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Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis

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Abstract

We conducted a multicenter, randomized, controlled trial to investigate the effect of long-term oral supplementation with branched-chain amino acids (BCAA) on the event-free survival in 622 patients with decompensated cirrhosis. In the present study, the development of liver cancer was analyzed as an endpoint in particular. Subjects received either treatment with BCAA at 12 g/day or dietary therapy containing the matched daily energy and protein intake. A Cox regression analysis was carried out to estimate the hazard ratios for different background factors stratified by treatment group. Liver cancer was noted in 89 patients. The risk for liver cancer was significantly higher for males, patients with concurrent diabetes mellitus, patients with an alpha-fetoprotein (AFP) level of 20 ng/mL or higher, patients with higher body mass index (BMI), and patients with lower serum albumin levels. When the BCAA group and the diet group were compared for factors that interacted with the treatment arms, the risk for liver cancer was significantly reduced in the BCAA group with a BMI of 25 or higher and with an AFP

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level of 20 ng/mL or higher. Oral supplemental treatment with BCAA may reduce the risk of liver cancer in cirrhotic patients with these specific factors.

Keywords: Liver cirrhosis; Liver cancer; Risk factor; Branched-chain amino acids; Body mass index

1. Introduction

Liver cancer is one of the major causes of death in patients with chronic hepatic disorders, and the disease has recently increased in incidence and mortality in Western countries and Japan [1–5]. The known causes of liver cancer are conditions such as hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, substantial alcohol consumption, and non-alcoholic fatty liver disease (NAFLD). Patients with these conditions often have a spontaneous clinical course associated with chronic hepatitis leading to liver cirrhosis and subsequent liver cancer. In Japan, particularly, there is a higher incidence of liver cancer resulting from HCV infection, and in this context, efforts should be made to reduce the development of this cancer in patients with positive anti-HCV antibodies [6]. To date, various epidemiological and other studies have extensively investigated possible risk factors of liver cancer due to chronic hepatic disorders, identifying sex, age, severity of hepatic inflammation and fibrosis, and race as some of these factors [3,5,7,8]. In addition, recent interest in NAFLD has prompted investigation of additional possible risk factors including diabetes mellitus [3,5,7,9–12], obesity as indicated by the body mass index (BMI) [3,13–15], and hyperinsulinemia [16,17].

Despite their adequate food consumption, patients with liver cirrhosis may have protein malnutrition such as reduced skeletal muscle mass and hypoalbuminemia [18–22]. Several studies have described that long-term supplementation with branched-chain amino acids (BCAA) was given to patients with decompensated cirrhosis for the treatment of protein malnutrition, and this therapy improved or maintained nutritional status, as well as increased survival rates [23–28]. However, these results have not been verified in larger studies, partly because of the lack of a BCAA preparation suitable for long-term treatment. In addition, the clinical effects of BCAA treatment have not been clarified particularly in terms of whether such effects are associated with either the development or prevention of liver cancer.

Based on the above background, we conducted a multicenter, randomized, controlled trial to investigate the effects of long-term oral supplementation with BCAA on the event-free survival in Japanese patients with decompensated cirrhosis who had hypoalbuminemia despite adequate food intake. The favorable results from this trial have already been described in our previous article [29], but presented here are the results obtained in an additional analysis using data from the same trial performed to evaluate the risk factors of liver cancer and the effects of BCAA treatment on cancer incidence in high-risk patients.

2. Materials and methods

2.1. Subjects, design, and protocol

Subjects included in this multicenter, randomized, controlled trial were Japanese patients with decompensated cirrhosis who had hypoalbuminemia despite adequate food intake.

A total of 646 subjects were enrolled in the trial and were randomized at the registration center to receive either a 4 g BCAA preparation containing 952 mg L-isoleucine, 1904 mg L-leucine, and 1144 mg L-valine (LIVACT Granules®; Ajinomoto Co., Inc., Tokyo) administered orally three times daily after meals or dietary therapy containing the matched daily energy and protein intake (25–35 kcal/kg/day and 1.0–1.4 g protein/kg/day including BCAA preparation). They continued their randomized therapies for at least 2 years.

Details of the trial design and protocol are presented in our previous article [29].

This study was a post-marketing clinical trial, and upon approval by the institutional review board of each institution, the trial was performed according to good clinical practice (GCP) and good post-marketing surveillance practice (GPMSP). All subjects gave written informed consent to participate in this trial.

2.2. Analytical endpoint

The primary endpoint of the trial was time to the earliest onset of any of the following events: (1) aggravation of hepatic failure (ascites, peripheral edema, hepatic encephalopathy, and jaundice), (2) rupture of esophageal or gastric varices, (3) development of liver cancer, and (4) death from any cause [29]. In the present analysis, we particularly focused on the development of liver cancer among the above events and other events were classified as censoring. Subjects underwent ultrasonography every 3 months. Any lesions suspected for liver cancer were further evaluated using computed tomography, magnetic resonance imaging, and celiac angiography to determine the development of liver cancer.

2.3. Analysis population

Of 646 patients enrolled in the trial, a total of 622 patients were included in the analysis (314 and 308 out of the BCAA and the diet groups, respectively). Excluded from the analysis were 21 patients for whom therapies other than trial treatment were selected at baseline and 3 patients who were not compliant with the randomized treatment [29].

Details of patient background characteristics are presented in our previous article [29].

2.4. Statistical analysis

All analyses were performed using the SAS Release 6.12 or 8.1 software (SAS Institute Inc., Cary, NC, USA).

