

Alteration of the serum myostatin level following L-carnitine treatment in patients with chronic liver disease: A pilot study

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Abstract. Muscle atrophy is an independent prognostic factor for patients with cirrhosis. Recently, L-carnitine treatment was reported to increase muscle mass in patients with liver cirrhosis. The aim of the present study was to investigate the effects of L-carnitine treatment on the serum levels of myostatin, a myokine regulating muscle mass, in patients with chronic liver disease. This was a retrospective before-after study. Seven patients with chronic liver disease treated with L-carnitine were enrolled in the present study [mean age, 66 years (39-75); female/male, 6/1; mean treatment period, 248 days (86-956)]. The serum levels of myostatin were measured by ELISA before and after L-carnitine treatment. In a stratification analysis, patients were classified into the high or low FIB-4 index groups according to the cut-off value of 3.25. No significant changes in the serum levels of myostatin were observed after L-carnitine treatment in the whole analysis. However, in the stratification analysis according to the FIB-4 index, the serum myostatin level was significantly decreased after L-carnitine treatment in the high FIB-4 index group (49.7 pg/ml vs. 41.8 pg/ml; $P=0.028$). In addition, L-carnitine treatment significantly increased the serum level of creatine kinase in the high FIB-4 group (89.0 U/l vs. 128 U/l; $P=0.044$). On the whole, the findings of the present study demonstrated that treatment with L-carnitine decreased the serum myostatin level in cirrhotic patients with a high FIB-4 index. It was also demonstrated that L-carnitine treatment increased the serum

level of creatine kinase. These findings suggest that L-carnitine may downregulate myostatin, leading to an improvement in muscle atrophy in patients with liver cirrhosis.

Introduction

Sarcopenia is the loss of muscle strength and muscle mass, which can be caused by various factors, including ageing, inflammation, malnutrition and chronic disease (1,2). Sarcopenia is observed in approximately 30% of patients with chronic liver disease and in approximately 40% of patients with liver cirrhosis (3). Sarcopenia is associated with an increased risk of falls (4) and muscle cramps (5). Sarcopenia is also a risk factor for hepatic encephalopathy (6), decompensated liver cirrhosis (7) and hepatocellular carcinoma (8). In addition, Hanai *et al* reported that sarcopenia impairs the prognosis of patients with liver cirrhosis (9).

One of causative factor for sarcopenia is carnitine deficiency and carnitine deficiency is frequently observed in patients with liver cirrhosis (10). Carnitine transports long-chain fatty acids into the mitochondria, leading to the production of energy through fatty acid oxidation. Carnitine deficiency is involved in the development of sarcopenia in patients with liver cirrhosis (10). On the other hand, L-carnitine treatment has been reported to suppress the loss of skeletal muscle mass in patients with liver cirrhosis (10). Furthermore, in cirrhotic patients, L-carnitine has been reported to improve hyperammonemia, which is associated with the loss of skeletal muscle mass (11). In addition, it has been demonstrated that L-carnitine treatment prevents the loss of skeletal muscle mass (12). Thus, L-carnitine treatment is beneficial for the prevention of sarcopenia in patients with liver cirrhosis. However, the mechanisms through which L-carnitine treatment affects muscle mass remain unclear.

Recently, skeletal muscle has been shown to secrete cytokines and proteoglycan peptides named 'myokines' by muscle contraction (13). Myokines perform various biological functions through the activation of receptors on the liver, bone, fat, brain and heart, as well as in muscle (14). Myostatin, irisin and decorin are myokines regulating muscle mass (15-17). In particular, serum levels of myostatin are known to be significantly elevated in patients with liver cirrhosis compared with

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Abbreviations: CK, creatine kinase; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; AMPK, AMP-activated protein kinase

Key words: L-carnitine, skeletal muscle, myokine, growth differentiation factor-8, liver cirrhosis

healthy controls (18). In addition, higher serum myostatin levels have been shown to be associated with hyperammonemia and muscle atrophy in patients with liver cirrhosis (19,20). Moreover, high serum myostatin levels are associated with a high mortality rate of patients with liver cirrhosis (19,20). However, alterations in the serum levels of myokines following L-carnitine treatment in patients with chronic liver disease have yet to be determined, at least to the best of our knowledge. The aim of the present study was to investigate alterations in the serum levels of myokines following L-carnitine treatment in patients with chronic liver disease.

