Heating improves poor compliance with branched chain amino acid-rich supplementation in patients with liver cirrhosis: A before-after pilot study

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Abstract. Although branched chain amino acid (BCAA) supplementation improves malnutrition in cirrhotic patients, patient compliance with the administration of BCAA-rich supplements is poor due to their bitter taste. Since temperature is an important factor affecting taste, we examined the effect of heating on the stability of BCAAs and on the compliance of patients with liver cirrhosis with BCAA-rich supplement administration. A thermal denaturation test was first conducted, in which the BCAA-rich supplement Aminoleban® EN was heated to 37, 60, or 80˚C for 30 or 60 min. The concentration of three amino acids, L-valine, L-leucine and L-isoleucine, was subsequently measured. The nutritional status of the cirrhotic patients was also evaluted. Patients presenting liver failure with a Child-Pugh class of A (n=2), B (n=2) or C (n=2) were hospitalized at Kurume University Hospital. Six patients with liver cirrhosis (HCV, n=3; HBV, n=1; alcohol, n=2) were enrolled. Venous blood samples were drawn in the morning after a 12-h overnight fast. The BCAA-rich supplement was administered to patients at room temperature (25˚C) or heat loaded at 60˚C for 10 min, with the temperature maintained above 45˚C. Each patient was interviewed by a nationally registered dietitian regarding food consumption and intake of the BCAA-rich supplement immediately after each meal. Nutritional status was evaluated according to serum albumin levels, blood hemoglobin, prothrombin time and total lymphocyte count. No significant decrease was noted in valine, leucine or isoleucine levels following the heating of the BCAAs to 80˚C. The caloric intake of the BCAA-rich supplement was significantly higher with administration after heating to 60˚C, compared to caloric intake with administration at 25˚C. In addition, heating of the BCAA-rich supplement significantly increased blood lymphocyte counts. In conclusion, heating did not affect the stability of the BCAAs, and may improve compliance with BCAA-rich supplement administration. As a result, the nutritional status of cirrhotic patients may be improved.

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Introduction

Branched chain amino acid (BCAA) supplementation improves not only hepatic encephalopathy, but also protein-energy malnutrition and quality of life (QOL) in patients with liver cirrhosis (1). However, the long-term compliance of cirrhotic patients with BCAA-rich supplement administration is often poor, as BCAAs have a bitter unpalatable flavor (2,3), and as foods with a predominantly bitter taste have been shown to be specifically less preferred by patients with liver disease compared to healthy controls (4). Although an increase in the particle size of BCAA crystals lowers their bitterness intensity score as evaluated by human gustatory tests and an artificial taste sensor (5), additional measures are required to improve the compliance of cirrhotic patients with BCAA-rich supplement administration.

The sensory properties of food can regulate appetite (6). Changes in taste, smell, texture and the temperature of foods influence food intake. Food temperature, in particular, has a strong influence on taste. Warm food changes the taste threshold on the tongue (7). Moreover, warming foods has recently been reported to activate thermosensitive molecules in the taste transduction pathway, leading to changes in taste (8). Nevertheless, the effects of warming BCAA-rich supplements have never been tested. In this study, we examined the effect of heating on the stability of BCAAs and on compliance with the administration of BCAA-rich supplements, and evaluated the resulting nutritional status of cirrhotic patients.

Materials and methods

Materials. All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Thermal denaturation test for BCAAs. A coffee-flavored BCAA-rich supplement (Aminoleban® EN, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was selected, and 50 g of the supplement were sonicated in 200 ml of water for 45 min at 25˚C in a beaker (solution A) (Table I). Solium citrate (39 g) and 32 ml of perchloric acid were diluted in 2000 ml of water (solution B). A mixture of 1.5 ml of solution A and 98.5 ml of solution B was heated in flasks to 37, 60 or 80˚C for 30 or 60 min in a thermostatic bath. For the stability testing of the BCAAs, 10 μl of each mixture were used.