2.4.1. Risk factors for liver cancer

A Cox proportional hazard model stratified by treatment group (BCAA or dietary treatment) was applied to estimate the hazard ratios (HR) of liver cancer, with their two-tailed *p*-values, for explanatory variables listed below. At first, the models containing treatment group as a stratifying factor and each explanatory variable were analyzed. The purpose of using these models was to confirm that factors reported as a risk of liver cancer similarly increased the incidence of liver cancer in this trial. Next, models containing an interaction term between the treatment groups and the explanatory variable were analyzed. The significance level of interaction term was 0.2. The purpose of adding interaction term was to determine whether degree of change in the HR for 1 unit in explanatory variables was related to differences in treatment groups. If the interaction term is significant and acts on reduction in HR, it would suggest that BCAA decreases the risk of liver cancer. For variables that were found to have a statistically significant regression coefficient when using the model without the interaction term, the correlation coefficients between variables were calculated with their *p*-values for checking of multicolinearity. Patients who had ascites or moderate to severe edema at baseline were excluded from the analysis for the parameter of BMI, since correct BMI could not be obtained from patients with such conditions. As previously described [29], a diagnosis of ascites was made using ultrasonography, and edema was graded physically according to the depth of pretibial pitting edema.

The explanatory variables used for the HR estimation were:

(1) sex (male or female),
(2) age (years; continuous variable),
(3) cause of liver cirrhosis (HCV or non-HCV),
(4) baseline serum albumin level (g/dL; continuous variable),
(5) baseline total bilirubin level (mg/dL; continuous variable),
(6) baseline platelet count (continuous variable),
(7) baseline plasma BCAA/L-tyrosine molar ratio (BTR) (continuous variable),
(8) baseline Child-Pugh score (class A, B, or C),
(9) concurrent diabetes mellitus as diagnosed according to the criteria described previously [30] (no or yes),
(10) baseline BMI (continuous variable),
(11) baseline alpha-fetoprotein (AFP) level (below 20 or 20 ng/mL and higher),
(12) baseline protein induced by Vitamin K absence-II (PIVKA-II) (below 40 or 40 mAU/mL and higher),
(13) baseline total energy intake (kcal/kg body weight/day; below 25, 25 to below 35, or 35 and higher),
(14) baseline protein intake (g/kg body weight/day; below 1.0, 1.0 to below 1.4, or 1.4 and higher),
(15) history of alcohol consumption (no or yes).

2.4.2. Effects of BCAA treatment

For variables that were found to have a significantly increased HR and to interact with the treatment arms, further analyses were performed as follows: cumulative proportion of cancer-free patients were estimated for each treatment group using the Kaplan–Meier method in the subgroup specified by the variables, differences between the two treatment groups were evaluated statistically using the log rank test, and the HRs between groups were estimated using a Cox proportional hazard model. Another similar comparison was also performed in a subset of patients with liver cirrhosis caused by HCV infection. In the subgroup analysis for BMI, patients were classified according to their baseline BMI: below 25 or 25 and higher [31].

3. Results

3.1. Risk factors for liver cancer

Table 1 shows the results of the analysis using a Cox proportional hazard model.

Variables that were found to have a significantly increased HR of liver cancer without the interaction term were sex, baseline serum albumin level, concurrent diabetes mellitus, baseline BMI, and baseline AFP level (see the column "two-tailed *p*-value for regression coefficient" in Table 1). The incidence of liver cancer was significantly higher in males, patients with concurrent diabetes mellitus, and patients with an AFP level of 20 ng/mL or higher. The incidence of liver cancer was also significantly increased in patients with higher BMI or lower serum albumin levels.

When using models with the interaction term of the treatment arms, BMI scores and AFP levels had a significantly stronger interaction with treatment. In particular, a markedly strong interaction was shown between BMI scores and treatment ($p = 0.005$ for BMI; $p = 0.150$ for AFP level, see Table 1).