Patients and methods

Study design. The present study was a retrospective observational study that aimed to investigate alterations in the serum levels of myokines following L-carnitine treatment in patients with chronic liver disease.

Ethics. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in the prior approval granted by the Institutional Review Board of Kurume University. An opt-out approach was used to obtain informed consent from the patients, and personal information was protected during data collection. None of the patients were institutionalized.

Patients. From July, 2017 to January, 2018, 7 patients were enrolled who met following the following inclusion and exclusion criteria. The inclusion criteria were patients with liver cirrhosis who: i) Were ≥ 20 years of age; ii) treatment with L-carnitine for 3 months; and iii) had undergone biochemical examination. The exclusion criteria were patients with: i) Hepatocellular carcinoma; and ii) severe heart, pulmonary, renal, or brain failure.

For stratification analysis, patients were classified into the low FIB-4 index ($n=3$) or high FIB-4 index groups ($n=4$) according to the cut-off value of >3.25 of the FIB-4 index. The cut-off value of the FIB-4 index for advanced fibrosis was based on a previous study (21).

Laboratory determinations. Venous blood samples were drawn before and 12 weeks after treatment of L-carnitine. Red blood cell count, hemoglobin, white blood cell count, lymphocytes, platelet count, prothrombin activity and serum levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total protein, albumin, total bilirubin, total cholesterol, triglycerides, α -fetoprotein, des- γ -carboxy prothrombin, blood urea nitrogen, creatinine, sodium, creatine kinase (CK) and ammonia were measured using standard clinical methods as previously described (9,11). The Child-Pugh score and FIB-4 index were calculated as previously described (22). The estimated glomerular filtration rate (eGFR) was also calculated as previously described (23).

Evaluation of myokines. In the present study, 3 myokines, namely as myostatin, irisin and decorin were evaluated. The serum levels of myostatin, irisin and decorin were evaluated by enzyme-linked immunosorbent assay (ELISA) (Irisin,

recombinant-ELISA kit, EK-067-29, Phoenix Pharmaceuticals Inc.; Decorin, Human Decorin ELISA, ab99998, Abcam; Myostatin, GDF-8/Myostatin Quantikine ELISA kit, DGDF80, Research and Diagnostic Systems Inc.).

Statistical analysis. Data are expressed as the median (interquartile range), range, or number. Changes in variables before and after treatment of L-carnitine were analyzed by Wilcoxon signed rank tests. The correlation between the FIB-4 index and changes in serum myostatin level was analyzed by Spearman's rank correlation analysis. The level of statistical significance was set at $P < 0.05$.

Results

Patient characteristics. The patient characteristics are summarized in Table I. The median age was 66 years, and the ratio of female to male was 6:1. The median body mass index was 24.7 kg/m^2 . Child-Pugh class B was observed in 71.4% of patients. The median level of blood ammonia and FIB-4 index were $79.5 \text{ }\mu\text{g/dl}$ and 5.35, respectively. The median level of serum CK was 94.5 U/l .

Alterations in biochemical examinations before and after L-carnitine treatment. Alterations in biochemical examinations before and after L-carnitine treatment are summarized in Table I. No significant differences were observed in the serum levels of aspartate aminotransferase, and lactate dehydrogenase before and after L-carnitine treatment (Fig. 1A and B). In addition, no significant differences were observed in the blood ammonia level before and after L-carnitine treatment (Fig. 1C). The serum CK level was significantly increased after L-carnitine treatment (Fig. 2).

Alterations in serum levels of myostatin, irisin, and decorin after L-carnitine treatment. Alterations in the serum levels of, irisin, decorin and myostatin after L-carnitine treatment were investigated. No significant differences were observed in the serum levels of irisin, decorin and myostatin before and after L-carnitine treatment (Table II).

Alterations in serum levels of irisin, decorin and myostatin after L-carnitine treatment in the high FIB-4 index group. In the stratification analysis according to the FIB-4 index, patients were classified into the low FIB-4 or high FIB-4 groups based on the cut off value of >3.25 of the FIB-4 index, which corresponds to the F4 stage. No significant differences were observed in the serum levels of irisin and decorin before and after L-carnitine treatment in the high FIB-4 group (Fig. 3A and B). In the high FIB-4 group, the serum level of myostatin was significantly decreased after L-carnitine treatment (Fig. 3C).

Correlation between the FIB-4 index and changes in the serum myostatin level after L-carnitine treatment. The correlation between the FIB-4 index and changes in the serum myostatin level was analyzed by Spearman's rank correlation analysis. A significant negative correlation was observed between the FIB-4 index and changes in the serum myostatin level (Fig. 4).

