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Original Article

Association of Omega-3 Polyunsaturated Fatty Acids with Sarcopenia in Liver Cirrhosis Patients with Hepatocellular Carcinoma

Akitoshi Sano[1], Jun Inoue[1*], Eiji Kakazu[1,2], Masashi Ninomiya[1], Mio Tsuruoka[1], Kosuke Sato[1], Masazumi Onuki[1], Satoko Sawahashi[1], Keishi Ouchi[1] and Atsushi Masamune[1]

[1]Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Department of Liver Disease, The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba, Japan

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Abstract

Background and Aims: Sarcopenia is associated with the prognosis of patients with liver cirrhosis and hepatocellular carcinoma (HCC). Given their diverse physiological activities, we hypothesized that plasma fatty acids might influence the progression of sarcopenia. This study aimed to clarify the association between fatty acids and sarcopenia in cirrhotic patients with HCC. ***Methods:*** In this single-center retrospective study, we registered 516 cases and analyzed 414 cases of liver cirrhosis and HCC. The skeletal muscle mass index was measured using a transverse computed tomography scan image at the third lumbar vertebra. The cutoff value for sarcopenia followed the criteria set by the Japan Society of Hepatology. Fatty acid concentrations were measured by gas chromatography. ***Results:*** Fatty acid levels, particularly omega-3 (n-3) polyunsaturated fatty acid (PUFA), were lower in patients with poor liver function (Child-Pugh grade B/C) and were negatively correlated with the albumin-bilirubin score ($p<0.0001$). The prognosis of HCC patients with low PUFA levels was significantly worse. Among the different fatty acid fractions, only n-3 PUFAs significantly correlated with skeletal muscle mass index ($p=0.0026$). In the multivariate analysis, the n-3 PUFA level was an independent variable associated with sarcopenia ($p=0.0006$). ***Conclusions:*** A low level of n-3 PUFAs was associated with sarcopenia in patients with liver cirrhosis and HCC.

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[*]**Correspondence to:** Jun Inoue, Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1, Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan; ORCID: https://orcid.org/0000-0003-1171-7835. Tel/Fax: +81-22-717-7171, E-mail: jinoue@med.tohoku.ac.jp

Introduction

Morbidity and mortality due to liver cirrhosis are increasing worldwide. Liver cirrhosis is the most potent risk factor for the development of hepatocellular carcinoma (HCC), the 6th most common cancer globally.[1] Cirrhosis is a significant predisposing condition for malnutrition, frailty, and sarcopenia.[2] Sarcopenia is defined by the European Working Group on Sarcopenia as "a progressive and generalized skeletal muscle disorder associated with an increased likelihood of adverse outcomes, including falls, fractures, disability, and mortality".[3] Pathophysiological factors contributing to sarcopenia include hepatocellular necrosis with cytokine release, host biomolecules such as danger-associated and pathogen-associated molecular patterns, portosystemic shunting resulting in hyperammonemia and endotoxemia, and the underlying etiology of liver disease (ethanol, cholestasis, insulin resistance, etc.).[2] Sarcopenia is associated with shorter survival and higher recurrence rates of HCC in patients with cirrhosis and HCC.[4] Exercise and nutritional therapy are vital treatments for sarcopenia. Several studies have reported that exercise therapy, rehabilitation, and branched-chain amino acids (BCAAs) have contributed to improved frailty and prolonged prognosis in sarcopenia patients with HCC.[5]

Fatty acids (FAs) are essential components of lipids and play important roles in cell and tissue metabolism and function. Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are a class of long-chain fatty acids with many beneficial biological effects. It has been shown that n-3 PUFAs suppress muscle protein degradation, enhance the rate of muscle protein synthesis in response to anabolic stimuli, oxidative stress, and inflammation, and improve insulin sensitivity and lipid profiles.[6,7] Although the impact of FAs on skeletal muscle systems has recently come to attention, few studies have been reported on the composition of plasma FAs in cirrhosis with sarcopenia. We hypothesized that plasma FAs influence sarcopenia in patients with cirrhosis and HCC. To verify this hypothesis, we retrospectively examined the status of sarcopenia and plasma FA profiles using our institution's database of patients with liver cirrhosis and HCC.

Methods

Study design

This is a single-center, retrospective observational study. We retrospectively enrolled 516 patients with FA data who were admitted to Tohoku University Hospital for the treatment of liver diseases from December 2017 to June 2021. Cases without skeletal muscle volume data, liver cirrhosis, or HCC were excluded (Supplementary Fig. 1). The diagnosis of HCC was performed by combining computed tomography (CT) and tumor markers (alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin). The Barcelona Clinic Liver Cancer (BCLC) staging system was used to evaluate tumor progression.[8] The diagnosis of liver cirrhosis was based on histological findings (F4) or imaging findings (presence of varices, liver deformity, splenomegaly, etc.) and serological findings.

Blood examination and plasma fatty acids measurement

Blood samples were obtained in the early morning after overnight fasting. The plasma specimens were separated, and FA concentrations were measured by gas chromatography at a central laboratory (SRL, Inc., Tokyo, Japan) according to the method described elsewhere.[9] Briefly, total lipids in plasma were extracted following Folch's procedure, followed by hydrolysis to free FAs. Free FAs were esterified with potassium methoxide/methanol and boron trifluoride–methanol. The methylated FAs were analyzed using a GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an omegawax-250 capillary column (SUPELCO, Sigma–Aldrich Japan, Tokyo, Japan). The Child-Pugh (CP) grade, albumin-bilirubin (ALBI) score, and model for end-stage liver disease (MELD)-Na score were calculated to assess the severity of liver dysfunction.[10,11] The fibrosis-4 (FIB-4) index was derived from aspartate aminotransferase, alanine aminotransferase, platelet count, and age to predict advanced fibrosis.[12]

Measurement of skeletal muscle volume for evaluating sarcopenia

CT scans were used to measure skeletal muscle mass. A transverse CT image at the level of the third lumbar spine was assessed from each scan. Skeletal muscle was identified, and the cross-sectional areas (cm^2) were quantified using Hounsfield unit (HU) thresholds of −29 to +150 with image analysis software (WeVIEW Z-edition, HITACHI, Japan) after calibration with air, water, and bone (air, water, and bone are defined as −1,000 HU, 0 HU, and 1,000 HU, respectively).[13] Multiple muscles, including the psoas, erector spinae, quadratus lumborum, abdominal obliques, and rectus abdominis, were quantified by manually tracing the CT images. The cross-sectional areas were then normalized for height (cm^2/m^2) for the skeletal muscle mass index (SMI),[14] Sarcopenia was defined as a third lumbar spine SMI value <42 cm^2/m^2 for males and <38 cm^2/m^2 for females according to the Japan Society of Hepatology guidelines for secondary sarcopenia in liver disease.[15]

