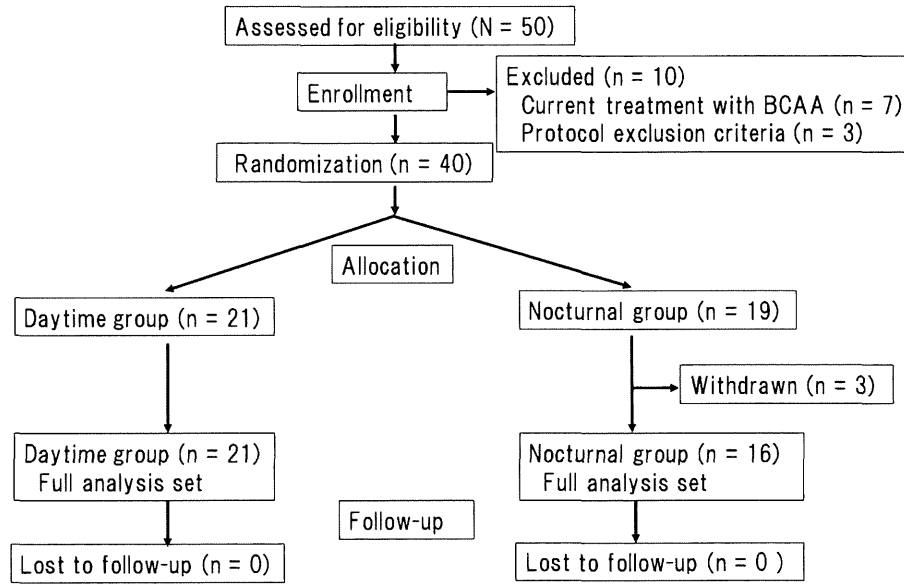


**Fig. 1** Flow diagram of study recruitment through follow up. *BCAA* branched-chain amino acid

**Table 1** Patients' baseline clinical and biochemical characteristics

| Patients | Daytime group $n = 21$ | Nocturnal group $n = 16$ | $P$ |
|---|---|---|---|
| Age (years) | 73 (41–83) | 72 (52–79) | 0.70 |
| Gender (male/female) | 8/13 | 7/9 | 0.78 |
| Etiology | | | 0.24 |
| Alcohol | 1 | 2 | |
| HBV | 1 | 1 | |
| HCV | 16 | 7 | |
| Others | 3 | 6 | |
| Liver function | | | |
| Child-Pugh class (A/B) | 12/9 | 9/7 | 0.96 |
| BMI (kg/m$^2$) | 22.2 (14.9–30.3) | 23.9 (19.2–29.2) | 0.21 |
| Ascites (yes/no) | 0/21 | 0/16 | |
| History of HCC therapy (yes/no) | 4/17 | 4/12 | 0.16 |

Values for age and BMI are expressed as medians and ranges

*HBV* hepatitis B virus, *HCV* hepatitis C virus, *BMI* body mass index

## QOL assessment

The therapy was well accepted and tolerated. There were no significant differences in any of the subjects according to the results of the questionnaire survey on either subjective or objective symptoms, or on the SF-8 between the daytime group and the nocturnal group after 3 months of treatment.

All the patients had a significant decrease in the occurrence of muscle cramps in the legs ($P = 0.004$) (Fig. 2a) and all exhibited significant effects on general health scores as revealed by the SF-8 after 3 months ($P = 0.01$) (Fig. 3a). After 3 months of treatment, the daytime group had a decrease in the occurrence of muscle cramps in the legs (Fig. 2b), and exhibited significant effects on general health ($P = 0.005$), vitality ($P = 0.049$), social functioning ($P = 0.016$), mental health ($P = 0.037$), and role emotional ($P = 0.029$) as revealed by the SF-8 (Fig. 3b). On the other hand, although the nocturnal group had a significant decrease in muscle cramps in the legs ($P = 0.014$) (Fig. 2c), there were no significant effects on any parameter of the SF8 in this group (Fig. 3c).

