |
| Total amino acids | 2100 - 3500 | 2770.7 | ± | 391.8 |
| Total BCAAs | 270 - 600 | 362.4 | ± | 66.4 |

Table 4. Plasma amino acid concentrations before the first exercise bout. Values are given as mean ± SD. BCAA, branched-chain amino acid.

Table 4 shows values for individual plasma amino acids. Total amino acids and total BCAAs were not reduced from the normal range. The mean glutamine concentration was approximately at the upper limit of the normal range. Concentrations of total BCAAs tended to correlate with BMI ($r = 0.6099$, $p < 0.1$) in patients. Concentrations of total BCAAs did not correlate with $FEV_1$(%of the predicted value), which is an index of COPD severity.

Plasma amino acid profiles at 1 h after BCAA ingestion were examined in 6 of 10 patients (Table 5). Valine, leucine, and isoleucine significantly increased by more than 3- to 5-times after BCAA ingestion. Glutamine and arginine were also slightly but significantly increased.

| Amino acid (nmol/L) | Fasting | | | After BCAA ingestion (n=6) | | |
|---|---|---|---|---|---|---|
| Taurine | 51.7 | ± | 6.0 | 52.6 | ± | 3.8 |
| Threonine | 112.4 | ± | 15.6 | 110.8 | ± | 19.4 |
| Serine | 101.7 | ± | 18.1 | 104.7 | ± | 20.5 |
| Asparagine | 45.5 | ± | 8.6 | 43.5 | ± | 7.3 |
| Glutamic acid | 32.6 | ± | 11.5 | 31.3 | ± | 14.2 |
| Glutamine | 749.2 | ± | 165.0 | 889.4 | ± | 167.4** |
| Proline | 133.8 | ± | 20.1 | 116.9 | ± | 23.2* |
| Glycine | 210.8 | ± | 47.7 | 190.7 | ± | 48.6* |
| Alanine | 351.1 | ± | 67.1 | 315.5 | ± | 42.7 |
| Citrulline | 40.8 | ± | 16.6 | 39.6 | ± | 18.0 |
| Aminobutyric acid | 14.6 | ± | 3.7 | 15.3 | ± | 3.4 |
| Valine | 203.3 | ± | 22.9 | 679.8 | ± | 160.1** |
| Cystine | 51.0 | ± | 6.4 | 51.0 | ± | 5.6 |
| Methionine | 25.1 | ± | 4.6 | 22.9 | ± | 4.2 |
| Isoleucine | 59.2 | ± | 12.2 | 322.6 | ± | 83.6** |
| Leucine | 103.6 | ± | 19.0 | 606.5 | ± | 163.1** |
| Tyrosine | 66.0 | ± | 14.9 | 61.5 | ± | 12.0 |
| Phenylalanine | 56.6 | ± | 9.8 | 53.0 | ± | 6.7 |
| Histidine | 74.2 | ± | 10.8 | 77.7 | ± | 10.4 |
| Tryptophan | 60.0 | ± | 9.1 | 53.4 | ± | 8.8** |
| Ornithine | 59.0 | ± | 17.1 | 59.3 | ± | 17.7 |
| Lysine | 216.8 | ± | 29.2 | 232.4 | ± | 38.6 |
| Arginine | 111.1 | ± | 29.7 | 128.1 | ± | 36.3* |
| Total amino acids | 2918.4 | ± | 332.0 | 4246.0 | ± | 320.3** |
| Total BCAAs | 366.0 | ± | 51.5 | 1609.0 | ± | 386.9** |

Table 5. Plasma amino acid concentration before and after ingestion of BCAAs. Valine, leucine, and isoleucine were increased more than 300% by 1 h after BCAA ingestion. Values are given as mean ± SD. *: $p < 0.05$ vs. before ingestion; **: $p < 0.01$ vs. before ingestion.