Statistical analysis

A comparison of variables between the two groups was made using the unpaired t-test. For comparisons involving more than three groups, Dunnett's test was used. Pearson's chi-square test was used to compare gender, etiology, CP grade, and BCLC staging. Data are expressed as mean±standard deviation. The linear association between FAs and other variables was quantified using Pearson's correlation coefficient. The cumulative overall survival rate was calculated using the Kaplan-Meier method, and differences between the curves were evaluated using the log-rank test. Differences were considered significant at $p<0.05$. Factors influencing prognosis were analyzed using the Cox proportional hazards model. We examined the association between sarcopenia and FAs by univariate and multivariate analysis using logistic regression analysis. Priori variables were selected based on previous literature and included age, etiology, cirrhosis status (CP grade, ALBI score, MELD-Na score), BCAA levels, liver fibrosis, and tumor progression.[16,17] The FIB-4 index and BCLC staging were used as hepatic fibrosis and tumor condition variables, respectively. The final multivariable model included variables with a p-value of <0.10 in the subsequent analysis stage. Propensity score-matching analysis was performed with the add-in package in JMP® Pro 17 software (SAS Institute, NC) using the 1:1 nearest available matching method. The covariates included age, gender, etiology, BCLC staging, CP grade, ALBI score, FIB-4 index, MELD-Na score, and BCAAs. Participants with missing data were excluded from the analysis. A p-value of <0.05 was considered statistically significant in all analyses. All statistical analyses were performed with JMP® Pro 17.

Results

Clinical characteristics of enrolled patients

Data from 516 cases were included in the analysis; the following cases were excluded: acute hepatitis (N=2), acute on chronic liver failure (N=1), and missing data (N=48) (Supplementary Fig. 1). Due to the small sample sizes of patients without HCC (N=24) and patients without liver cirrhosis (N=27), we included only patients with both HCC and liver cirrhosis (N=414) in our primary analysis. The baseline characteristics are shown in Table 1. The average age of patients with liver cirrhosis and HCC was 71.5 years, with males predominant (81%). Regarding liver disease etiology, patients with HCC due to viral hepatitis were in the majority (46%), followed by alcohol-associated liver disease (26%). Most cases were in BCLC stage A (47%) or B (42%). The high FIB-4 index and ALBI score in this group suggested the progression of hepatic fibrosis and decreased liver reserve capacity. Sarcopenia was observed in 247 patients (60%). Liver cirrhosis patients without HCC were younger and had a higher FIB-4 index than the HCC group. Additionally, patients in the chronic hepatitis group were younger than the HCC group and had lower FIB-4 indexes and ALBI scores, indicating less fibrosis and deterioration of liver reserve function. In the chronic hepatitis group, sarcopenia cases were fewer than in the HCC group, but this was not significant ($p=0.12$).

Plasma fatty acids were correlated with liver dysfunction in subjects with liver cirrhosis

We analyzed the relationship between plasma FA composition and liver function. Saturated fatty acids (SFAs), n-3 PUFAs, and n-6 PUFAs were lower in patients with severe cirrhosis (CP grade B and C) compared to those with CP grade A (Fig. 1, Table 2). There was no significant difference in monounsaturated fatty acid (MUFA) levels. Focusing on the relative amount of each FA fraction, only n-3 PUFAs were significantly lower in Child-Pugh grade B/C patients. Evaluating ALBI scores as another index of liver function, Pearson correlation analysis revealed negative correlations between each FA fraction and ALBI score, especially n-3 PUFAs ($R=-0.33$, $p<0.0001$) (Supplementary Fig. 2). Using propensity score matching for patient backgrounds (age, gender, etiology, BCLC staging), decreases in n-3 PUFAs ($p=0.007$) and n-6

Table 1. Characteristics of the analyzed patients in this study

Mean±S.D.	Cirrhosis with HCC	Cirrhosis without HCC		Chronic hepatitis without HCC	
	N=414	N=24	*p*-value	N=27	*p*-value
Age, years	71.5±11.2	59.4±10.6	<0.0001*	54.3±19.1	<0.0001*
Gender, N	79/335	11/13	0.0041*	14/13	0.0003*
F/M (%)	(19/81)	(46/54)		(52/48)	
Etiology, N	137/53/107/68/49	12/1/1/6/4	0.0442*	8/2/0/10/7	0.0002*
HCV/HBV/alcohol/MASLD/others (%)	(33/13/26/16/12)	(46/4/4/31/15)		(34/3/3/34/26)	
BCLC staging, N	194/172/48	NA		NA	
A/B/C (%)	(47/42/11)				
Child-Pugh grade, N	365/46/3	19/5/0	0.3564	NA	
A/B/C (%)	(88/11/1)	(79/21/0)			
HCC treatment, N	153/155/66/40	NA		NA	
TACE/RFA/chemotherapy/others (%)	(37/37/16/10)				
T-Bil, mg/dL	1.1±0.5	1.5±0.5	0.0002*	0.8±0.3	0.0481*
AST, U/L	37.6±25.4	88.6±102.8	<0.0001*	49.0±32.5	0.1783
ALT, U/L	28.4±20.1	78.8±83.4	<0.0001*	63.3±51.5	<0.0001*
Albumin, g/dL	3.6±0.5	3.5±0.6	0.3093	3.9±0.5	0.0264*
PT-INR	1.0±0.1	1.1±0.1	0.4124	1.0±0.1	0.178
PLT, $\times 10^3/\mu L$	143.7±66.3	96.5±37.5	0.0012	214.6±82.7	<0.0001*
Sodium, mEq/L	140.4±2.7	140.4±3.4	0.9974	140.6±2.1	0.9
Creatinine, mg/dL	1.1±1.2	0.7±0.3	0.3806	0.8±0.2	0.4495
α-fetoprotein, ng/mL	10,332.4±67,525.5	11.6±14.1	<0.0001*	3.3±2.4	<0.0001*
DCP, mAU/mL	12,612.0±58,071.5	NA		NA	
ALBI score	−2.3±0.4	−2.1±0.6	0.0409*	−2.6±0.4	0.0049*
FIB-4 index	4.3±2.8	7.5±7.0	<0.0001*	2.1±1.9	0.0010*
MELD-Na	8.7±3.6	9.0±3.7	0.8202	7.1±1.0	0.0424*
BCAA, nmol/mL	481.4±102.4	463.0±177.7	0.6462	462.8±119.0	0.6064
Triglyceride, mg/dL	107.6±65.8	100.7±48.5	0.8471	106.9±46.7	0.9985
Total Cholesterol, mg/dL	170.9±54.5	156.9±46.1	0.3683	181.5±46.7	0.5546
Total FAs, μg/mL	2,827.9±568.7	2,993.4±727.8	0.3216	2,987.7±568.7	0.308
SFAs, μg/mL	915.0±191.5	1,007.0±250.3	0.0505	945.8±163.4	0.6766
Relative amount, %	32.3±1.5	33.6±1.8	0.0001*	31.7±1.6	0.0481
MUFAs, μg/mL	675.9±182.2	793.9±199.2	0.0043*	708.5±128.8	0.6034
Relative amount, %	23.7±3.1	26.6±1.9	<0.0001*	23.9±3.3	0.9790
n-3 PUFAs, μg/mL	231.9±83	202.2±87.0	0.1851	257.0±111.7	0.2689
Relative amount, %	8.2±2.4	6.9±2.3	0.0184*	8.6±3.1	0.6806
n-6 PUFAs, μg/mL	967.9±200	990.4±270.8	0.8505	1,076.3±226.4	0.0184*
Relative amount, %	34.4±3.7	32.9±3.4	0.1379	35.9±4.0	0.0833
CRP, mg/dL	0.4±0.7	0.3±0.6	0.7264	0.3±0.4	0.6941
SMI, cm^2/m^2	40.8±8.1	35.4±10.6	0.0039*	40.8±7.1	1.0000
Presence of Sarcopenia	247/167	17/7	0.2679	12/15	0.1231
sarcopenia/non-sarcopenia (%)	(60/40)	(71/29)		(44/56)	