Table I. Patient characteristics and differences in variables before and after L-carnitine treatment.

Variable	Reference value	L-carnitine treatment				P-value
		Before		After		
		Median (IQR)	Range (min-max)	Median (IQR)	Range (min-max)	
No. of patients	N/A	7	N/A	N/A	N/A	
Age (years)	N/A	66 (39-75)	31-75	N/A	N/A	
Sex (female/male)	N/A	6/1	N/A	N/A	N/A	
Body mass index (kg/m ²)	18.5-24.9	24.7 (21.7-31.7)	18.9-34.2	N/A	N/A	
Child-Pugh class (A/B/C)	N/A	14.3/71.4/14.3% (1/5/1)	N/A			
FIB-4 index	<1.45	5.35 (2.48-6.91)	0.93-11.04			
Performance status	0-4	1 (0-2)	0-3	N/A	N/A	
HCV/NASH/ALD/PBC/Wilson	N/A	2/1/1/2/1	N/A	N/A	N/A	
Biochemical examinations						
Red blood cell count (x10 ⁴ /μl)	435-555	410 (295-474)	257-506	380 (287-462)	246-471	0.85
Hemoglobin (g/dl)	13.7-16.8	13.4 (9.5-13.9)	7.3-13.9	12.6 (9.5-13.8)	9.1-14.8	0.94
White blood cell count (/μl)	3,300-8,600	4,850 (2,775-8,075)	2,100-8,900	4,200 (1,900-8,600)	1,800-13,900	0.58
Platelet count (x10 ³ /mm ³)	3.3-8.6	10.1 (7.7-23.4)	7.0-28.1	24.7 (7.9-24.7)	6.8-25.7	0.47
ALT (IU/l)	10-30	35 (24-114)	12-135	41 (20-131)	16-225	0.63
GGT (IU/l)	13-64	37 (12-70)	12-125	50 (14-86)	14-124	0.27
Total protein (g/dl)	6.6-8.1	6.77 (6.27-6.90)	4.42-7.22	7.23 (49.00-7.58)	4.38-8.18	0.48
Albumin (g/dl)	4.1-5.1	2.95 (2.65-3.99)	2.10-4.28	2.83 (2.22-4.27)	2.08-4.44	0.67
Prothrombin activity (%)	80-120	71 (65-92)	59-104	65 (62-100)	53-115	0.30
Ammonia (μg/dl)	12-66	79.5 (60.0-106.8)	48.0-133.0	120.5 (57-160.5)	39-162	0.60
Total bilirubin (mg/dl)	0.40-1.20	1.09 (0.73-1.40)	0.70-11.54	1.21 (0.68-1.93)	0.45-17.47	0.36
Total cholesterol (mg/dl)	142-219	144 (127-170)	103-242	124 (104-162)	94-243	0.10
Triglyceride (mg/dl)	40-149	115.5 (45.0-258.0)	45.0-258.0	95.5 (39.8-208.8)	36-217	0.34
AFP (ng/ml)	≤7.0	5.0 (0.2-144.3)	0.95-40.425	6.15 (1.6-2539)	3.4-734.5	0.37
Des-γ-carboxy prothrombin (mAU/ml)	<40	20.0 (8.0-57.5)	13.5-44.8	79.3 (9.0-290.0)	17.25-167.8	0.35
BUN (mg/dl)	8.0-20.0	11.8 (8.2-15.1)	1.0-23.6	12.2 (9.1-17.9)	0.7-24.8	0.21
Creatinine (mg/dl)	0.65-1.07	0.50 (0.46-0.63)	0.43-0.65	0.62 (0.55-0.75)	0.55-0.82	0.06
eGFR (ml/min/1.73 m ²)	>90.0	97.1 (72.1-111.5)	66.8-126.3	76.1 (66.4-90.3)	51.2-112.1	0.07
Creatine kinase (U/l)	59-248	94.5 (60.0-117.5)	45-131	123 (101-153)	71-205	0.03

Data are expressed as the median [interquartile range (IQR)], range, or number. Changes in variables before and after treatment with L-carnitine were analyzed by Wilcoxon signed rank tests. N/A, not applicable; HCV, hepatitis C virus; HBV, hepatitis B virus; ASH, alcoholic liver disease; NASH, non-alcoholic liver disease; PBC, primary biliary cholangitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; AFP, alpha-fetoprotein; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

Discussion

The present study investigated the association between L-carnitine and the serum levels of myostatin, irisin and decorin in patients with chronic liver disease. However, no significant differences in the serum levels of myostatin, irisin and decorin were observed before and after L-carnitine treatment in patients with chronic liver disease. A stratification analysis was also performed according to the FIB-4 index and it was found that the serum myostatin levels were decreased after L-carnitine treatment in patients with a high FIB-4 index.