### 3.3 Muscle energy metabolism

Representative $^{31}$P-MR spectra obtained from a 75-year-old patient with COPD at the end of exercise are shown in Figure 1. The peak for Pi was higher than that for PCr, and shifted

toward that of PCr during the control bout of exercise (Fig. 1A). In contrast, the peak of Pi was lower than that of PCr, and the Pi peak went back toward the normal chemical shift (-4.9 ppm). The values of pHi were calculated as 6.39 (Fig. 1A) and 6.84 (Fig. 1B) during the control and BCAA bouts of exercise, respectively. Values of PCr/(PCr+Pi) during the control (Fig. 1A) and BCAA bout (Fig. 1B) of exercise were 0.467 (Fig. 1A) and 0.630 (Fig. 1B), respectively.

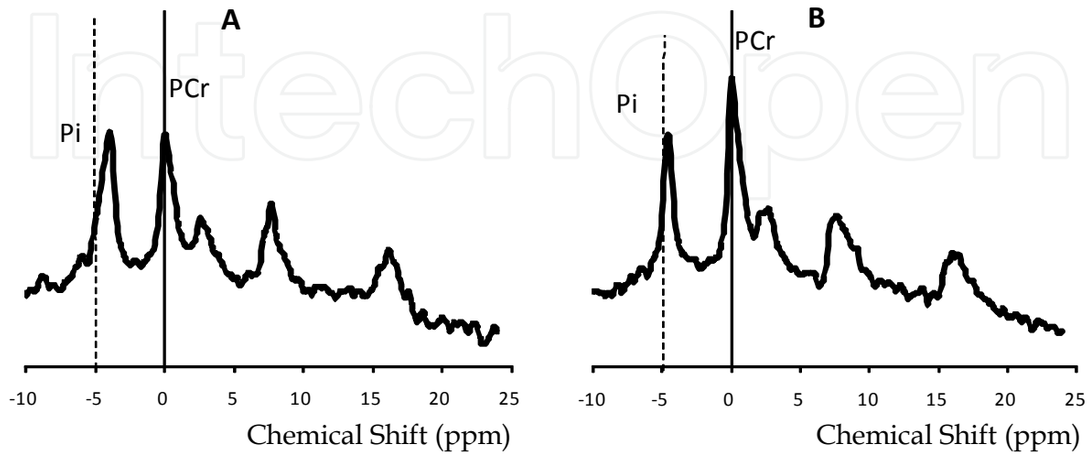



Fig. 1. $^{31}$P-MR spectra after 3 min of exercise, obtained from a 75-year-old patient with COPD. A and B, First (control) and second (after BCAA ingestion) bouts of exercise, respectively; Pi, inorganic phosphate; PCr, phosphocreatine.

Calculated values of pHi and PCr/(PCr+Pi) in the control bout of exercise were 6.39 and 0.467, respectively, and those in the BCAA bout were 6.84 and 0.630, respectively.

Mean values of pHi and PCr/(PCr+Pi) at rest and at the end of each exercise are shown in Tables 6 and 7, respectively. Both pHi and PCr/(PCr+Pi) were significantly decreased at the end of each exercise bout. Mean values for pHi and PCr/(PCr+Pi) were significantly higher at the end of the BCAA bout than at the end of the control bout. Thus, ΔpHi and ΔPCr/(PCr+Pi) were significantly smaller in the BCAA bout. In addition, acidic changes in pHi were attenuated in 6 of the 10 patients in the BCAA bout (Fig. 2).

| | Control bout | | | BCAA bout | | |
|---|---|---|---|---|---|---|
| At rest | 7.00 | ± | 0.08 | 7.03 | ± | 0.09 |
| End of exercise | 6.70 | ± | 0.19 # | 6.86 | ± | 0.14 * † |
| ΔpH | -0.31 | ± | 0.19 | -0.17 | ± | 0.17 * |

Table 6. Changes in muscle pH. *, $p < 0.05$ vs. control bout; †, $p < 0.05$ vs. resting; #, $p < 0.01$ vs. resting. ΔpH, difference in pHi between resting and end of exercise.

| | Control bout | | | BCAA bout | | |
|---|---|---|---|---|---|---|
| At rest | 0.870 | ± | 0.023 | 0.876 | ± | 0.032 |
| End of exercise | 0.423 | ± | 0.056 | 0.502 | ± | 0.103 * # |
| ΔPCr/(PCr+Pi) | -0.447 | ± | 0.059 # | -0.373 | ± | 0.094 * |

Table 7. Changes in PCr/(PCr+Pi). *, $p < 0.05$ vs. control bout; #, $p < 0.01$ vs. resting. ΔPCr/(PCr+Pi), difference in PCr/(PCr+Pi) between resting and end of exercise.