*p-value <0.05 (vs Cirrhosis with HCC). S.D., standard deviation; HCV, hepatitis C virus; HBV, hepatitis B virus; MASLD, metabolic dysfunction-associated steatotic liver disease; BCLC, Barcelona Clinical Liver Cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; PLT, platelet; DCP, des-γ-carboxy prothrombin; ALBI, albumin-bilirubin; FIB-4, fibrosis-4 score; MELD, the model of end-stage liver disease; BCAA, branched-chain amino acid; FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; CRP, C-reactive protein; SMI, skeletal muscle mass index; HCC, hepatocellular carcinoma.

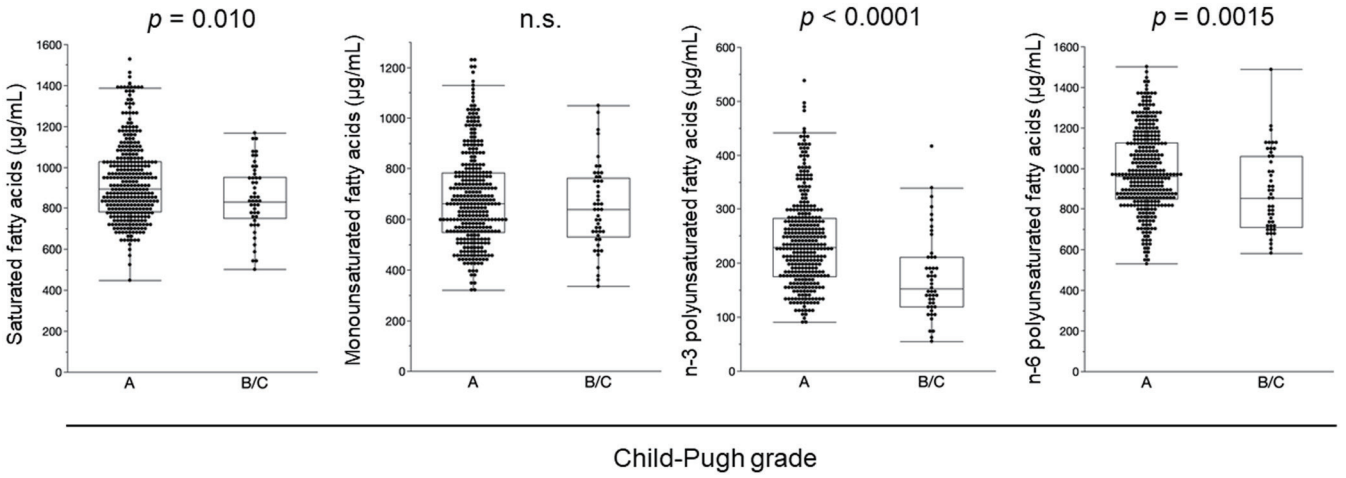


Fig. 1. Comparison of plasma fatty acid profiles in liver cirrhosis and hepatocellular carcinoma patients according to Child-Pugh grade. n.s., not significant.

PUFAs (p=0.0305) were observed in the CP B/C group (Supplementary Table 1). Additionally, comparing liver cirrhosis without HCC and chronic hepatitis without HCC using propensity score matching, n-3 PUFAs were decreased in the cirrhosis group (p=0.0441), with a significant relative amount decrease (p=0.0289) (Supplementary Table 2).

Plasma fatty acids were not associated with the progression of hepatocellular carcinoma

To evaluate whether HCC progression affects plasma FA levels, we examined the association between FA levels and HCC status using BCLC staging, number of liver tumors, and maximum tumor diameter. Each FA fraction showed no differences with BCLC staging or number of tumors (Supplementary Fig. 3A, B). Similarly, no correlations were found between FA levels and tumor diameter (Supplementary Fig. 3C). In addition, we compared liver cirrhosis patients with and without HCC by using propensity score matching to account for patient backgrounds (age, gender, etiology, CP grade, ALBI score, FIB-4 index, MELD-Na score) (Supplementary Table 3). While the relative amount of MUFAs showed a significant difference, the levels of each fatty acid fraction were not different between those groups.

Comparison of patients according to the presence of sarcopenia and outcome

The clinical characteristics of HCC patients with and without sarcopenia are shown in Table 3. Patients with sarcopenia had a higher proportion of females, aspartate aminotransferase, alanine aminotransferase, AFP, and FIB-4 index, and a lower number of BCLC staging A cases. Nutritionally, total BCAAs were significantly lower in subjects with sarcopenia, as previously described.[18] Notably, total FAs were significantly lower in patients with sarcopenia than those without. The absolute amounts of SFAs, MUFAs, and n-3 PUFAs were significantly lower in patients with sarcopenia. Focusing on relative amounts of FAs, there was no significant difference between SFAs and MUFAs, but the relative amount of n-3 PUFAs was significantly lower in subjects with sarcopenia. The relative amount of n-6 PUFAs was higher in patients with sarcopenia.

We analyzed the presence of sarcopenia and FA fractions in relation to the prognosis of HCC. The Kaplan-Meier curves in Figure 2 show that patients with sarcopenia had significantly worse overall survival rates than those without sarcopenia. To evaluate the relationship between FA levels and mortality, patients with low levels of each FA were defined as those with FA levels lower than the quartile. Although low relative amounts of SFAs and MUFAs were associated with better overall survival rates, worse survivals were observed in subjects with low relative amounts of n-3 and n-6 PUFAs (Log-rank: p=0.011, p=0.030, respectively). Using the Cox proportional hazards model, an analysis of variables related to prognosis was conducted (Supplementary Table 4). The levels of n-3 PUFAs and the relative amount of n-6 PUFAs were found to be independent variables related to the prognosis of HCC in the multivariate analysis.