Thus, L-carnitine treatment may suppress the serum myostatin level in patients with liver cirrhosis.

Blood ammonia levels did differ significantly after L-carnitine treatment in the present study. L-carnitine is known to enhance ammonia detoxification in the urea cycle by increasing *N*-acetylglutamate, leading to a decrease in blood ammonia levels in patients with liver cirrhosis (24). Abbasnezhad *et al* performed a meta-analysis on 799 patients from 9 randomized clinical trials and demonstrated that L-carnitine treatment significantly reduced the blood ammonia level (25). Thus, the results of the present study

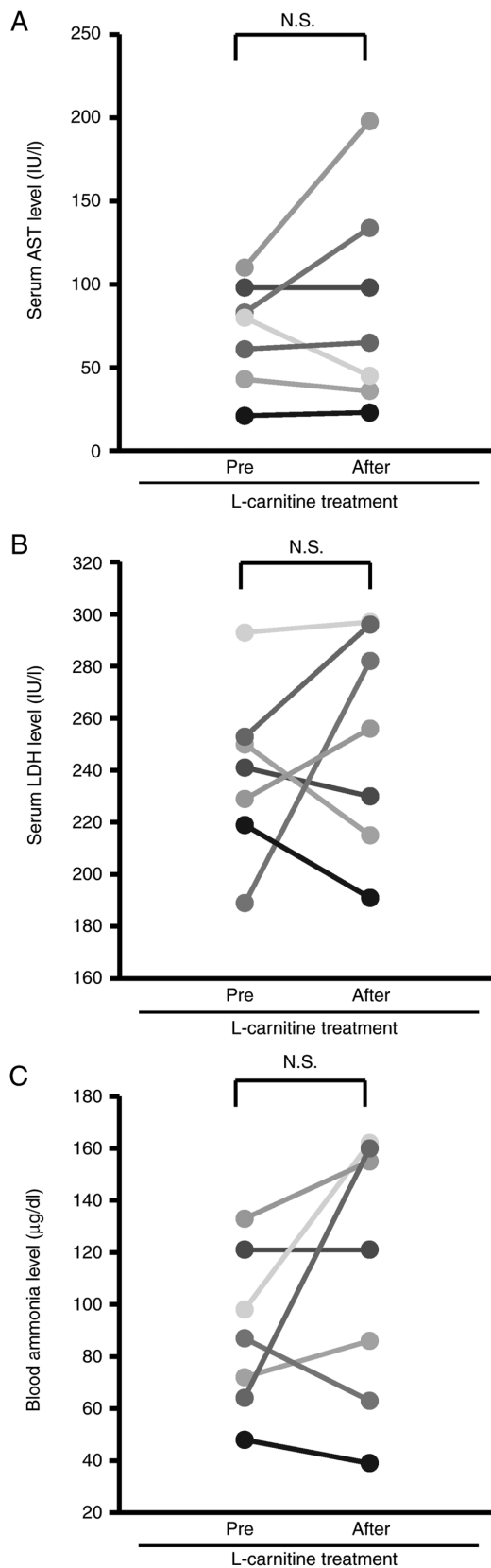


Figure 1. Alterations in biochemical examinations before and after L-carnitine treatment (A) serum aspartate aminotransferase level, (B) lactate dehydrogenase level, and (C) blood ammonia level. N.S., not significant; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

differ from those of that previous study. Although the reason for this discrepancy remains unclear, the small sample size

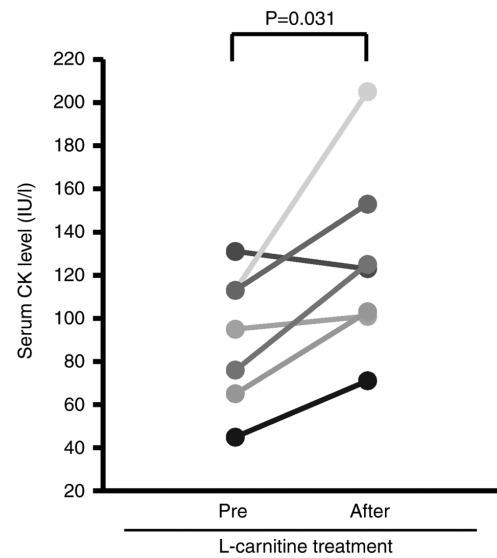


Figure 2. Alterations in the serum CK level before and after L-carnitine treatment. CK, creatine kinase.