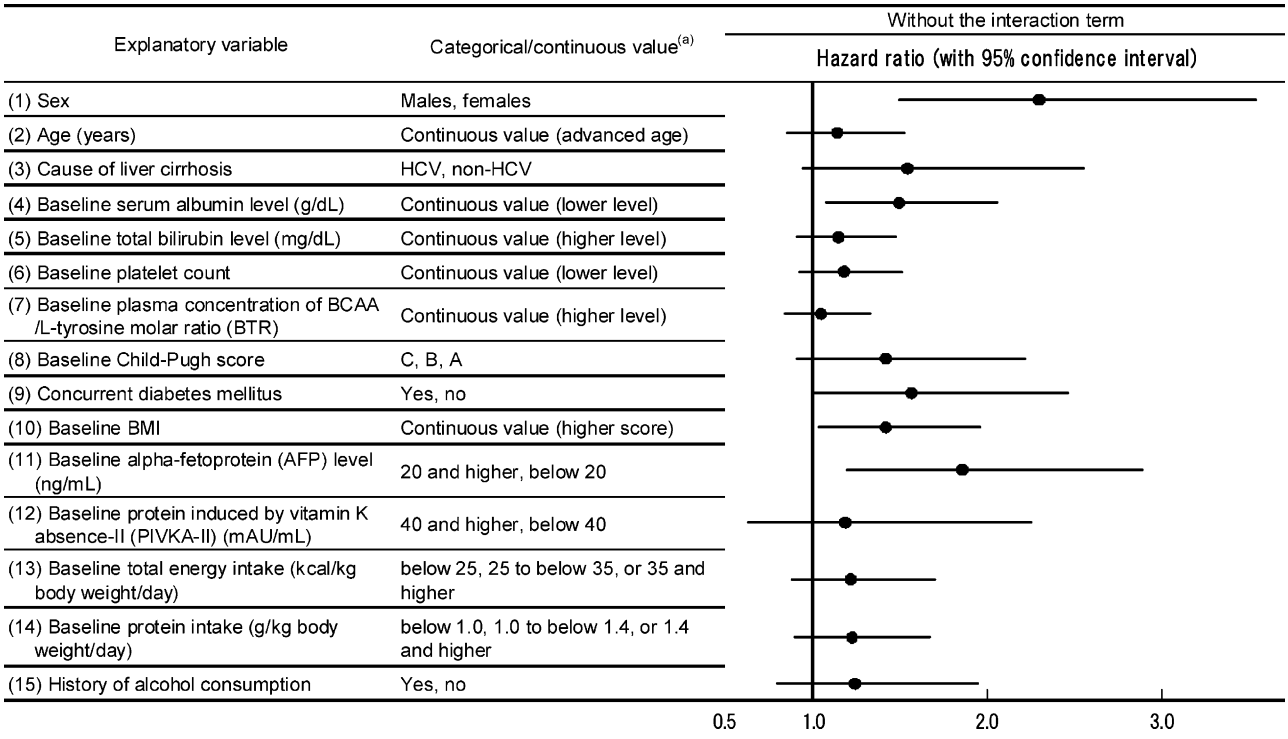
The correlation coefficients were calculated between variables that were found to have a statistically significant regression coefficient when using the model without the interaction term. The absolute correlation coefficient was not so high and was below 0.2 for all relationships of such variables (Table 2).

3.2. Effects of BCAA treatment

The incidence of liver cancer between the BCAA group and the diet group were compared with regard to variables that

Table 1
Analysis of risk for liver cancer in cirrhotic patients using Cox regression model

Explanatory variable	Categorical/continuous value[a]	Without the interaction term				With the interaction term
		Hazard ratio	95% Confidence interval		Two-tailed *p*-value for regression coefficient	Two-tailed *p*-value of the interaction between treatment and each variable
			Lower limit	Upper limit		
(1) Sex	Male, female	2.30	1.50	3.53	0.000[b]	0.574
(2) Age (years)	Continuous value (advanced age)	1.14	0.86	1.52	0.377	0.618
(3) Cause of liver cirrhosis	HCV, non-HCV	1.54	0.93	2.55	0.096	0.261
(4) Baseline serum albumin level (g/dL)	Continuous value (lower level)	1.49	1.08	2.06	0.014[b]	0.355
(5) Baseline total bilirubin level (mg/dL)	Continuous value (higher level)	1.16	0.91	1.47	0.227	0.086[c]
(6) Baseline platelet count	Continuous value (lower level)	1.18	0.93	1.51	0.174	0.474
(7) Baseline plasma concentration of BCAA/L-tyrosine molar ratio (BTR)	Continuous value (higher level)	1.05	0.84	1.32	0.669	0.884
(8) Baseline Child-Pugh score	C, B, A	1.42	0.91	2.21	0.122	0.802
(9) Concurrent diabetes mellitus	Yes, no	1.57	1.00	2.45	0.048[b]	0.952
(10) Baseline BMI	Continuous value (higher score)	1.42	1.03	1.96	0.035[b]	0.005[c]
(11) Baseline alpha-fetoprotein (AFP) level (ng/mL)	20 and higher, below 20	1.86	1.20	2.89	0.006[b]	0.150[c]
(12) Baseline protein induced by Vitamin K absence-II (PIVKA-II) (mAU/mL)	40 and higher, below 40	1.19	0.63	2.25	0.588	0.673
(13) Baseline total energy intake (kcal/kg body weight/day)	Below 25, 25 to below 35, or 35 and higher	1.22	0.88	1.70	0.088	0.510
(14) Baseline protein intake (g/kg body weight/day)	Below 1.0, 1.0 to below 1.4, or 1.4 and higher	1.23	0.90	1.68	0.203	0.699
(15) History of alcohol consumption	Yes, no	1.24	0.80	1.93	0.335	0.716



[a] Categorical values are listed in order from those with higher risk for liver cancer to lower risk, while higher-risk continuous values are shown in parenthesis. For explanatory variables (2), (4), (5), (6), (7), and (10), hazard ratio is presented for values between 25 and 75 percentiles of each variable. For explanatory variable (8), hazard ratio is presented for one-grade increase in Child-Pugh scores when grades 1–3 are given to Child-Pugh scores A, B, and C, respectively. For explanatory variables (11)–(14), hazard ratio is presented for values within and outside the normal range.