The level of n-3 polyunsaturated fatty acids was correlated with skeletal muscle mass index in liver cirrhosis patients with hepatocellular carcinoma

We analyzed the correlation between SMI values and each FA fraction in liver cirrhosis patients with hepatocellular carcinoma. Although SFAs, MUFAs, and n-6 PUFAs did not show significant correlations, only n-3 PUFAs were positively correlated with SMI (R=0.15, p=0.0026) (Fig. 3A). Focusing on the relative amount of each FA fraction, n-3 PUFAs were positively correlated (R=0.19, p=0.0003), and n-6 PUFAs were negatively correlated (R=−0.14, p=0.011) with SMI (Fig. 3B). This result was similar to the analysis of all patients, including cirrhosis patients without HCC and chronic hepatitis patients (Supplementary Fig. 4). Calculating the n-6/n-3 ratio also showed a negative correlation with SMI (R=−0.15, p=0.0041) (Supplementary Fig. 5).

Since we found a significant association between liver function and FA levels, as shown in Figures 1 and 2, we performed the same analysis only in the CP grade A cohort (Supplementary Fig. 6). Even in the CP grade A population, only n-3 PUFAs were positively correlated with SMI (R=0.20, p=0.0003).

Logistic regression analysis revealed that the level of n-3 PUFAs was the independent variable related to sarcopenia

Given the significant correlation between n-3 PUFAs and skeletal muscle mass, we analyzed the association between the level of n-3 PUFAs and the risk of sarcopenia using logistic regression analysis. Table 4 shows the odds ratio for the presence of sarcopenia in patients with liver cirrhosis

Table 2. Comparison of clinical characteristics and fatty acid levels of liver cirrhosis and hepatocellular carcinoma patients with Child-Pugh grade A and B/C

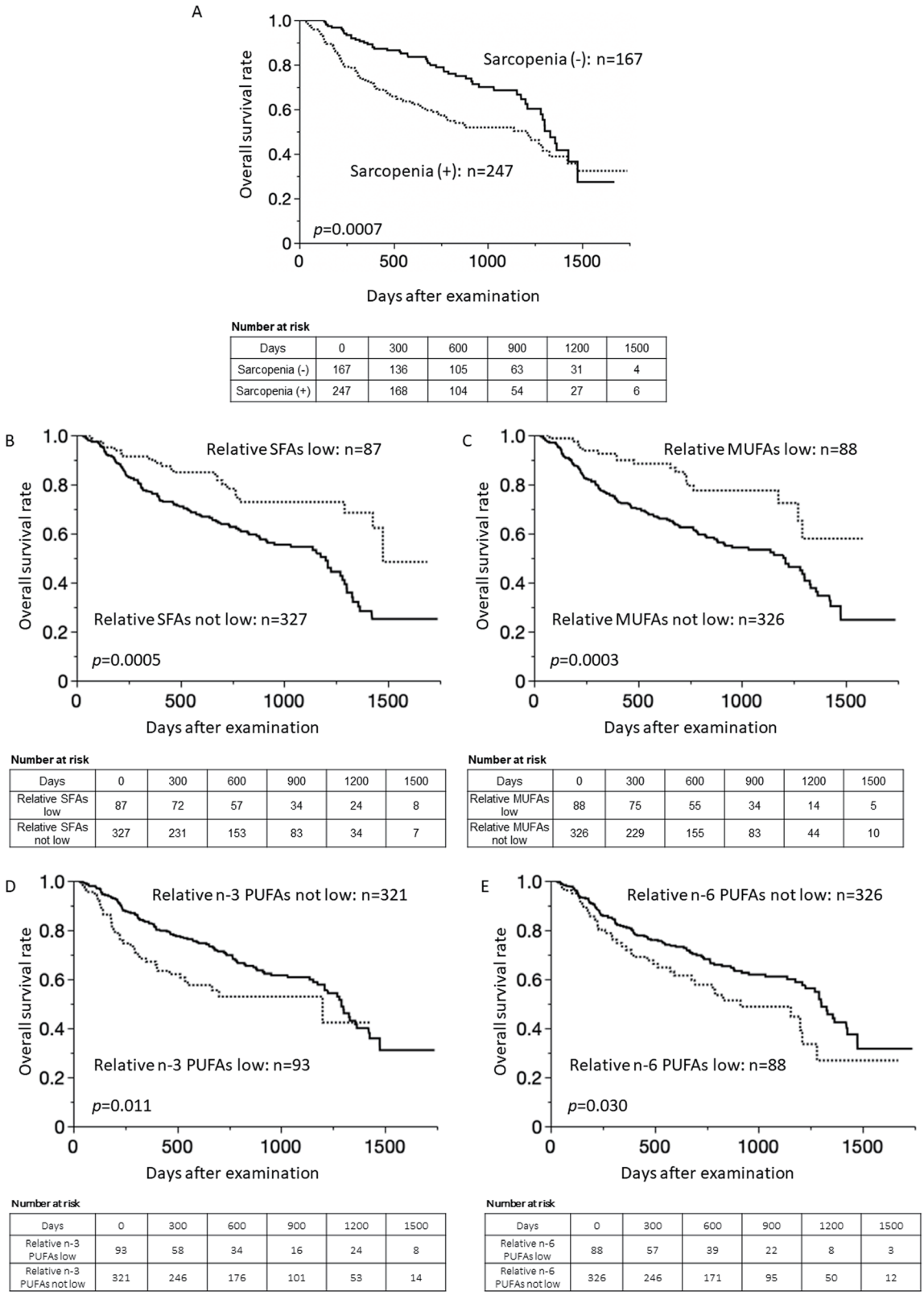
Mean±S.D.	Child-Pugh grade A	Child-Pugh grade B/C	*p*-value
	N=365	N=49	
Age, years	72.2±10.9	66.3±12.3	0.0005*
Gender, N	67/298	12/37	0.4577
F/M (%)	(18/82)	(24/76)	
Etiology, N	121/48/91/64/41	16/5/16/4/8	0.2485
HCV/HBV/alcohol/MASLD/others (%)	(33/13/25/18/11)	(33/10/32/8/17)	
BCLC staging, N	172/152/41	21/20/7	0.7834
A/B/C (%)	(47/42/11)	(43/41/16)	
HCC treatment, N	133/147/56/29	20/8/10/11	0.1018
TACE/RFA/chemotherapy/others (%)	(36/40/15/9)	(41/16/20/23)	
T-Bil, mg/dL	1.0±0.4	1.7±0.9	<0.0001*
AST, U/L	35.2±23.9	58.6±28.4	<0.0001*
ALT, U/L	27.5±19.4	35.8±23.7	0.0148*
Albumin, g/dL	3.7±0.4	3.0±0.4	<0.0001*
PT-INR	1.0±0.07	1.1±0.1	<0.0001*
PLT, $\times 10^3/\mu L$	145.4±61.2	129.8±99.6	0.2359
Sodium, mEq/L	140.6±2.5	139.1±3.8	0.0005*
Creatinine, mg/dL	1.0±1.0	1.2±2.5	0.3374
α-fetoprotein, ng/mL	8,698.2±60,161.5	24,663.7±113,245.6	0.1476
DCP, mAU/mL	11,658.7±56,563.7	20,935.5±70,143.5	0.3332
ALBI score	−2.4±0.4	−1.6±0.5	<0.0001*
FIB-4 index	4.0±2.5	7.0±3.9	<0.0001*
MELD-Na	8.3±3.2	10.8±4.6	<0.0001*
BCAA, nmol/mL	489.6±98.7	412.8±108.4	<0.0001*
Triglyceride, mg/dL	107.9±48.5	104.3±152.6	0.7131
Total Cholesterol, mg/dL	169.7±35.6	182.5±136.8	0.0941
Total FAs, μg/mL	2,858.4±566.4	2,568.1±527.3	0.0043*
SFAs, μg/mL	923.2±192.7	845.6±168.4	0.023*
Relative amount, %	32.3±1.5	33.0±1.3	0.0086*
MUFAs, μg/mL	680.3±183.3	638.3±170.9	0.2199
Relative amount, %	23.6±3.2	24.7±3.2	0.0558*
n-3 PUFAs, μg/mL	238.0±81.7	180.7±77.8	<0.0001*
Relative amount, %	8.3±2.3	7.0±2.6	0.0013*
n-6 PUFAs, μg/mL	980.1±197	864.4±198.3	0.0011*
Relative amount, %	34.4±3.7	33.8±3.7	0.3052
CRP, mg/dL	0.4±0.7	0.8±1.2	0.0005*
SMI, cm^2/m^2	40.9±8.0	40.0±9.2	0.4808
Presence of Sarcopenia, N	216/149	30/18	0.6581
sarcopenia/non-sarcopenia (%)	(59/41)	(61/39)	