may be a possible reason. Another possible reason may be renal dysfunction. The kidneys are involved in ammonia metabolism by removing a significant amount of ammonia in urine in cirrhotic patients with hyperammonemia (26). The meta-analysis by Abbasnezhad *et al* demonstrated an improvement of renal function by L-carnitine treatment (25). However, in the present study, the tendency of an increase in the serum creatinine level and a decrease in eGFR was observed during the study period. Thus, an impairment of removing ammonia in the urine is a possible reason for the blood ammonia level exhibiting no change in the present study.

The serum CK level was significantly increased by L-carnitine treatment in the present study. However, the serum aspartate aminotransferase and lactate dehydrogenase levels were not significantly increased by L-carnitine treatment, indicating that the increase in the serum CK level was not due to muscle damage. L-carnitine is known to improve fatigue and muscle cramps (27,28), which can result in an increase in physical activity, and the serum CK level is known as a marker for physical activity (29). In addition, CK is an enzyme, which catalyzes the reversible phosphorylation of creatine to phosphocreatine and of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (30). The expression of CK is known to be increased during muscle hypertrophy in order to adapt to energy demands (30). Although the present study did not evaluate the effect of L-carnitine on muscle mass, L-carnitine has been reported to cause muscle hypertrophy (10,11). Thus, an increase in the serum CK level may be a response to an increase in physical activity and/or muscle hypertrophy in the present study.

L-carnitine has been reported to cause muscle hypertrophy even in patients with no improvement of hyperammonemia (10). The present study examined the effects L-carnitine treatment on serum myokine levels, which are associated with muscle hypertrophy. It was found that the serum level of myostatin, but not that of irisin and decorin, was decreased 12 weeks after L-carnitine treatment in patients with a high FIB-4 index. In addition, there was a significant negative

Table II. Changes in serum levels of myokines before and after L-carnitine treatment.

Myokine	L-carnitine treatment				P-value
	Before		After		
	Median (IQR)	Range (min-max)	Median (IQR)	Range (min-max)	
Irisin (ng/ml)	5.87 (5.23-6.71)	4.41-9.43	5.18 (4.77-7.52)	4.58-9.49	0.61
Decorin (pg/ml)	7479 (6031-15037)	2977-26523	9642 (5756-14080)	2454-32901	0.41
Myostatin (pg/ml)	68.56 (49.42-86.87)	49.15-98.87	68.55 (41.73-86.87)	38.94-96.90	0.86

Data are expressed as the median [interquartile range (IQR)], range, or number. Changes in variables before and after treatment with L-carnitine were analyzed by Wilcoxon signed rank tests.