Measurement of BCAAs. Using high-performance liquid chromatography, the concentration of three amino acids, L-valine (Val), L-leucine (Leu) and L-isoleucine (Ile), was compared in each of the heated samples with the manufacturer's specifications for Aminoleban EN.

Patients. Patients presenting liver failure with a Child-Pugh class of A (n=2), B (n=2) or C (n=2) were hospitalized at Kurume University Hospital. Six patients with liver cirrhosis (HCV, n=3; HBV, n=1; alcohol, n=2) were enrolled. Patient demographic data were collected on the same day as blood collection. Venous blood samples were collected in the morning after a 12-h overnight fast. The BMI of patients was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). The BCAA-rich supplement was administered to patients at room temperature (25˚C) or heat loaded at 60˚C for 10 min with the temperature maintained above 45˚C. None of the subjects were institutionalized. Informed consent for participation was obtained from each patient. The study protocol was approved by the Ethics Committee of the Kurume University School of Medicine. All experiments were carried out in accordance with the Declaration of Helsinki.

Food and BCAA-rich supplement intake. Each patient was interviewed by a nationally registered dietitian regarding food consumption and intake of the BCAA supplement Aminoleban EN immediately after each meal.

Evaluation of nutritional status. Nutritional status was evaluated according to serum albumin levels, blood hemoglobin, prothrombin time and total lymphocyte count. These nutritional parameters were determined using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital).

Statistical analysis. Data were expressed as the mean ± SD. Statistical comparisons among multiple groups were performed by the Friedman test. Statistical comparisons between two groups were performed by analysis of variance (ANOVA) followed by the Wilcoxon signed-rank test using StatView Power PC software for Macintosh (version 5.1; SAS, Cary, NC). P-values <0.05 were considered significant.

Table I. Composition of Aminoleban EN (per 50 g in 200 ml of water).

Total energy	210 kcal
Protein	13.5 g
Amino acids (Fischer's ratio = 38)	
L-valine	1.60 g
L-leucine	2.04 g
L-isoleucine	1.92 g
L-thereonine	0.13 g
L-tryptophan	0.07 g
L-arginine hydrochloride	0.30 g
L-histidine hydrochloride	0.19 g
L-lysine hydrochloride	0.24 g
Fat (rice oil)	3.50 g
Carbohydrates (dextrin)	31.05 g
Vitamins[a]	
Minerals[b]	
Deionized water	180 ml
pH	5.5-7.0

[a]Vitamins include retinol palmitate, ergocalciferol, bisbentiamine, riboflavin, pyridoxine HCl, cyanocobalamin, folic acid, sodium l-ascorbate, tocopherol acetate, phytonadione, calcium pantothenate, nicotinamide and biotin. [b]Minerals include trace amounts of magnesium sulphate, calcium glycerophosphate, potassium iodide, potassium chloride, sodium dihydrogen phosphate dihydrate, sodium ferrous citrate, cupric sulphate, zinc sulphate and manganese sulphate.

Results

Degradation of L-valine, L-leucine and L-isoleucine by heating. No significant decrease in the concentration of Val, Leu or Ile was observed in the BCAA supplement under any of the test conditions (Table II). Compared to baseline concentrations, there was no significant change in Val concentrations following heating of the supplement to 37 or 60˚C for 30 or 60 min, while heating the BCAA supplement to 80˚C for 30 or 60 min resulted in a significant increase in Val concentrations compared to the baseline. Leu and Ile concentrations were significantly increased by heating of the supplement to 37, 60 or 80˚C for 30 or 60 min, compared to baseline values (Table III).