*p-value <0.05. S.D., standard deviation; HCV, hepatitis C virus; HBV, hepatitis B virus; MASLD, metabolic dysfunction-associated steatotic liver disease; BCLC, Barcelona Clinical Liver Cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; PLT, platelet; DCP, des-γ-carboxy prothrombin; ALBI, albumin-bilirubin; FIB-4, fibrosis-4 score; MELD, the model of end-stage liver disease; BCAA, branched-chain amino acid; FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; CRP, C-reactive protein; SMI, skeletal muscle mass index.

Table 3. Comparison of clinical characteristics of patients with and without sarcopenia

Mean±S.D.	Sarcopenia (-)	Sarcopenia (+)	*p*-value
	N=167	N=247	
Age, years	71.7±9.0	71.4±12.5	0.8010
Gender, N	20/147	59/188	0.0019*
F/M (%)	(12/88)	(24/76)	
Etiology, N	49/17/45/36/20	88/36/62/32/29	0.0705
HCV/HBV/alcohol/MASLD/others (%)	(29/10/27/22/12)	(36/14/25/13/12)	
BCLC staging, N	93/65/9	101/107/39	0.0005*
A/B/C (%)	(56/39/5)	(41/43/16)	
Child-Pugh grade, N	149/17/1	216/29/2	0.8527
A/B/C (%)	(89/10/1)	(87/12/1)	
HCC treatment, N	58/76/17/16	95/79/49/24	0.2033
TACE/RFA/chemotherapy/others (%)	(35/45/10/10)	(38/32/20/10)	
T-Bil, mg/dL	1.2±0.6	1.0±0.4	0.4833
AST, U/L	34.2±17.8	39.9±29.3	0.0248*
ALT, U/L	29.3±15.9	27.7±22.5	0.4381*
Albumin, g/dL	3.7±0.4	3.6±0.5	0.0882
PT-INR	1.0±0.09	1.0±0.08	0.7076
PLT, $\times 10^3/\mu L$	140.4±57.3	146.0±71.8	0.5113
Sodium, mEq/L	140.7±2.2	140.3±3.0	0.1476
Creatinine, mg/dL	1.1±1.0	1.0±1.4	0.9343
α-fetoprotein, ng/mL	1,958.8±11,318.7	16,030.6±86,648.4	0.0394*
DCP, mAU/mL	5,294.0±38,196.8	17,540.8±67,909.6	0.0380
ALBI score	−2.3±0.4	−2.3±0.5	0.4728
FIB-4 index	3.9±2.5	4.5±3.0	0.0416*
MELD-Na	8.8±3.5	8.6±3.7	0.5311
BCAA, nmol/mL	505.1±101.5	465.3±100.1	0.0002*
Triglyceride, mg/dL	118.9±56.2	99.9±70.7	0.0054*
Total Cholesterol, mg/dL	170.1±33.9	171.5±64.9	0.7983
Total FAs, μg/mL	2,938.9±590.3	2,749.2±540.7	0.0020*
SFAs, μg/mL	954.6±202	887.0±179	0.0010*
Relative amount, %	32.5±1.7	32.3±1.3	0.1949
MUFAs, μg/mL	709.7±189.8	651.9±173.2	0.0033*
Relative amount, %	24.0±3.0	23.6±3.4	0.2345
n-3 PUFAs, μg/mL	254.0±92.7	216.3±71.6	<0.0001*
Relative amount, %	8.6±2.5	7.9±2.3	0.0093*
n-6 PUFAs, μg/mL	984.7±193.7	956.1±204	0.9070
Relative amount, %	33.7±3.7	34.8±3.6	0.0051*
CRP, mg/dL	0.3±0.5	0.5±0.8	0.0068*
SMI, cm^2/m^2	48.5±5.8	35.5±4.4	<0.0001*

*p-value <0.05. S.D., standard deviation; HCV, hepatitis C virus; HBV, hepatitis B virus; MASLD, metabolic dysfunction-associated steatotic liver disease; BCLC, Barcelona Clinical Liver Cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; PLT, platelet; DCP, des-γ-carboxy prothrombin; ALBI, albumin-bilirubin; FIB-4, fibrosis-4 score; MELD, the model of end-stage liver disease; BCAA, branched-chain amino acid; FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; CRP, C-reactive protein; SMI, skeletal muscle mass index.