## Changes in laboratory data

After 3 months' treatment, there were no significant differences in any laboratory parameters between the daytime group and the nocturnal group. After 3 months, Fisher's ratio was significantly increased in the nocturnal group ($1.48 \pm 0.04$ vs. $2.32 \pm 0.71$, $P = 0.04$) compared with the value in the daytime group ($1.42 \pm 0.24$ vs.

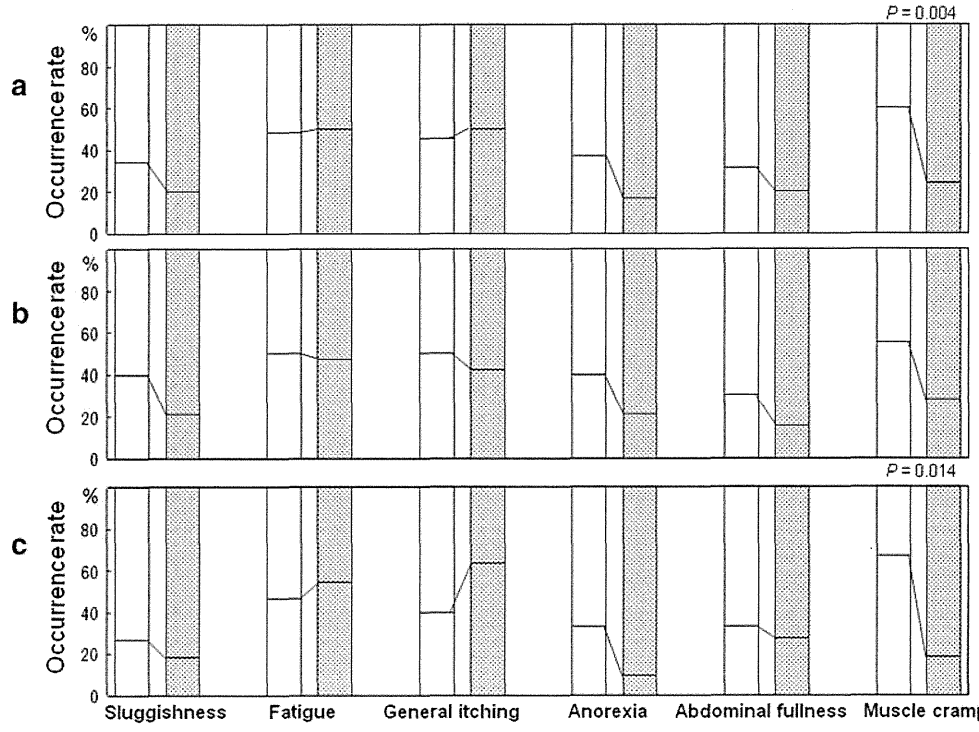


**Fig. 2** Occurrence rates from the Japanese version of questionnaires on subjective and objective symptoms in patients with liver cirrhosis. All the patients had a significant decrease in the occurrence of muscle cramps in the legs ($P = 0.004$) (nocturnal group, $P = 0.014$). *White bars*, pretreatment; *dotted bars*, after 3 months of treatment. **a** All the patients, **b** daytime group, **c** nocturnal group