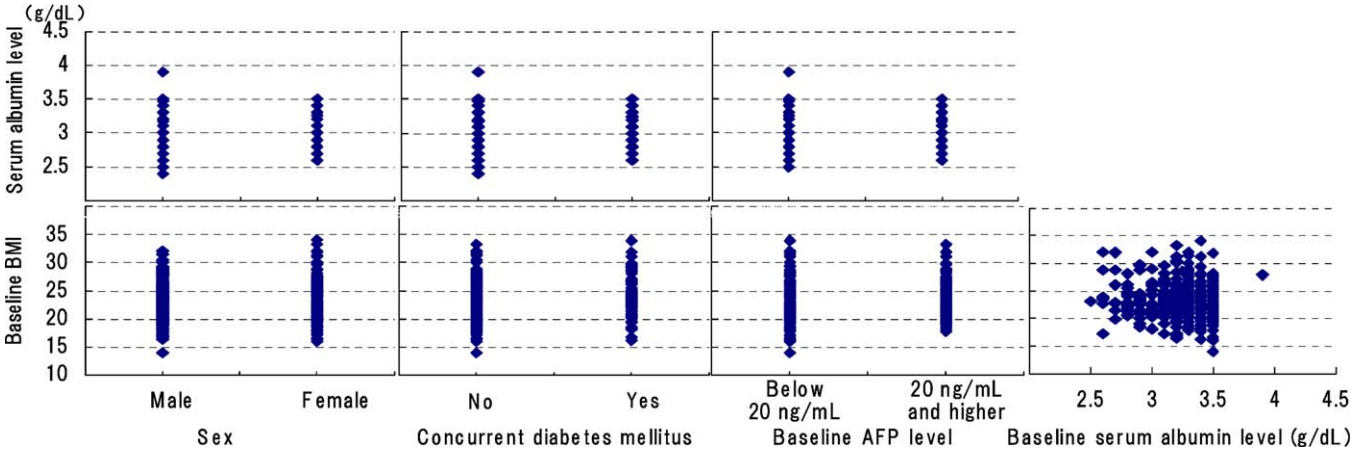
[b] $p < 0.05$.

[c] $p < 0.20$ for data analyzed with the interaction term.

Table 2
Correlation between variables with a significant regression coefficient without the interaction term

	Sex		Concurrent diabetes mellitus		Baseline AFP level		Baseline serum albumin level (g/dL)	Baseline BMI
	Male	Female	No	Yes	Below 20 ng/mL	20 ng/mL and higher		
Sex								
Male			216	78	150	102	294	186
Female			259	69	135	141	328	217
Concurrent diabetes mellitus								
No	$r=-0.065$				205	196	475	306
Yes	$p=0.108$				80	47	147	97
Baseline AFP level								
Below 20 ng/mL	$r=0.106$		$r=-0.102$				285	183
20 ng/mL and higher	$p=0.015$		$p=0.019$				243	165
Baseline serum albumin level (g/dL)	$r=0.037$		$r=-0.077$		$r=-0.066$			403
	$p=0.357$		$p=0.055$		$p=0.131$			
Baseline BMI	$r=0.050$		$r=0.026$		$r=0.163$		$r=-0.082$	
	$p=0.314$		$p=0.597$		$p=0.002$		$p=0.100$	

No. of patients, Spearman rank-order correlation coefficient.



had a significantly increased HR and also significantly interacted with the treatment arms. For BMI scores, the incidence of liver cancer was significantly lower in the BCAA group with a BMI of 25 or higher (Table 3 and Fig. 1; $p=0.008$), whereas there was no difference between two groups with a BMI below 25 (Table 3 and Fig. 2; $p=0.860$). The baseline characteristics of patients with a BMI below 25 and those with a BMI of 25 or higher are summarized in Table 4. Similarly, patient characteristics of the BCAA and diet groups with a BMI of 25 or higher are summarized in Table 5. Of these background factors, body weight and the incidence of slight peripheral edema was significantly higher in patients with a BMI of 25 or higher than in patients with a BMI below 25 ($p=0.000$ and $p=0.032$, respectively) (Table 4). The incidence of hepatic encephalopathy was significantly higher in the BCAA group than in the diet group ($p=0.016$) (Table 5).

For the AFP level, which had lesser interaction with treatment, the incidence of liver cancer was also significantly

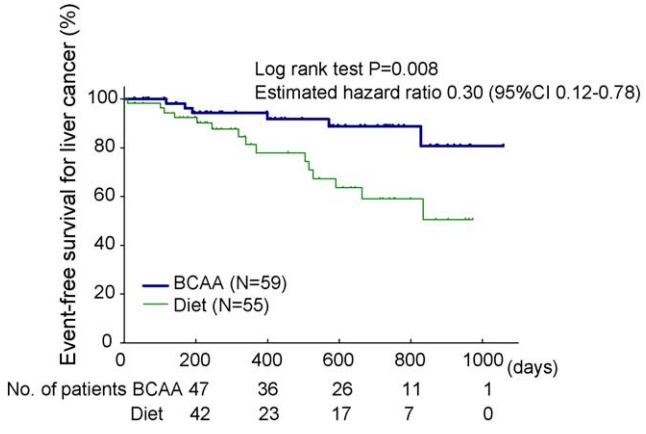
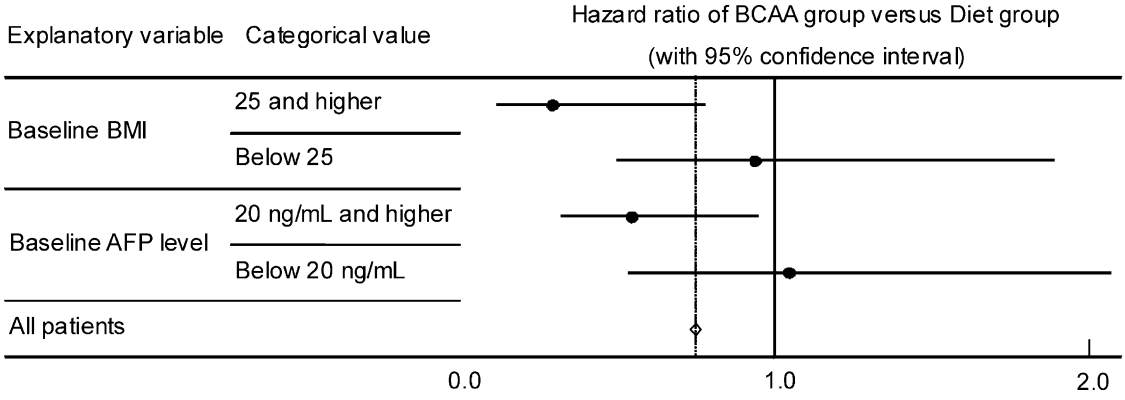


Fig. 1. Kaplan–Meier estimates of event-free survival for liver cancer in patients with a BMI score of 25 or higher.