Number at risk

Days	0	300	600	900	1200	1500
Sarcopenia (-)	167	136	105	63	31	4
Sarcopenia (+)	247	168	104	54	27	6

Number at risk

Days	0	300	600	900	1200	1500
Relative SFAs low	87	72	57	34	24	8
Relative SFAs not low	327	231	153	83	34	7

Number at risk

Days	0	300	600	900	1200	1500
Relative MUFAs low	88	75	55	34	14	5
Relative MUFAs not low	326	229	155	83	44	10

Number at risk

Days	0	300	600	900	1200	1500
Relative n-3 PUFAs low	93	58	34	16	24	8
Relative n-3 PUFAs not low	321	246	176	101	53	14

Number at risk

Days	0	300	600	900	1200	1500
Relative n-6 PUFAs low	88	57	39	22	8	3
Relative n-6 PUFAs not low	326	246	171	95	50	12

Fig. 2. Kaplan-Meier survival curves for the length of time until death. (A) Analysis for patients with and without sarcopenia. (B-E) Analysis for patients with and without low levels of each fatty acid fraction. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; +, with; -, without.

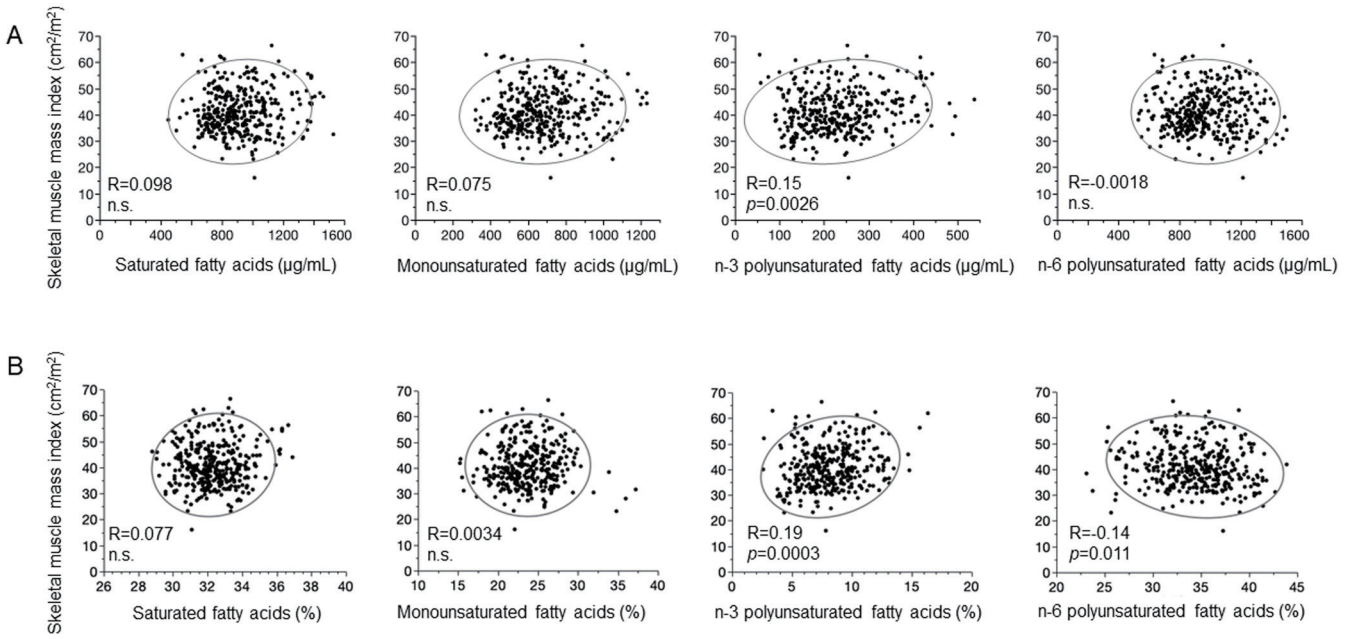


Fig. 3. The correlation between various fatty acid fraction levels and skeletal muscle mass index. (A) Analysis of absolute amounts of fatty acids. (B) Analysis of relative amounts of fatty acids. n.s., not significant.

Table 4. Logistic regression analysis for variates associated with sarcopenia in patients with liver cirrhosis

	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	***p*-value**	**Odds ratio (95% CI)**	***p*-value**
Age	0.9977 (0.9803–1.0155)	0.8002		
Gender				
women	1 (Reference)		1 (Reference)	
men	0.4335 (0.2498–0.7523)	0.0019*	0.3323 (0.1585–0.6964)	0.0035*
Etiology				
virus	1 (Reference)		1 (Reference)	
alcohol	0.7333 (0.4509–1.1926)	0.2113	0.7258 (0.3936–1.3383)	0.3046
MASLD	0.4731 (0.2697–0.8300)	0.0091*	0.3432 (0.1751–0.6723)	0.0018*
others	0.7718 (0.4056–1.4684)	0.3728	0.4330 (0.1848–1.0149)	0.0541
BCLC staging				
A	1 (Reference)		1 (Reference)	
B	1.5309 (1.0079–2.3253)	0.0458*	1.8315 (1.1070–3.0300)	0.0185*
C	4.0300 (1.8514–8.7724)	0.0004*	4.3626 (1.5686–12.1327)	0.0048*
Child-Pugh grade				
A	1 (Reference)			
B-C	1.1880 (0.6409–2.2022)	0.6595		
ALBI score	1.1777 (0.7542–1.8392)	0.5914		
FIB-4 index	1.0821 (1.0018–1.1687)	0.0365*	1.0581 (0.9624–1.1633)	0.2303
MELD-NA score	0.9752 (0.9223–1.0311)	0.3773		
BCAA	0.9961 (0.9934–0.9982)	0.0002*	0.9993 (0.9966–1.0021)	0.631
n-3 PUFAs	0.9944 (0.9917–0.9971)	<0.0001*	0.9947 (0.9915–0.9978)	0.0006*

*p-value <0.05. CI, confidence interval; n.s., not significant; MASLD, metabolic dysfunction-associated steatotic liver disease; BCLC, Barcelona Clinical Liver Cancer; ALBI, albumin-bilirubin; FIB-4, fibrosis-4 score; BCAA, branched chain amino acid; PUFA, polyunsaturated fatty acid.