Caloric intake of BCAA-rich supplement and compliance with its administration. Heating the BCAA-rich supplement to 60˚C resulted in a longer period of compliance (17.5±10.1 days) compared to the period of compliance observed when the supplement was administered at 25˚C (1.8±0.8 days) (Fig. 1A). Caloric intake of the BCAA-rich supplement was significantly higher following heating to 60˚C (380.3±41.9 kcal/day) than when the supplement was administered at 25˚C (145.8±0.8 kcal/day) (Fig. 1B). No significant difference was noted in total caloric intake between the 25 and 60˚C BCAA-rich supplement (Fig. 1C).

Table II. Effect of heating on L-valine, L-leucine and L-isoleucine concentrations.

Amino acid (%)	Temperature	Time of heating load (min)			P-value
		0	30	60	
L-valine	37˚C	98.7±0.3	99.3±0.6	99.3±0.3	0.09
	60˚C	98.7±0.3	99.4±0.4	99.3±0.3	0.24
	80˚C	98.7±0.2	99.5±0.3	99.4±0.2	0.02
L-leucine	37˚C	97.4±0.6	100.1±0.6	99.7±0.4	0.02
	60˚C	97.3±0.6	100.3±1.0	100.2±1.1	0.02
	80˚C	97.3±0.6	99.8±0.4	99.5±0.4	0.02
L-isoleucine	37˚C	96.1±1.4	98.1±1.2	99.4±1.1	0.02
	60˚C	96.1±1.4	99.4±0.7	100.0±1.1	0.02
	80˚C	96.1±1.4	99.3±0.6	99.0±1.0	0.02

Values are expressed as the mean ± SD. Contents of L-valine, L-leucine and L-isoleucine show the w/v of BCAA-rich supplement samples.

Table III. Baseline characteristics of the subjects.

Gender (no. male/female)	5/1
Age (years)	55.5±11.0
Body mass index (kg/m^2)	23.9±5.1
Blood hemoglobin (g/dl)	9.6±2.5
Serum albumin level (mg/dl)	2.73±0.53
Blood lymphocyte (count/10^3 l)	796.4±172.8
Serum cholinesterase (U/l)	57.6±36.6
Prothrombin time (INR)	1.67±0.75
Serum total bilirubin (mg/dl)	4.21±7.83

Data are expressed as the mean ± SD or number of subjects. INR, international normalized ratio.

Evaluation of nutritional status. No significant changes were noted in serum albumin levels before and after the intake of the heated BCAA-rich supplement (Fig. 2A), nor was a significant decrease in blood hemoglobin levels observed (Fig. 2B). Likewise, no significant changes were observed in cholinesterase levels (Fig. 2C) or in prothrombin time (Fig. 2D). The blood lymphocyte count was significantly higher after administration of the heated BCAA-rich supplement than before ingestion (Fig. 2E).

Discussion

In this study, we demonstrated that L-valine, L-leucine and L-isoleucine concentrations in a BCAA-rich supplement were not degraded by heating. Compliance with administration in patients with liver cirrhosis was improved when the supplement was heated to 60˚C. During treatment with the heated BCAA-rich supplement, serum albumin, hemoglobin and serum cholinesterase levels as well as prothrombin time were maintained, although the patients were in a wasting state. Moreover, blood lymphocyte counts, one of the key parameters indicating nutritional status, were