Table 3
Analysis of risk for liver cancer in cirrhotic patients (comparison between BCAA group and Diet group)

Explanatory variable	Categorical value		BCAA group	Diet group	Hazard ratio	95% Confidence interval		Two-tailed p-value[a]	Cox regression model (with the interaction term)
						Lower limit	Upper limit		Two-tailed p-value of the interaction between treatment and each variable
Baseline BMI	25 and higher	No. of patients	59	55	0.30	0.12	0.78	0.008[b]	0.005[c]
		No. of events	6	15					
	Below 25	No. of patients	145	144	0.94	0.50	1.79	0.860	
		No. of events	19	19					
Baseline AFP level	20 ng/mL and higher	No. of patients	131	112	0.55	0.32	0.95	0.030[b]	0.150[c]
		No. of events	23	29					
	Below 20 ng/mL	No. of patients	138	147	1.05	0.53	2.08	0.885	
		No. of events	16	17					
All patients		No. of patients	314	308	0.76	0.51	1.15	0.197	
		No. of events	42	49					



[a] p-Values were estimated using the log rank test.
[b] $p < 0.05$.
[c] $p < 0.20$ for data analyzed Cox regression model with the interaction term.

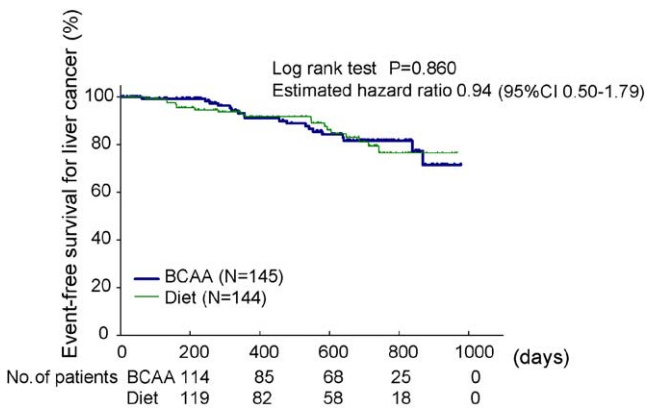


Fig. 2. Kaplan–Meier estimates of event-free survival for liver cancer in patients with a BMI score below 25.

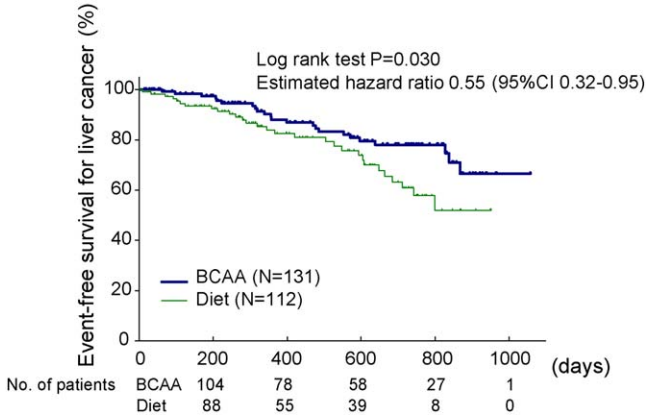


Fig. 3. Kaplan–Meier estimates of event-free survival for liver cancer in patients with an AFP level of 20 ng/mL or higher.

Table 4
Demographic and clinical characteristics of the patients at entry into the study (comparison between baseline BMI of 25 and higher or below 25)

Characteristic	Baseline BMI		Statistics[a]
	25 and higher	Below 25	
No. of patients	114	289	–
Sex (male/female)	46/68	140/149	n.s.[b]
Age (years)	62 ± 8	62 ± 9	n.s.
Body weight (kg)	68.0 ± 10.0	54.7 ± 7.8	$p = 0.000$
Etiology (HCV/HBV/alcoholic/others)	87/12/5/10	215/27/19/28	n.s.
Serum albumin (g/dL)	3.3 ± 0.3	3.3 ± 0.3	n.s.
Total bilirubin (mg/dL)	1.2 ± 0.8	1.2 ± 0.7	n.s.
Platelet count ($\times 10^4$ mm^{-3})	8.8 ± 4.3	8.9 ± 5.6	n.s.
Child-Pugh grade (A/B/C)	55/39/1	133/95/0	n.s.
Presence of varices	62 (54%)	146 (51%)	n.s.
Hepatic coma (grade 0/1/2/3/4)	108/4/1/1/0	277/8/3/0/0	n.s.
Ascites (absent/slight/moderate/severe)	114/0/0/0	289/0/0/0	n.s.
Peripheral edema (absent/slight/moderate/severe)	52/62/0/0	166/123/0/0	$p = 0.032$

Data are expressed as number of patients or mean ± S.D.
[a] Statistical analysis was performed by Fisher exact test, chi-square test, Wilcoxon test or Student's *t*-test.
[b] n.s., not significant.