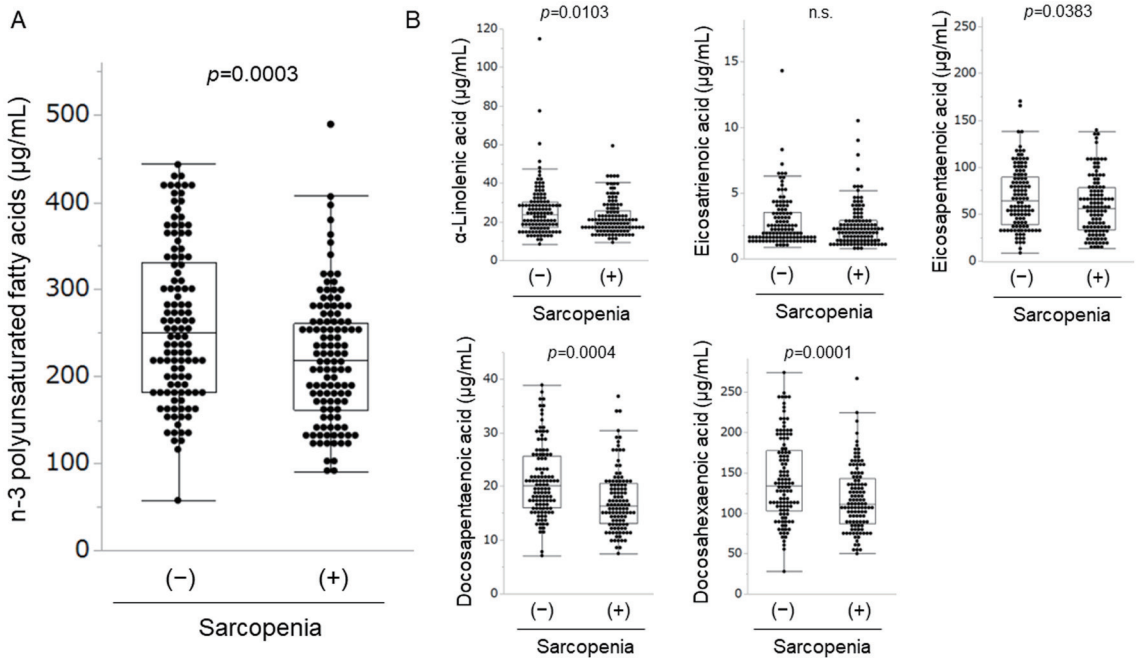


Fig. 4. The comparison of n-3 polyunsaturated fatty acids with and without sarcopenia in matched patients with liver cirrhosis. (A) The level of n-3 polyunsaturated fatty acids is shown. (B) The levels of each fatty acid in n-3 polyunsaturated fatty acids are shown. n.s., not significant; +, with; -, without.

for each variable. In the univariate analysis, gender, metabolic dysfunction-associated steatotic liver disease (MASLD), BCLC staging, FIB-4 index, BCAA level, and n-3 PUFA level were associated with the presence of sarcopenia. Multivariate analysis showed that the n-3 PUFA fraction was associated with the presence of sarcopenia. In addition to n-3 PUFAs, gender, etiology (MASLD), and BCLC staging B and C were identified as factors associated with sarcopenia. In the univariate analysis, every n-3 PUFA except C20:3 (eicosatrienoic acid) was a significant variable: C18:3 (alpha-linolenic acid), C20:5 (eicosapentaenoic acid (EPA)), C22:5 (docosapentaenoic acid) and C22:6 (docosahexaenoic acid (DHA)) (Supplementary Table 5).

The comparison of n-3 polyunsaturated fatty acids with and without sarcopenia in matched patients with liver cirrhosis

Finally, the n-3 PUFAs level was compared between patients with sarcopenia and those without, using propensity score matching to align their backgrounds. The covariates included age, gender, etiology, BCLC staging, CP grade, ALBI score, FIB-4 index, MELD-Na score, and BCAA. It was confirmed that there were no significant differences in each factor among the two groups (Supplementary Table 6). The total levels of n-3 PUFAs were significantly lower in the group of patients with sarcopenia, even among patients with matched backgrounds (Fig. 4A). Additionally, among the n-3 PUFA fractions, the levels of alpha-linolenic acid, EPA, docosapentaenoic acid, and DHA were significantly lower in patients with sarcopenia than in those without (Fig. 4B). We also analyzed only the CP grade A patients, with the same results as above (Supplementary Table 6, Supplementary Fig. 7).

Discussion

In this single-center retrospective study, we analyzed factors related to sarcopenia in patients with HCC and liver cirrhosis, with a particular focus on various FA levels. Our findings indicated that various FA fractions decreased with declining liver reserve capacity. Among these FAs, the level of n-3 PUFAs showed a correlation with skeletal muscle mass and remained an independent variable associated with sarcopenia, even after adjusting for various confounding factors. Therefore, the level of n-3 PUFAs is considered a factor involved in sarcopenia in patients with liver cirrhosis and HCC.

Sarcopenia is a prevalent muscle abnormality in patients with cirrhosis. The presence of sarcopenia is a major predictor of mortality pre- and post-liver transplantation,[19,20] longer hospital stays,[21] and hepatic encephalopathy.[22] Sarcopenia is also associated with poor survival in patients with HCC.[23] The pathogenesis of sarcopenia is multifactorial, involving factors such as hyperammonemia,[24,25] increased autophagy,[24] proteasomal activity,[26] myostatin,[27] and impaired mitochondrial function.[26]

Nutritional factors are also critical in the pathogenesis of sarcopenia. Nutritional deficits, especially BCAAs, are common in patients with liver cirrhosis. Accelerated skeletal muscle consumption of BCAAs in liver cirrhosis leads to muscle protein breakdown, resulting in sarcopenia.[28] While BCAAs are widely known to be important in the progression of sarcopenia, the association between sarcopenia and FAs in liver diseases is unclear. In the present study, we analyzed the relationship between sarcopenia and plasma FA levels in patients with liver cirrhosis and HCC and found that lower levels of n-3 PUFAs were associated with sarcopenia.

This study revealed that FA levels were lower in patients with reduced liver reserve function (CP grade B/C). Notably, only the level of n-3 PUFAs was lower in relative amounts in CP grade B/C patients and was most negatively correlated with the ALBI score. This result is consistent with previous reports showing that patients with liver cirrhosis lack crucial FAs, including n-3 PUFAs, which could be explained by reduced dietary intake, impaired liver synthesis, and increased degradation of PUFAs due to lipid peroxidation.[29–31] It was

expected that FA levels would decrease with worsening cancer due to deteriorating nutritional status. However, there was no association between HCC status and FA levels in this study. This might be because the patients in this study mainly had good hepatic reserve and could be treated for cancer. Further case studies are needed on the FA composition in patients with HCC and impaired hepatic reserve.

In this study, patients with sarcopenia had a significantly worse prognosis, which is consistent with previous studies.[17] Past studies have reported that PUFA intake improves mortality in diabetes[32] and cancer.[33] We found that although a low relative amount of SFAs and MUFAs was associated with a better overall survival rate, significantly worse survival rates were observed in subjects with low relative amounts of n-3 and n-6 PUFAs. In the Cox proportional hazards model, the following independent variables were associated with prognosis: etiology, BCLC staging, tumor marker (des-γ-carboxy prothrombin), the presence of sarcopenia, and PUFA levels. Although HCC treatment was a significant variable in univariate analysis, it became non-significant in multivariate analysis, likely due to confounding between HCC treatment and other variables. These results suggested that there may be a relationship between FA levels and mortality in HCC patients.