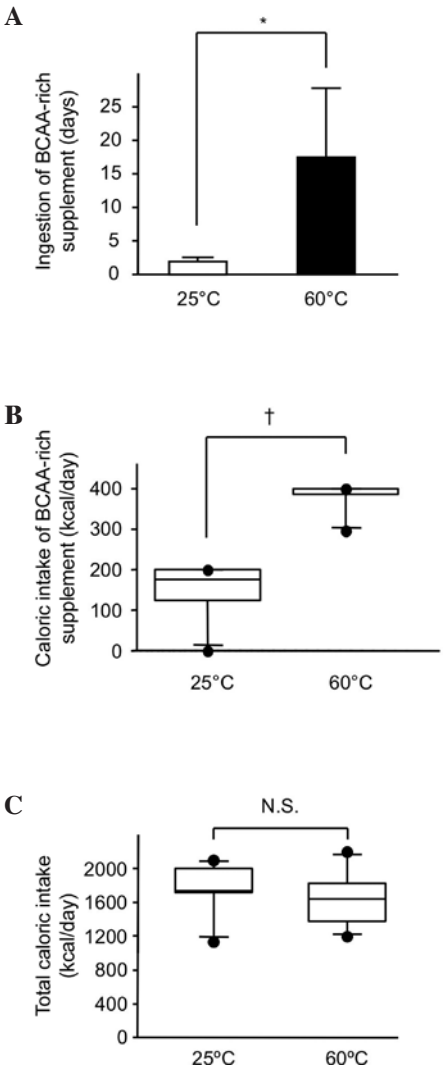

Figure 1. Heating effects on a BCAA-rich supplement for patients with liver cirrhosis. Days of ingestion (A), caloric intake of the BCAA-rich supplement (B) and total caloric intake (C) at 25 and 60˚C. Values are expressed as the mean ± SD. Differences between two groups were analyzed using the paired t-test. Statistical comparisons between two groups were performed by analysis of variance (ANOVA) followed by the Wilcoxon signed-rank test. *P<0.05, †P<0.001.

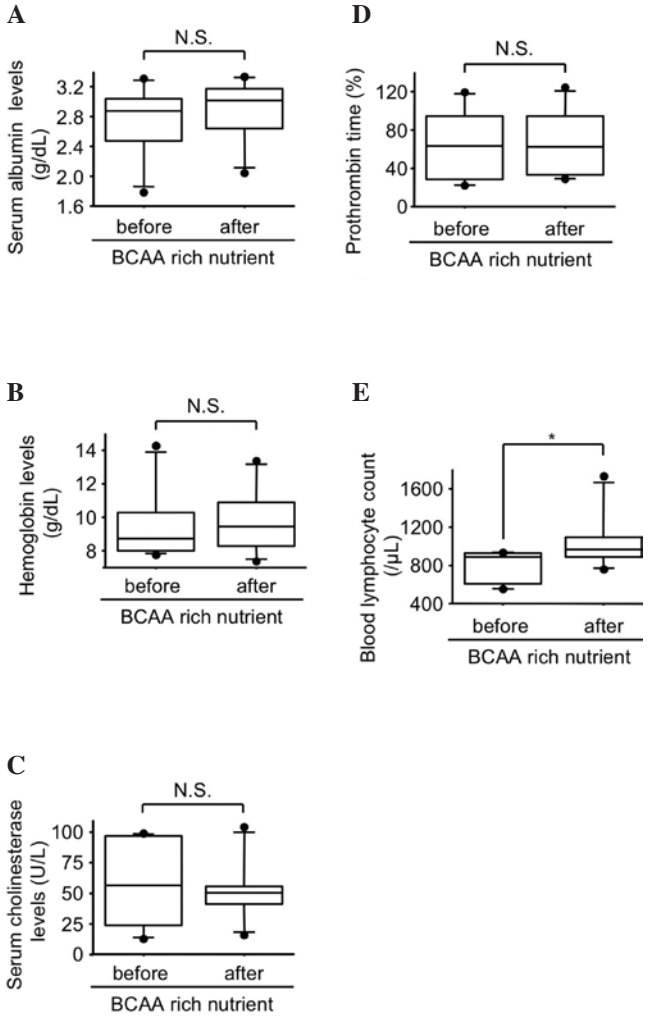

Figure 2. Evaluation of nutritional status before and after the intake of BCAA-rich supplement. Serum albumin levels (A), blood hemoglobin levels (B), serum cholinesterase levels (C), prothrombin time (D) and blood lymphocyte count (E) before and after administration of the BCAA-rich supplement. Values are expressed as the mean ± SD. Differences between two groups were analyzed using the paired t-test. Statistical comparisons between two groups were performed by analysis of variance (ANOVA) followed by the Wilcoxon signed-rank test. N.S., not significant. *P<0.05.

significantly increased by treatment with the heated BCAA-rich supplement compared to treatment with the BCAA-rich supplement administered at 25˚C. Thus, heating may be a key to improving patient compliance with BCAA-rich supplement administration and, as a result, nutritional status.