Table 5
Demographic and clinical characteristics of the patients at entry into the study (comparison between BCAA group and Diet group with a BMI of 25 or higher)

Characteristic	BCAA group	Diet group	Statistics[a]
Number of patients	59	55	–
Sex (male/female)	22/37	24/31	n.s.[b]
Age (years)	62 ± 8	62 ± 9	n.s.
Body weight (kg)	66.3 ± 8.4	69.7 ± 11.3	n.s.
Etiology (HCV/HBV/alcoholic/others)	46/7/1/5	41/5/4/5	n.s.
Serum albumin (g/dL)	3.3 ± 0.3	3.3 ± 0.3	n.s.
Total bilirubin (mg/dL)	1.3 ± 1.0	1.2 ± 0.5	n.s.
Platelet count ($\times 10^4$ mm^{-3})	8.3 ± 3.7	9.3 ± 4.8	n.s.
Child-Pugh grade (A/B/C)	28/21/1	27/18/0	n.s.
Presence of varices	33 (56%)	29 (53%)	n.s.
Hepatic coma (grade 0/1/2/3/4)	53/4/1/1/0	55/0/0/0/0	$p = 0.016$
Ascites (absent/slight/moderate/severe)	59/0/0/0	55/0/0/0	n.s.
Peripheral edema (absent/slight/moderate/severe)	29/30/0/0	23/32/0/0	n.s.

Data are expressed as number of patients or mean ± S.D.
[a] Statistical analysis was performed by Fisher exact test, chi-square test, Wilcoxon test or Student's *t*-test.
[b] n.s., not significant.

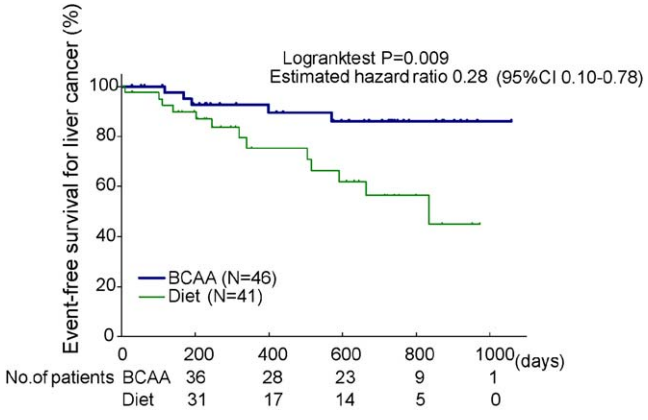


Fig. 4. Kaplan–Meier estimates of event-free survival for liver cancer in patients with cirrhosis caused by HCV infection and a BMI score of 25 or higher.

decreased in the BCAA group with an AFP level of 20 ng/mL or higher (Table 3 and Fig. 3; $p = 0.030$).

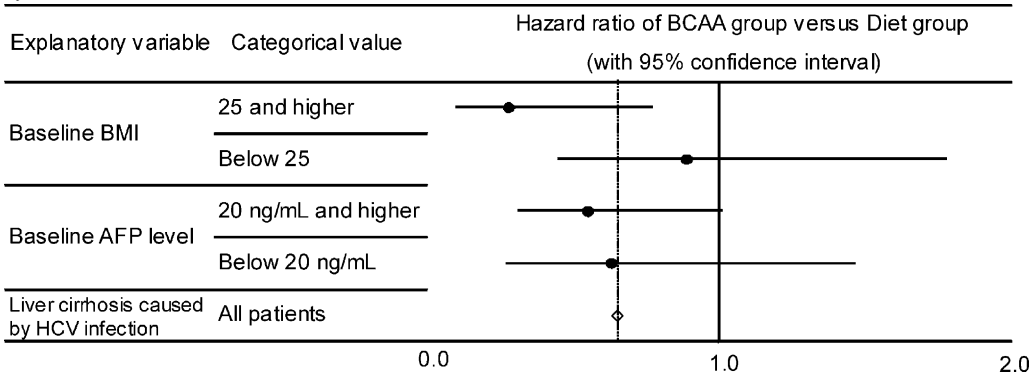
In a similar comparison performed in a subset of patients with liver cirrhosis caused by HCV infection, the BCAA group with a BMI of 25 or higher had a significantly lower incidence of liver cancer (Table 6 and Fig. 4; $p = 0.009$).

4. Discussion

Decompensated liver cirrhosis can now be managed to some extent after the introduction of treatment methods for complications of the disease such as ruptured esophageal varices, ascites, and hepatic encephalopathy. Under such circumstances, one important issue to be addressed is how to reduce the incidence of liver cancer in patients with cirrhosis. The known causes of liver cancer include HBV infection, HCV infection, substantial alcohol consumption, NAFLD,

Table 6
Analysis of risk for liver cancer in patients with cirrhosis caused by HCV infection (comparison between BCAA group and Diet group)

Explanatory variable	Categorical value		BCAA group	Diet group	Hazard ratio	95% Confidence interval		Two-tailed p-value[a]
						Lower limit	Upper limit	
Baseline BMI	25 and higher	No. of patients	46	41	0.28	0.10	0.78	0.009[b]
		No. of events	5	13				
	Below 25	No. of patients	106	109	0.89	0.44	1.78	0.739
		No. of events	15	17				
Baseline AFP level	20 ng/mL and higher	No. of patients	109	91	0.56	0.31	1.01	0.052
		No. of events	20	24				
	Below 20 ng/mL	No. of patients	88	77	0.63	0.27	1.47	0.280
		No. of events	9	14				
Liver cirrhosis caused by HCV infection	All patients	No. of patients	227	204	0.66	0.42	1.06	0.080
		No. of events	32	40				