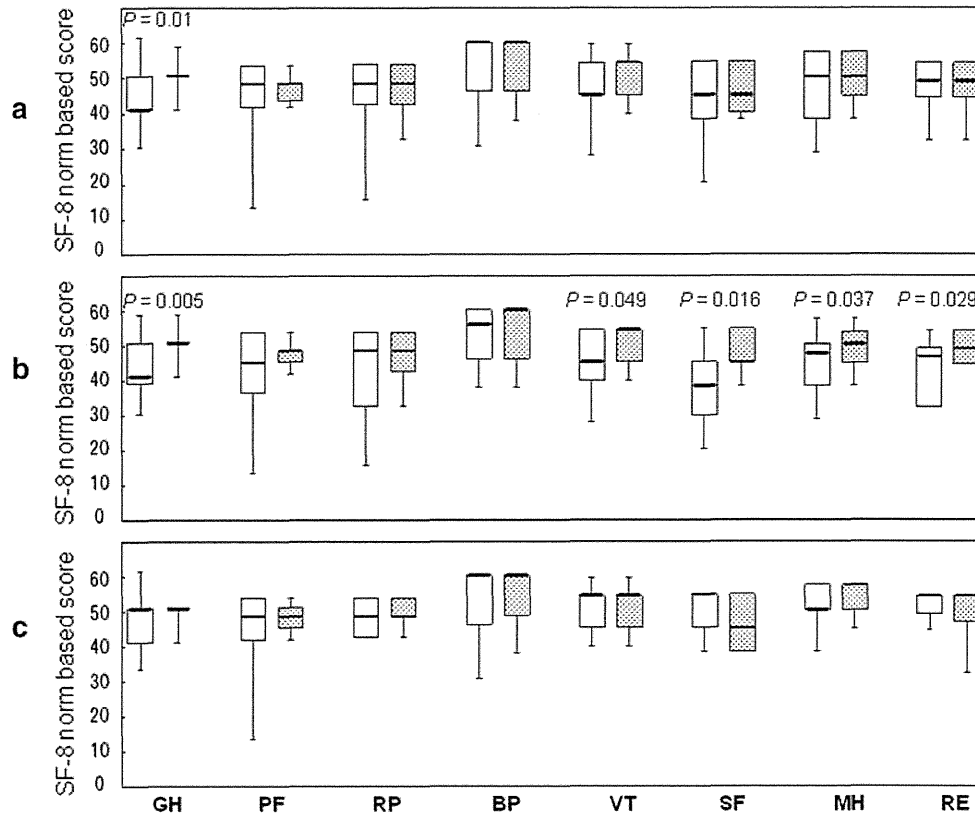


**Fig. 3** Results from the Japanese version of the Medical Outcomes Study Short-Form 8-Item Health Survey (SF-8). All the patients showed a significant effect revealed by the general health scores on the SF-8 ($P = 0.01$). The daytime group showed a significant effect on general health ($P = 0.005$), vitality ($P = 0.049$), social functioning ($P = 0.016$), mental health ($P = 0.037$), and role emotional ($P = 0.029$) as revealed on the SF-8. Conversely, the nocturnal group did not show any significant effects on any parameters of the SF8. *GH* general health, *PF* physical functioning, *RP* role limitation due to physical problems, *BP* body pain, *VT* vitality, *SF* social functioning, *MH* mental health, *RE* role limitation due to emotional problems. *White bars*, pretreatment; *dotted bars*, after 3 months of treatment. **a** All the patients, **b** daytime group, **c** nocturnal group

1.75 ± 0.16, $P = 0.18$). However, serum albumin after 3 months was not changed in either group (3.34 ± 0.15 vs. 3.42 ± 0.19 g/dl, $P = 0.85$ in the nocturnal group, and 3.35 ± 0.18 vs. 3.40 ± 0.35 g/dl, $P = 0.59$ in the daytime group) (Table 2). Moreover, glycated albumin was not changed by BCAA administration.