The pHi of all participants in the control bout of exercise correlated with leucine concentration (r=0.6713) and tended to correlate with BCAA concentration (r=0.6015).

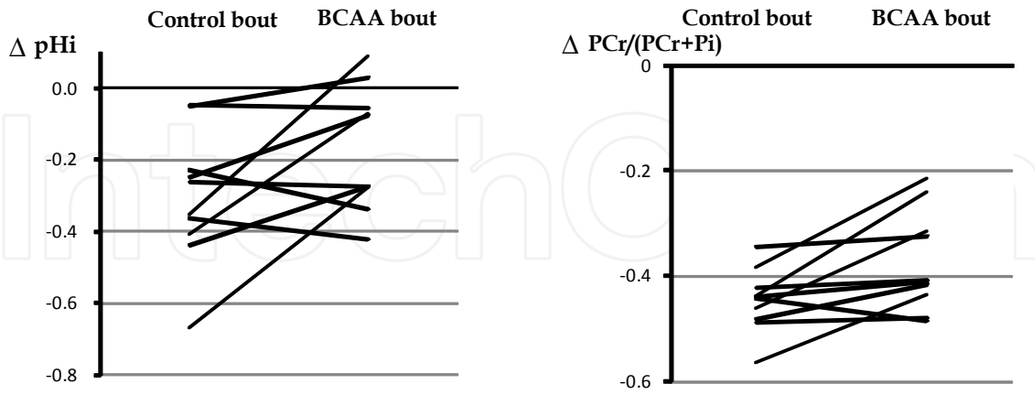



Fig. 2. Differences in pHi (ΔpHi) and PCr/(PCr+Pi) (ΔPCr/(PCr+Pi)) of each patient in control and BCAA bout. Six of 10 patients showed that ΔpHi and ΔPCr/(PCr+Pi) were reduced in the BCAA bout.

## 4. Discussion

We investigated the acute effects of a single BCAA ingestion on muscle energy metabolism during hand-grip exercise in patients with COPD and found a decrease in ΔpHi and ΔPCr/(PCr+Pi) for the BCAA bout. The finding suggests that BCAA supplementation before exercise can cause an attenuation of acidosis in exercising muscle among COPD patients, probably due to an increase in oxidative capacity and a decrease in lactate production.

### 4.1 Muscle pH

Values of pHi can be measured using $^{31}$P-MRS as the pH-dependence of a chemical shift between cellular Pi and PCr peaks (Sapega et al., 1987; Taylor et al., 1983). In addition, pHi can be determined as the $H^+$ balance of consumption (PCr hydrolysis, buffering capacity and $H^+$ efflux) and lactate production processes (Kemp, 2004). A decrease in pHi during exercise suggests that lactic acid accumulates in exercising muscle cells due to anaerobic glycolysis (Kemp, 2004; Sapega et al., 1987; Taylor et al., 1983). The line width frequently increased and the Pi peak often split during exercise in patients, suggesting that the pH among muscle fibers is heterogeneous, and supporting the suggestion that this phenomenon reflects metabolic heterogeneity among muscle fiber types (Vandenborne et al., 1993).

The $H^+$ and lactate produced can either be buffered and removed intracellularly or released to the interstitium. Several transport systems as well as muscle buffering remove $H^+$ during intense skeletal muscle contraction. Buffers include free Pi, PCr and histidine residues in both proteins and some dipeptides, and the capacity of such buffers is decreased by high-intensity exercise via a decrease in protein buffering (Bishop et al., 2009). Supplementation with β-alanine, which increases muscle protein buffer capacity, attenuates the fall in blood pH during exercise (Baguet et al., 2010). Whether BCAA supplementation affects muscle buffer capacity remains unclear.