[a] p-Values were estimated using the log rank test.
[b] $p < 0.05$.

and aflatoxin. In Japan, the incidence of liver cancer due to HCV infection is particularly high [6]. Many possible risk factors of liver cancer due to chronic hepatic disorders have been identified as follows: males, advanced age, severe hepatic disorders, races (Asian and African), and substantial alcohol consumption [3,5,7,8]. Some studies have described no relationship between the development of liver cancer and concurrent diabetes mellitus, but taking into account its association with NAFLD, recent studies have pointed out that diabetes mellitus is one of the important risk factors of liver cancer [3,5,7,9–12]. Other epidemiological studies have shown that there are additional risk factors such as concurrent hyperinsulinemia and a high BMI score [3,13–17].

From 1997 to 2003, we conducted a multicenter, randomized, controlled trial to investigate the effect of supplemental BCAA therapy on the protein-energy malnutrition and on the event-free survival in Japanese patients with decompensated cirrhosis. This trial was performed using a much larger sample size and longer observation period than other previous studies conducted based on a similar concept [19,26]. The primary composite endpoints of the trial were: (1) aggravation of hepatic failure, (2) rupture of esophageal or gastric varices, (3) development of liver cancer, and (4) death from any cause. The trial results demonstrated that BCAA treatment significantly decreased the events as compared with dietary treatment, and that long-term treatment with BCAA granules could reduce the incidence of common associated conditions of liver cirrhosis [29,32]. In the present analysis, we particularly focused on one of the primary endpoints, the development of liver cancer, and investigated various risk factors and the effect of BCAA treatment on this endpoint in high-risk patients.

Using a Cox proportional hazard model, we estimated the HRs of liver cancer for background factors available for evaluation. The HR was significantly higher for males, a low serum albumin level, concurrent diabetes mellitus, a high BMI score, and an AFP level of 20 ng/mL or higher. These results were consistent with other studies as for variables of males [5,7,8], concurrent diabetes mellitus [3,5,7,9–12], and a high BMI score [3,13–15]. As a lower albumin level is associated with severer liver cirrhosis, the significantly high HR of patients with a low serum albumin level may also be consistent with previous studies that have shown that cirrhosis of high severity is one of the risk factors of liver cancer [5,7,8]. Patients with an AFP level of 20 ng/mL or higher might also have severer liver cirrhosis or a very minute cancer that was not detected by imaging of the liver performed at the time of enrollment, which possibly increased the risk for clinically detectable liver cancer. The correlation coefficients were calculated between variables that were found to have a statistically significant regression coefficient when using the model without the interaction term. There was no

strong correlation between such variables, indicating a high independency of them. In addition, it is quite important that, in Japan and presumably in other developed countries, one quarter of cirrhotic patients (114/403, 28%, Table 3) might already be overweight or obese with a BMI score of 25 or higher, even after excluding those with ascites or moderate to severe edema.

In the comparison between BCAA and dietary treatments, it is important to note that the BCAA group had a significantly decreased incidence of liver cancer if they had a BMI score of 25 or higher. Moreover, BMI as a continuous variable significantly interacted with the treatment arms, indicating a marked effect of BCAA treatment to reduce the incidence of liver cancer as suggested by the HRs of 0.94 and 0.30 against dietary treatment in patients with a BMI below 25 and a BMI of 25 or higher, respectively. Cirrhotic patients often have impaired glucose tolerance associated with hyperinsulinemia and peripheral insulin resistance. It has been suggested that insulin may promote the development of cancer [16,33], and a prospective cohort study has demonstrated a higher mortality of liver cancer due to hyperinsulinemia [17]. Another study has also described that postprandial hyperinsulinemia stimulates the growth of human hepatocellular carcinoma [34].

A recent study on the CCl4 rat model of liver cirrhosis has shown that some BCAA (L-leucine and L-isoleucine) decreased blood glucose level after glucose loading without any alteration of insulin level. This effect of BCAA could be explained by the underlying mechanisms of insulin-independent glucose uptake into muscle cells and subsequent storage of glucose in skeletal muscles through stimulation of glycogen synthesis via the mammalian target of rapamycin (m-TOR) [35,36]. Based on these findings, the effect of BCAA treatment to improve glucose metabolism and hyperinsulinemia may be associated with an action of these amino acids to store glycogen in skeletal muscles in cirrhotic patients with impaired glucose tolerance who have decreased glycogen storage in the liver. Indeed, a preliminary clinical study has supported this hypothesis in patients with liver cirrhosis [37].

As described above, the effect of BCAA on glucose metabolism may contribute to a decrease in the liver cancer incidence in cirrhotic patients with a BMI score of 25 or higher, since they may have a particularly higher incidence of hyperinsulinemia and peripheral insulin resistance. It has also been described that BCAA inhibited in vitro the growth of human liver cancer cell lines [38]. Further investigation will be necessary to evaluate the relationship between BCAA and development of liver cancer.

We performed another comparison in a subset of patients with liver cirrhosis caused by HCV infection, which accounts for a substantial proportion of causes of liver cirrhosis in Japan. As observed in data obtained in all patients, BCAA again significantly decreased the incidence of liver cancer in patients with a BMI score of 25 or higher.