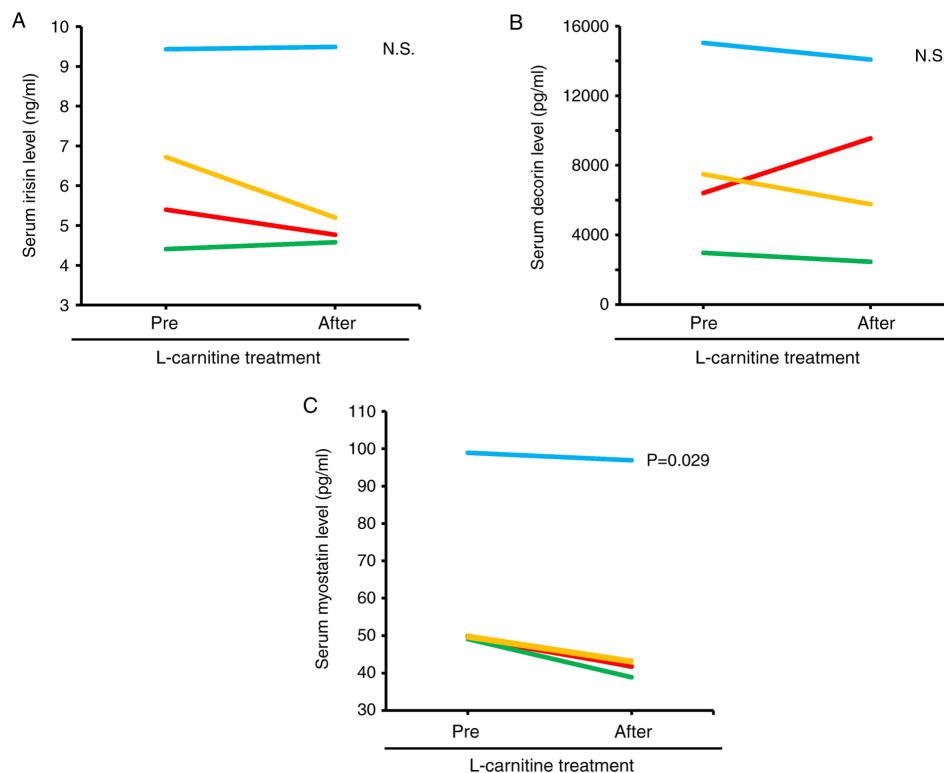


Figure 3. Alterations in the serum levels of irisin, decorin and myostatin after L-carnitine in the high FIB-4 index group. (A) Serum irisin level, (B) serum decorin level, and (C) serum myostatin level. N.S., not significant.

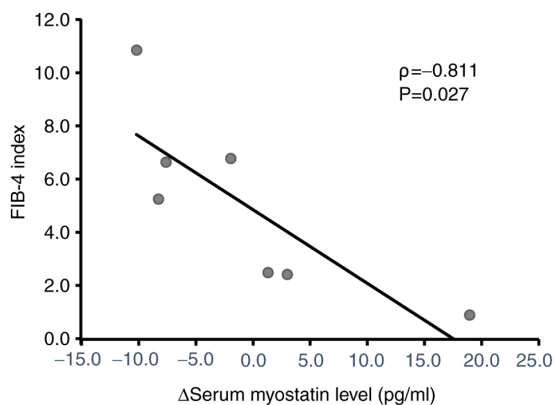


Figure 4. Correlation between the FIB-4 index and changes in the serum myostatin level after L-carnitine treatment.

correlation between the FIB-4 index and the changes in the myostatin level after L-carnitine treatment. Since myostatin is a negative regulator of muscle hypertrophy (17), L-carnitine may suppress myostatin, leading to muscle hypertrophy. The mechanisms responsible for the L-carnitine-induced suppression of myostatin remain unclear; the expression of myostatin is regulated by AMP-activated protein kinase (AMPK) (31). L-carnitine transports long-chain fatty acids from the cytosol to the mitochondrial matrix, leading to an increase in ATP production through β -oxidation (32). An increase in intracellular ATP level is known to downregulate AMPK (33). Thus, L-carnitine may suppress myostatin expression via the suppression of AMPK.

There are several limitations to the present study. First, this was a retrospective pilot study with a small sample size,

which conducted in a single center. Second, the etiology and severity of liver cirrhosis vary, suggesting the heterogeneity of the enrolled subjects. Third, no information was available for dietary habits, as L-carnitine is an abundant nutrient found in red meat (34). Thus, a multi-center randomized controlled trial with information on dietary habits is required to prove the effects of L-carnitine on the serum myostatin level.

In conclusion, the present study investigated an association between L-carnitine treatment and the serum levels of myostatin, irisin and decorin in patients with chronic liver disease. No significant differences were observed in the serum levels of these 3 myokines. However, it was found that the serum level of myostatin, but not that of irisin and decorin, was decreased 12 weeks after L-carnitine treatment in patients with a high FIB-4 index. Thus, L-carnitine may downregulate myostatin, leading to improved muscle atrophy in patients with liver cirrhosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

DN and TK participated in the study conception and design, data acquisition and interpretation, and manuscript drafting. TTs, SY, KS and RH participated in data acquisition and interpretation. TTs, SY, KS, HK and TTo participated in the analysis and interpretation of data. HK and TTo participated in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, as reflected in the prior approval given by the institutional review board of Kurume University. An opt-out approach was used to obtain informed consent from patients, and personal information was protected during data collection. None of the patients were institutionalized.

Patient consent for publication

Not applicable.

Competing interests

TK discloses honoraria (lecture fees) from Mitsubishi Tanabe Pharma Corporation, MSD K.K, and Otsuka Pharmaceutical Co., Ltd. The other authors disclose no competing interests.

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