**Table 2** Changes in chemical markers in the daytime and nocturnal groups

| | Daytime group | | | Nocturnal group | | |
|---|---|---|---|---|---|---|
| | Baseline | 3 Months | *P* | Baseline | 3 Months | *P* |
| Albumin (g/dl) | 3.4 (3.1–3.8) | 3.4 (3.0–4.0) | 0.59 | 3.4 (3.1–3.5) | 3.4 (3.0–3.8) | 0.85 |
| Platelets ($\times 10^4/\mu l$) | 6.8 (3.8–38.4) | 7.35 (3.3–16.6) | 0.17 | 7.95 (4.0–12.8) | 9.05 (4.4–18.7) | 0.46 |
| AST (IU/l) | 65 (24–139) | 72 (26–149) | 0.28 | 60 (21–139) | 53 (31–115) | 0.99 |
| ALT (IU/l) | 42 (11–105) | 49 (17–123) | 0.32 | 41 (14–112) | 32 (17–96) | 0.57 |
| T. Bil. (mg/dl) | 1.1 (0.4–2.8) | 1.1 (0.4–2.2) | 0.46 | 1.0 (0.5–2.7) | 0.9 (0.4–2.8) | 0.08 |
| PT (%) | 73.5 (58.1–90.9) | 76.0 (54.0–87.0) | 0.65 | 63.4 (50.0–81.4) | 70.8 (54.5–82.2) | 0.32 |
| $NH_3$ (μg/dl) | 70 (19–108) | 51 (19–104) | 0.33 | 29 (10–138) | 19 (17–158) | 0.61 |
| Free fatty acid (Eq/l) | 652 (99–2437) | 261 (87–2736) | 0.83 | 281 (39–2685) | 579 (39–2850) | 0.72 |
| Cholinesterase (mg/dl) | 144 (59–187) | 149 (41–252) | 0.59 | 128 (87–273) | 150 (87–246) | 0.79 |
| Glycated albumin (%) | 21.0 (15.5–44.8) | 19.3 (16.6–47.8) | 0.91 | 20.0 (16.0–42.3) | 19.5 (16.8–39.4) | 0.73 |
| Fisher's ratio | 1.27 (1.00–2.23) | 1.60 (1.33–2.30) | 0.18 | 1.57 (1.14–1.72) | 2.12 (1.15–3.52) | 0.04 |
| AFP (ng/ml) | 5.65 (3.6–222.0) | 9.0 (2.9–59.5) | 0.35 | 11.1 (2.1–432.0) | 14.7 (1.8–954.0) | 0.36 |

All data values are expressed in medians and ranges

*T. Bil.* total bilirubin, *PT* prothrombin time, *AFP* alpha-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

### Treatment compliance

Overall, 20 (95.2 %) patients in the daytime group and 15 (93.8 %) patients in the nocturnal group received more than 80 % of the planned daily dose of the study drug.

### Adverse reactions

Adverse reactions to BCAA administration were identified in 4 patients in the nocturnal group: 2 patients developed abdominal distension (grade 2), and 2 patients developed general itching (grade 1). However, all 4 patients recovered with conservative treatment.

### HCC and esophageal varices

Two patients in each arm of the present study developed HCC after 3 months of follow up. There were no patients who bled from esophageal varices during the observation period.

## Discussion

To our knowledge, this is the first multicenter randomized controlled trial to have investigated the efficacy of nocturnal BCAA granule administration in improving QOL in patients with cirrhosis. In this study, the administration of BCAA granules significantly reduced the occurrence of muscle cramps in the leg for all the patients and the nocturnal group. Generally, muscle cramps are caused by a variety of factors, including diuretic treatment, intolerance of amino acids, and deficiency of vitamin E and taurine [18, 19]. Marchesini et al. [6] reported that the frequency of muscle cramps, which was associated with poor QOL in patients with decompensated cirrhosis, was dramatically reduced by BCAA supplementation over a period of 3 months; however, the BCAA supplementation was associated with poor QOL in patients with decompensated cirrhosis. Of note, in our study, QOL in the daytime group was significantly improved after 3 months of treatment compared with that in the nocturnal group. In particular, the components of GH, VT, SF, MH, and RE improved significantly in the daytime group. We could not explain this discrepancy between the QOL findings in our study and those of Marchesini et al. [6]. However, in our nocturnal group, Fisher's ratio, which is the most important index of an amino acid imbalance, was significantly increased compared with that in the daytime group. In the matter of the results of the plasma Fisher's ratio, it is necessary to consider the influence of the interval between the last BCAA intake and blood sampling in the fasting state. In a single-administration study of BCAA in healthy rats, the mean maximum blood concentration of BCAA was observed after about 4 h and the concentration gradually decreased after that, and the mean half-life was about 58 h [20]. However, it is considered that the preservation of a higher BCAA concentration until morning is particularly important in patients with decompensated cirrhosis. Patients with liver cirrhosis cannot constantly maintain the concentration of BCAA administered in the daytime until nighttime because the BCAA is consumed for their

daytime physical activity [16]. We speculated that nocturnal BCAA administration would complement the deficiency experienced during the night. However, we found that nocturnal administration tended to induce more abdominal distension and general itching at night than daytime administration, thus lowering the QOL in nocturnal group.