The limitation of the present analysis is that the subpopulation with a BMI score of 25 or higher included patients with mild edema, although we totally excluded those with ascites or moderate to severe edema. Thus, a BMI score could have possibly been overestimated in this study as suggested in Table 4. However, in the subpopulation of patients with a BMI score of 25 or higher, there was no difference between the BCAA group and diet group in the incidence of mild edema and other factors except for hepatic coma (Table 5). Therefore, these findings may also support the idea that BCAA inhibits liver carcinogenesis in cirrhotic patients with a BMI score of 25 or higher.

In conclusion, the results of this analysis suggest the possibility that long-term BCAA treatment decreases the risk for liver cancer in patients with liver cirrhosis if they have a specific background factor. Further fundamental and clinical studies will be needed to confirm the above hypothesis.

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Appendix A

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References

[1] El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.

[2] El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–23.

[3] El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127(Suppl 1):S27–34.

[4] La Vecchia C, Lucchini F, Franceschi S, Negri E, Levi F. Trends in mortality from primary liver cancer in Europe. Eur J Cancer 2000;36:909–15.

[5] Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127(Suppl 1):S17–26.

[6] Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 2006;49:7–17.

[7] Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127(Suppl 1):S35–50.

[8] Ikeda K, Saitoh S, Suzuki Y, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930–8.

[9] Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. J Natl Cancer Inst 2000;92:1096–9.

[10] Hassan MM, Hwang LY, Hatten CJ, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206–13.

[11] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–8.

[12] Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005;54:533–9.

[13] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.

[14] Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. Gastroenterology 2004;127(Suppl 1):S97–S103.

[15] Oh SW, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. J Clin Oncol 2005;23:4742–54.

[16] Moore MA, Park CB, Tsuda H. Implications of the hyperinsulinaemia–diabetes–cancer link for preventive efforts. Eur J Cancer Prev 1998;7:89–107.

[17] Balkau B, Kahn HS, Courbon D, Eschwege E, Ducimetiere P. Hyperinsulinemia predicts fatal liver cancer but is inversely associated with fatal cancer at some other sites: the Paris Prospective Study. Diabetes Care 2001;24:843–9.

[18] Alberino F, Gatta A, Amodio P, et al. Nutrition and survival in patients with liver cirrhosis. Nutrition 2001;17:445–50.

[19] Marchesini G, Bianchi G, Merli M, et al. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology 2003;124:1792–801.

[20] Tajika M, Kato M, Mohri H, et al. Prognostic value of energy metabolism in patients with liver cirrhosis. Nutrition 2002;18:229–34.

[21] Moriwaki H, Miwa Y, Tajika M, Kato M, Fukushima H, Shiraki M. Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. Biochem Biophys Res Commun 2004;313:405–9.

[22] Kato M, Moriwaki H. Metabolic disorders in patients with liver cirrhosis. Hepatol Res 2004;30(Suppl 1):59–62.

[23] Suzuki K, Kato A, Iwai M. Branched-chain amino acid treatment in patients with liver cirrhosis. Hepatol Res 2004;30(Suppl 1):25–9.

[24] Nishiguchi S, Habu D. Effect of oral supplementation with branched-chain amino acid granules in the early stage of cirrhosis. Hepatol Res 2004;30(Suppl 1):36–41.

[25] Sato S, Watanabe A, Muto Y, et al. Clinical comparison of branched-chain amino acid (L-leucine, L-isoleucine, L-valine) granules and oral nutrition for hepatic insufficiency in patients with decompensated liver cirrhosis (LIV-EN study). Hepatol Res 2005;31:232–40.

[26] Yoshida T, Muto Y, Moriwaki H, Yamato M. Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. J Gastroenterol 1989;24:692–8.

[27] Muto Y, Yoshida T. Effect of oral supplementation with branched-chain amino acid granules on improvement of protein nutrition in decompensated liver cirrhosis: a cross-over controlled study. In: Ogoshi S, Okada A, editors. Parenteral and enteral hyperalimentation. Amsterdam: Excerpta Medica; 1984. p. 280–92.

[28] Muto Y, Yoshida T, Sato S, Suzuki K, Watanabe A. Effect of oral supplementation with BCAA-G on the prognosis of liver cirrhosis. JJPEN 1992;14:765–75.

[29] Muto Y, Sato S, Watanabe A, et al. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 2005;3:705–13.

[30] Kuzuya T, Nakagawa S, Satoh J, et al. Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002;55:65–85.

[31] The Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity. New Criteria for 'Obesity Disease' in Japan. Circ J 2002;66:987–92.

[32] Ghanta RK, Salvino RM, Mullen KD. Branched chain amino acid supplements in liver disease. Clin Gastroenterol Hepatol 2005;3:631–2.

[33] Boyd DB. Insulin and cancer. Integr Cancer Ther 2003;2:315–29.

[34] Saito K, Inoue S, Saito T, et al. Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 2002;51:100–4.

[35] Nishitani S, Takehana K. Pharmacological activities of branched-chain amino acids: augmentation of albumin synthesis in liver and improvement of glucose metabolism in skeletal muscle. Hepatol Res 2004;30S:19–24.

[36] Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 2005;288:G1292–300.

[37] Seko S, Nonami M, Fujiwara M, et al. [Usefulness of branched-chain amino acids in cirrhotic patients through evaluation of their glucose tolerance.] Diagn Treat 2006;94(6), in press [in Japanese].

[38] Saito Y, Saito H, Nakamura M, et al. Effect of the molar ratio of branched-chain to aromatic amino acids on growth and albumin mRNA expression of human liver cancer cell lines in a serum-free medium. Nutr Cancer 2001;39:126–31.