When the association between the composition of FAs and skeletal muscle mass was analyzed, it was revealed that SMI was significantly correlated only with the n-3 PUFA level. Because we elucidated the association between hepatic reserve (CP score and ALBI score) and plasma FA levels, it was essential to address the confounding relationship between FA composition and hepatic reserve in our analysis of skeletal muscle mass and FA composition. Hence, we also analyzed the correlation only in patients with CP grade A and conducted a multivariate analysis. The only FA that showed a significant correlation with muscle mass, even in patients with CP grade A, was n-3 PUFAs. In the multivariate analysis, a lower level of n-3 PUFAs was associated with an increased risk of sarcopenia among patients with liver cirrhosis, adjusted for patient backgrounds (gender, etiology, BCLC staging, FIB-4 index, and plasma BCAA level). Furthermore, we found lower n-3 PUFA levels in patients with sarcopenia when patient backgrounds were aligned using propensity score matching. These results showed an association between loss of skeletal muscle and lower levels of the n-3 FA fraction in patients with cirrhosis and HCC.

It has been known that n-3 PUFAs, especially EPA and DHA, play important roles in decreasing inflammatory processes[34] and the impact on skeletal muscle systems has recently come to attention. The nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) is a transcription factor that induces a pro-inflammatory response. The nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, alpha (IκBα), is a vital inhibitor of NF-κB. In C2C12 mouse skeletal myoblast cells, DHA and EPA were effective in inhibiting protein degradation, and DHA was able to increase the total protein IκBα level, which reduced NF-κB DNA-binding activity.[35,36] In addition, in mouse myoblasts, EPA was able to preserve cell viability by inhibiting mitogen-activated protein kinase apoptosis and stimulating MyoD, potentially reducing catabolic activity and increasing anabolic activity in skeletal muscle.[37] Further studies in C2C12 myoblasts indicate that EPA was able to restore insulin signaling when cells were co-incubated with lipopolysaccharide. This study demonstrated that EPA might help preserve the phosphorylation of mammalian targets of rapamycin, a pathway important for the activation of translation and muscle protein synthesis when cells face LPS exposure.[38] In this study, NF-κB was inhibited by EPA, providing a potential mechanism whereby mammalian targets of rapamycin phosphorylation could persist even during LPS exposure.[38] In addition, n-3 PUFAs were reported to improve intestinal function with altered intestinal microbiome.[39] Since a leaky gut may cause sarcopenia in patients with liver cirrhosis,[40] n-3 PUFA supplementation may be effective in preventing and treating sarcopenia in patients with HCC.

There are several clinical reports on the relationship between PUFAs and sarcopenia. A cross-sectional study of 363 people aged 60 years and above assessed the relationship between dietary fish oil intake and frailty and found that fish oil intake had a positive effect on the frailty status of younger subjects.[41] Studies have also demonstrated a relationship between n-3 PUFAs and sarcopenia in patients with cancer. For example, the change in muscle mass during chemotherapy was calculated in 41 patients with non-small cell lung cancer receiving chemotherapy, and patients with muscle loss had lower plasma EPA and DHA compared to those who were gaining muscle.[42] Itoh *et al*. showed that low EPA and DHA levels were associated with preoperative sarcopenia in patients with HCC.[43] Furthermore, Kitagawa *et al*. reported that the n-6/n-3 ratio and arachidonic acid (AA)/EPA ratio were associated with skeletal muscle depletion in cachexic patients with advanced gastrointestinal cancers.[44] In our study, we also found a negative correlation between the n-6/n-3 ratio and AA/EPA ratio and skeletal muscle mass. The effect of n-3 PUFA supplementation on sarcopenia is controversial, but randomized controlled trials have been conducted to elucidate the impacts of n-3 PUFAs on sarcopenia in older individuals.[45,46] Although the association between FAs and skeletal muscle mass has been reported in various diseases, the present study is novel as there have been no reports focusing on liver cirrhosis.

There are some limitations in this study. Firstly, this study was a single-center retrospective design. Prospective multicenter studies are needed. Additionally, the subjects of this study were patients aiming for liver cancer treatment, and almost all cases had relatively good liver reserve function. Decreased liver reserve function is a significant risk factor for sarcopenia,[17] but this study did not demonstrate any association between CP score, MELD score, and sarcopenia. The absence of cases with poor liver reserve function is also a limitation of this study. Similarly, the small sample size of patients without HCC and those without cirrhosis is a limitation. Although correlations between the various FA fractions and SMI were similar for patients overall (Supplementary Fig. 4) and for patients with HCC and cirrhosis (Fig. 3), the sample sizes of patients without HCC and cirrhosis were too small to adequately reflect these patient groups. Therefore, in the present study, we limited our analysis to the group of patients with cirrhosis and HCC. A further collection of cases is necessary for thorough examination. Furthermore, we could not assess factors such as daily eating and exercise habits and medications for dyslipidemia and diabetes mellitus. Since these factors, especially daily diet may influence plasma lipid concentrations, a database including these data should be established in the future. The lack of grip strength data is also a limitation. The sarcopenia guidelines of the Japan Society of Hepatology, the Asian Working Group for Sarcopenia, and the European Working Group on Sarcopenia in Older People include grip strength criteria.[15,47,48] These factors should be evaluated in future studies. This study evaluated FAs in total plasma and not solely free FAs, which is a limitation since it is not clear to what extent these FAs are unbound and possess signaling capacity. However, another publication showed that the total plasma FA profile was comparable to the free FA profile,[49] and it is assumed that the

overall plasma FA profile may also account for the physiological activity potential.

Despite these limitations, the strength of this study is that it included a large number of patients with FA profiles and performed detailed analyses such as multivariate analysis and Propensity Score matching. We believe that the present study provides a basis for future prospective studies to determine the effects of adding n-3 PUFAs for clinical application in the nutritional therapy of sarcopenia in patients with liver cirrhosis and HCC.

Conclusion

This retrospective study elucidated that FA levels, especially n-3 PUFAs, were decreased with impaired hepatic reserve, and a low n-3 PUFA level was associated with sarcopenia in patients with liver cirrhosis and HCC. Further prospective and multicenter studies are needed to elucidate whether intervention with n-3 PUFAs can prevent sarcopenia and improve the prognosis and quality of life in patients with HCC and cirrhosis.

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Conflict of interest

The authors have no conflict of interest related to this publication.

Author contributions

Study design (AS), analysis and interpretation of data (AS, JI), acquisition of data (AS, MN, MT, KS, MO, SS, KO), manuscript writing (AS, JI), critical revision (JI, AM), statistical analysis (AS, JI, EK), critical funding (AS, EK), and study supervision (AM). All authors have made significant contributions to this study and have approved the final manuscript.

Ethical statement

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Ethics Committee of Tohoku University Graduate School of Medicine (APPROVAL NUMBER: 2018-1-049, 2021-1-207, 2021-1-540). Informed consent was obtained in the form of an opt-out method.

Data sharing statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author (jinoue@med.tohoku.ac.jp).

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