### 4.2 Plasma BCAA profile in COPD

Several studies have investigated plasma amino acid profiles in COPD patients (Engelen et al., 2000b; Hoffored et al., 1990; Pouw et al., 1998; Yoneda et al., 2001). No specific plasma amino acid profile has been identified, probably due to the heterogeneity of disease stages in COPD (for example, severity of airflow limitation, hypoxemia, and malnutrition). One study of Japanese patients with COPD (Yoneda et al., 2001) found that concentrations of glutamic acid and glutamine were elevated, whereas those of BCAAs were decreased except for leucine. We have previously investigated plasma amino acid levels in fasting venous blood among COPD patients and age-matched healthy subjects, revealing that plasma glutamine was elevated without overall changes in BCAAs (Kutsuzawa et al., 2009). The present study also showed that levels of plasma BCAA were within normal limits. One previous study (Yoneda et al., 2001) demonstrated that decreased concentrations of BCAAs in COPD are specifically related to weight loss and decreased muscle mass. Our previous (Kutsuzawa et al., 2009) and present studies have shown that the concentration of BCAAs correlated or tended to correlate with BMI (r=0.54488, r=0.6099), respectively, so nutritional status relates to a low BCAA concentration. However, severity of COPD did not correlate with the concentration of BCAAs, since no correlation was seen with $FEV_1/FEV_1$pred.

We measured BCAA levels immediately before the BCAA bout in 6 of 10 patients. Each BCAA concentration after BCAA ingestion increased significantly, by more than 3- to 5-fold compared to fasting values and to a consistent 2- to 3-fold more than the upper limit of the normal range. Another study demonstrated that plasma levels of BCAAs at 2 h after BCAA supplementation increase 2-fold after ingesting > 8 g of BCAAs (Hamada et al., 2005). Plasma levels of BCAA by the BCAA bout have thus been sufficiently increased. Increased plasma levels of BCAAs after administration subsequently enhance BCAA uptake by muscle during exercise (MacLean et al., 1996).

### 4.3 Plasma BCAAs and muscle energy metabolism

We have previously reported that plasma BCAA (leucine, isoleucine, valine) concentrations correlate with both intracellular pH and the PCr index during exercise (Kutsuzawa et al., 2009). The present study also showed the relationship between muscle pH and leucine (r=0.6713, $p<0.05$). Total BCAAs tended to correlate with muscle pH (r=0.6015, $p<0.1$). A previous study (Doi et al., 2004) investigated muscle energy metabolism in patients with liver cirrhosis accompanied by low plasma BCAA concentrations using $^{31}$P-MRS and found an acidic pHi during exercise.

Low plasma BCAA concentrations might affect lactate metabolism through two mechanisms. Firstly, BCAAs expand the pool of TCA cycle intermediates (Rutten et al., 2005; Wagenmakers, 1998) and react with α-ketoglutarate to produce branched-chain α-keto acids and glutamic acid in the presence of BCAA aminotransferase (Vandenborne et al., 1993; Wagenmakers, 1998):

$$\text{BCAA} + \alpha\text{-ketoglutarate} \leftrightarrow \text{branched-chain } \alpha\text{-keto acids} + \text{glutamic acid}$$

BCAAs would therefore affect intracellular glutamic acid concentrations. During the first minutes of exercise, glutamic acid generates TCA cycle intermediates via the alanine aminotransferase reaction in skeletal muscle (Rutten et al., 2005):

pyruvate + glutamic acid → alanine + α-ketoglutarate

The accumulated pyruvate can generate alanine through this reaction instead of lactate during exercise. Engelen et al. discovered that glutamic acid concentrations in the quadriceps femoris muscles of COPD patients are reduced and might relate to a reduced lactic threshold (Engelen et al., 2000a).

Secondly, BCAAs can be oxidized during moderate to heavy exercise. Several studies have reported that endurance exercise activates the branched-chain α-keto acid dehydrogenase (BCKDH) complex, which catalyzes branched-chain α-keto acid to form coenzyme A compounds in human and rat skeletal muscle (Shimomura et al., 1995; Wagenmakers et al., 1989). Shimomura et al. (Shimomura et al., 1993) reported that activity of the BCKDH complex increases in rat hindlimb muscles after 5 min of electrically induced contraction. Rates of leucine oxidation were almost double from rest to moderate intensity in healthy individuals performing steady-state exercise (Lamont et al., 2001). The oxidation of BCAAs does not relate to glycolysis or produce lactate. This mechanism might also explain the correlation between pHi and total BCAA after exercise.