To evaluate the influence of heat loading on BCAAs, we tested the stability of Val, Leu and Ile after heating for 30 and 60 min at 37, 60 or 80˚C. No decrease in the concentration of Val, Leu or Ile was observed, indicating that the BCAA-rich supplement remained stable under heat loading. In contrast, Yeung *et al* examined the effect of autoclaving on the stability of BCAAs and found that it decreased the concentration of BCAA in an infant formula (9). The reason for this discrepancy is unclear; however, it is possible that the Maillard reaction – nonenzymatic glycation, rather than heating – caused the degradation of BCAAs (10). In general, the Maillard reaction occurs at high temperatures (>100˚C) and under alkaline conditions. The rate of the formation of Maillard reaction products accelerates with increasing temperature. Yeung *et al* conducted their experiments under high temperature conditions (9), whereas our experiment was designed to deliver a maximum temperature of no more than 80˚C at a pH of 5.5-7.0. It is therefore likely that the BCAAs were not degraded by the Maillard reaction in our experiment, but rather remained stable.

BCAAs have an inherently strong bitter taste, frequently resulting in poor patient compliance. In order to address this issue, several methods have been developed. An increase in the particle size of BCAAs (5) or the addition of various flavorings to BCAA-rich supplements is reported to suppress their bitter taste. However, patients in these studies still show poor compliance with BCAA-rich supplements. In the present study, the mean administration period for a coffee-flavored supplement administered at 25˚C was 1.8 days, due to its bitter taste. It is known that temperature has a strong influence on perceived taste (10). In addition, the effect of heat on the tongue is a sweet taste sensation (11). Therefore, we studied the effects of heating on patient compliance with BCAA-rich supplement administration.

It is unclear why heating BCAA-rich supplements improves compliance in patents with liver cirrhosis; however, there are two possible hypotheses. Since elevated tongue temperature enhances the perception of sweetness in humans, heating BCAA-rich supplements may reduce their inherently bitter taste. Alternatively, a cation channel of the transient receptor potential (TRP) superfamily, TRPM5, is highly expressed in the taste buds of the tongue, where it plays a key role in the perception of sweet and bitter tastes. Talavera *et al* investigated the heat activation of TRPM5 and found that it caused thermal sensitivity regarding sweet taste (10). Heated BCAA-rich supplements may therefore enhance a sweet taste through up-regulation of the TPRM5 receptor in the taste buds of the tongue.

Patients with decompensated liver cirrhosis undergoing invasive therapy experience poor nutrition due to an increase in their energy requirements. In this study, by heating the BCAA-rich supplement, sufficient patient compliance to the supplement administration was obtained, while serum albumin, hemoglobin and serum cholinesterase levels, as well as prothrombin time, were maintained. Moreover, following treatment with the heated BCAA-rich supplement, the total lymphocyte count was increased compared to that observed after administration of the BCAA-rich supplement at 25˚C.

BCAAs are not just the structural constituents of proteins. Their administration increases immune function parameters, including total lymphocyte count (12). Moreover, BCAAs are known to stimulate the production of HGF (13). Thus, some relevant pharmacological properties of BCAAs may contribute to maintaining nutritional status in patients with liver cirrhosis.

In conclusion, the key BCAAs, Val, Leu and Ile, remained stable in a BCAA-rich supplement under a heating load. By heating the supplement prior to administration, improvements in both compliance and nutritional status were observed in patients with liver cirrhosis. Heating is a simple method; thus, the heating of BCAA-rich supplements may serve as a routine strategy for improving patient compliance with supplement administration, which may in turn lead to improvments in the nutritional status of the patients.

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