In the present study, the glycated albumin level was not changed by BCAA administration. Sako et al. [18] reported that the administration of the BCAA supplement, Aminoleban EN® (210 kcal), in the evening decreased the number of muscle cramps in 8 outpatients with advanced liver cirrhosis. However, excess elevation of blood glucose occurs after meals in liver cirrhosis patients [21]. Aoyama et al. [15] reported that patients with a glucose level of more than 200 mg/dl 2 h after a 75-g OGTT experienced impaired glucose tolerance after 3 months' administration of BCAA supplements. However, impaired glucose tolerance did not occur with the administration of BCAA granules that consisted of only 4 g BCAA. A previous clinical trial has reported that BCAA administration decreased plasma glucose levels in patients with advanced liver cirrhosis [22]. Furthermore, Kawaguchi et al. [23] reported that BCAA improved insulin resistance in patients with chronic liver disease. Therefore, it is possible that BCAA granules are more useful for muscle cramps in patients with glucose intolerance than are BCAA supplements. On the other hand, Nakaya et al. [24] reported that BCAA supplements improved the nutrition state (serum albumin, energy metabolism) in patients with decompensated cirrhosis. Accordingly, in decompensated patients who cannot eat enough, it seems to be more feasible for them to take BCAA supplements.

HCC was noted in 2 patients with hepatitis C in each arm of the present study after 3 months of treatment with BCAA granules. It seems that the HCC occurrence rate (11.1 % per 3 months) in this study was much higher than the general rate (2–5 %) in Japan [25]. All 4 patients for whom HCC was noted had received HCC therapy previously, and their serum albumin levels had decreased after 3 months of the BCAA administration. The present study might have been better if we had not included these 4 patients who had previously received HCC therapy. However, a recent clinical study reported that long-term BCAA administration inhibited the development of HCC in liver cirrhosis patients with hepatitis C [5]. Furthermore, one study from Japan showed that BCAA administration in patients with liver cirrhosis suppressed HCC recurrence after treatment with radiofrequency ablation [26]. Further studies are needed to clarify whether or not there are clinical benefits of BCAA administration that would help prevent HCC.

There are three limitations of the present study. First, we could not establish a control group (i.e., a no-treatment group). However, large trials of BCAA supplements show that they decrease the progression of hepatic failure and are associated with improved survival in patients with decompensated cirrhosis [5, 6]. Therefore, we believe that it is not necessary to compare the BCAA administration results with a no-treatment control group. The second limitation is the short-term (3 months) administration. If we had administered BCAA granules for a longer term (e.g., 1 year), the serum albumin level might have increased significantly. On the other hand, the high rate of compliance in our study might have been due to the short-term administration. Finally, after the randomization, 3 patients in the nocturnal group chose early withdrawal because their regular pharmacy could not keep up with our treatment schedule. Accordingly, our study did not follow ITT (intention-to-treat) analyses because we omitted these patients from analysis in this study. However, it is considered that this withdrawal was caused by institutional error and not because of the patients' lack of compliance.

In conclusion, the daytime administration of BCAA granules significantly improved the QOL in patients with cirrhosis. And, while nocturnal administration significantly reduced patients' leg muscle cramps, it did not seem to improve their QOL.

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**Conflict of interest** H. Hidaka has served as a consultant and speaker for Ajinomoto Pharmaceutical Co., Inc., Tokyo, Japan; all the other authors have no personal interests to disclose in relation to this article.

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