### 4.4 Effects of amino acid supplementation on patients with COPD

The effects of BCAA ingestion before and/or after exercise on muscle damage and on muscle metabolism have been investigated in athletes since the 1990s. Several studies have demonstrated that BCAA supplementation attenuates the increase in blood lactate dehydrogenase (Coombes et al., 2000; Koba et al., 2007) and creatine kinase (Coombes et al., 2000) after prolonged exercise. Another study has shown that supplementation with amino acids including BCAAs attenuates delayed-onset muscle soreness (Nosaka et al., 2006). These findings suggested that BCAAs attenuate the degree of exercise-induced muscle damage.

Intense muscle activity generates intracellular protons and lactate accumulation. Several investigators have studied the effects of BCAA supplementation on lactate accumulation during exercise (Blomstrand et al., 1996; De Palo et al., 2001; MacLean et al., 1996; Matsumoto et a., 2009; Vukovich et al., 1992) in healthy subjects and athletes. MacLean et al. (MacLean et al., 1996) examined the effects of a large oral dose (308 mg/kg) of BCAAs on muscle amino acid metabolism during 90 min of dynamic knee extensor exercise. They reported that long-term exercise after BCAA administration resulted in significantly greater muscle $NH_3$, alanine and glutamine levels, as well as lower lactate production. Matsumoto et al. (Matsumoto et al., 2009) studied the effects of BCAA supplementation on the lactate threshold during incremental exercise. They demonstrated that BCAA ingestion immediately before an incremental exercise test after 6 days of BCAA supplementation increased oxygen uptake and workload at the lactate threshold, the onset of blood lactate accumulation. Blomstrand et al. reported a smaller decrease in muscle glutamic acid levels in healthy volunteers who consumed BCAAs and then performed a prolonged submaximal exercise test when compared to a control group (Blomstrand et al., 1996).

Supplementation with amino acids, particularly BCAAs and/or glutamic acid, should increase exercise capacity in COPD patients, as this capacity may be affected by altered muscle and plasma amino acid profiles. A small number of studies have examined

supplementation with BCAAs or glutamic acid during exercise in COPD patients. Menier et al. studied the effects of BCAA administration to COPD patients during rehabilitation and showed no effects of BCAA supplementation on maximal oxygen uptake or maximal work (Menier et al., 2001). Rutten et al. studied the metabolic and functional effects of glutamic acid intake in COPD patients. Oral glutamic acid ingestion (30 mg/kg body weight every 20 min for 80 min) did not increase muscle glutamic acid concentration or reduce plasma lactate levels in COPD patients or healthy controls (Rutten et al., 2008).

The effects of a single administration of BCAAs on muscle energy metabolism in COPD patients have not been investigated. The present study showed that BCAA ingestion attenuated ΔpHi and ΔPCr/(PCr+Pi) at the end of exercise compared with the control bout. The findings suggested that BCAA supplementation before exercise could increase oxidative phosphorylation and decrease lactate production, then attenuate acidosis in exercising muscle in COPD patients. We have previously investigated the effects of BCAA ingestion on muscle pH during similar repeated bouts of short-term (3-min) exercise in healthy young participants (Kutsuzawa et al., 2011). The pHi at the second exercise bout without BCAA ingestion was significantly decreased compared with the first bout. In contrast, neither pHi after 3 min of exercise nor ΔpHi differed significantly between the first and second exercise bouts after BCAA ingestion in healthy participants. Effects of BCAA ingestion on muscle pH may be intense in COPD patients, probably due to low BCAA concentrations. Muscle energy metabolism might be affected by BCAAs during exercise acting as an energy source and as substrates that expand the pool of TCA cycle intermediates.

Patients with COPD frequently develop hypoxemia at rest and/or during exercise, which can influence muscle energy metabolism. $PaO_2$ during exercise was not evaluated in the present study, although $PaO_2$ at rest ranged from 67 to 90 mmHg. Our previous study evaluated $PaO_2$ during the same hand-grip exercise in COPD patients, finding no significant decrease during exercise (Kutsuzawa et al., 1992). This suggests that reduced oxygen availability might not contribute to lactate accumulation in a control bout in COPD patients.

### 4.5 Limitations of study

The present study did not investigate muscle energy metabolism during two consecutive short-term bouts of exercise with a 1-h interval without BCAA supplementation. We previously studied the effects of a single administration of BCAAs on muscle pH during repeated short-term exercise (with or without BCAAs) in healthy young males (Kutsuzawa et al., 2011). In that study, healthy individuals performed repeated hand-grip exercises that consisted of the same weight, same distance, and double the frequency of the present study to induce low muscle pH. All participants who did not take BCAAs showed a pHi at completion of the second bout that was significantly decreased compared to that of the first bout. These findings suggested that the protocol resulted in decreased pHi at the end of the second bout of exercise as compared with the first bout. Therefore, pHi and PCr/(PCr+Pi) at the end of the second bout of hand-grip exercise in the present study might have been decreased.

This study did not measure concentrations of amino acids in muscle. Glutamic acid and BCAAs are taken up by skeletal muscle after consuming meals containing protein and the carbon skeletons are used for *de novo* glutamine synthesis (Wagenmakers, 1998). A few studies (Pouw et al., 1998; Engelen et al., 2000b) have measured concentrations of muscle amino acids in patients with COPD. Pouw et al. (Pouw et al., 1998) reported that levels of BCAAs in plasma and muscle do not differ between patients with COPD and healthy individuals. However, Engelen et al. (Engelen et al., 2000b) showed that plasma and muscle levels of leucine and isoleucine are significantly decreased in COPD subtypes with emphysema, indicating that leucine metabolism is altered in COPD patients. Both studies demonstrated a decrease in glutamic acid in muscles in such patients. Muscle concentrations of glutamic acid and BCAAs might thus be decreased in patients with COPD and low plasma BCAA concentrations.

Furthermore, we did not measure plasma concentrations of lactate, because the magnet in our MR equipment was too narrow to collect venous blood from the exercising forearm during exercise. We also did not determine whether attenuation of metabolic acidosis in muscle resulted in a decrease in plasma lactate levels. However, the present exercise model involved a small muscle mass, which produced only limited changes in arterial $H^+$ and lactate concentrations.

## 5. Conclusions

BCAA ingestion before a second bout of hand-grip exercise in patients with COPD resulted in a significant decrease in ΔpHi and ΔPCr/(PCr+Pi) at the end of the second bout of exercise. These findings suggest that BCAAs can help to prevent metabolic acidosis in exercising muscle among COPD patients, probably by increasing oxidative capacity and by decreasing lactate production. BCAA ingestion should thus improve exercise capacity in COPD patients.

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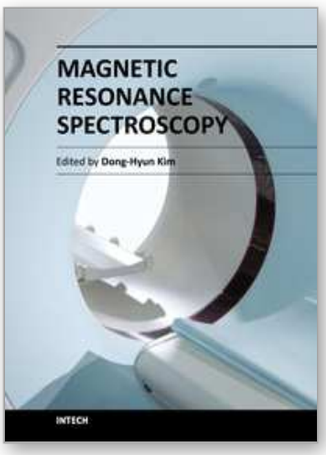
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Magnetic Resonance Spectroscopy (MRS) is a unique tool to probe the biochemistry in vivo providing metabolic information non-invasively. Applications using MRS has been found over a broad spectrum in investigating the underlying structures of compounds as well as in determining disease states. In this book, topics of MRS both relevant to the clinic and also those that are beyond the clinical arena are covered. The book consists of two sections. The first section is entitled 'MRS inside the clinic' and is focused on clinical applications of MRS while the second section is entitled 'MRS beyond the clinic' and discusses applications of MRS in other academic fields. Our hope is that through this book, readers can understand the broad applications that NMR and MRS can offer and also that there are enough references to guide the readers for further study in this important topic.

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