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L-carnitine supplementation vs cycle ergometer exercise for physical activity and muscle status in hemodialysis patients: A randomized clinical trial

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Abstract

Serum carnitine is decreased in hemodialysis patients, which induces muscle atrophy. Thus, we examined the different effects of L-carnitine and exercise on exercise activity and muscle status in hemodialysis patients. Twenty patients were divided into L-carnitine and cycle ergometer groups and were followed for 3 months. Muscle and fat mass, physical activities, and muscle status were evaluated by an impedance, physical function test, and magnetic resonance imaging, respectively. The L-carnitine significantly increased muscle mass ($P = .023$) and thigh circumference ($P = .027$), decreased fat mass ($P = .007$), and shortened chair stand-up time ($P = .002$) and 10-m walk test ($P = .037$). The fat fraction was improved by the L-carnitine ($P = .047$). Compared with the exercise group, L-carnitine improved the changes in 10-m walk test ($P = .026$), chair stand-up time ($P = .014$), and thigh circumference ($P = .022$). Baseline fibroblast growth factor-21 and myostatin levels predicted the L-carnitine-associated changes in exercise activities. L-carnitine, rather than exercise, improved physical activity and muscle status in hemodialysis patients.

KEYWORDS

L-carnitine, exercise, hemodialysis, myokine

1 | INTRODUCTION

A decrease in exercise activity and an impairment in exercise capacity are associated with derangements in activities of daily living and the quality of life (QOL) of hemodialysis (HD) patients.[1] Low physical activity increases the risk of cardiovascular disease (CVD) and is linked to all-cause and CVD mortality in patients with chronic kidney disease.[2] Further, low exercise activity induces mental disorders, such as depression.[3] Therefore,

improving physical activity is a crucial therapeutic strategy for HD patients.

Carnitine is a natural substance, which plays a pivotal role in fatty acid β-oxidation and energy production by transporting long-chain fatty acids from the cytoplasm to the mitochondria.[4] A decrease in circulating carnitine levels is associated with muscle carnitine deficiency,[5] suggesting that circulating carnitine levels could predict muscle carnitine content. We recently reported that serum carnitine levels are significantly decreased in HD patients due to the elimination of serum carnitine from the blood via HD.[6,7] Further, carnitine depletion has been associated with decreased soleus muscle weight in a rat model of carnitine deficiency.[8] Accordingly, carnitine deficiency by HD may be one of the causative factors for the progression of sarcopenia in end-stage renal disease patients. Since L-carnitine (LC) supplementation ameliorates slow-twitch skeletal muscle fiber atrophy in HD patients,[9] LC treatment may yield protective effects on muscle weakness and atrophy in these patients.

Recently, cycle ergometer (Ergo) exercise during HD sessions has focused on the prevention of sarcopenia.[10] However, the efficacy of Ergo exercise during HD session is not well established and it is sometimes difficult to achieve adequate exercise capacity in HD patients. Further, whether LC or Ergo treatment could improve the physical activity and muscle quality of HD patients remains unclear. Herein, we prospectively examined the efficacy of LC and Ergo exercise treatments by measuring several myokines in HD patients.

2 | PATIENTS AND METHODS

2.1 | Patients and study protocol

This single-center, open label, parallel-group study conducted at Kurume University Hospital recruited a total of 37 HD patients from November 2015 to June 2016. We could not determine a large sample size because of the single-center study with stable HD patients. Patients over 20 years of age with end-stage renal disease undergoing HD able to provide written informed consent for study participation were enrolled in this study. Exclusion criteria included being under 20 years of age; no carnitine deficiency, defined as having both free carnitine levels >36 μmoL/L, and an acyl/free (acylcarnitine free carnitine ratio) ratio < 0.4; contraindications for levocarnitine; pregnant women, or those possibly pregnant; patients deemed inadequate by a physician; or those suffering from symptomatic CVD or musculoskeletal disorders interfering with exercise training. Four patients had heart disease (one pacemaker implantation, two CVD, one cyanosis renal disease due to tricuspid atresia), one had hepatocellular carcinoma, three had orthopedic problems (two osteoarthritis of the knee, one toe amputation), seven could not participate in daytime research, one transferred to another clinic, and one supplemented diet medicine including LC (Figure 1). The remaining 20 patients (mean age: 55.5 ± 13.8 years old; mean duration of HD: 144 ± 84 months) were finally included and randomly assigned using simple randomization procedures (computer-generated list of random numbers) to either an LC (n = 10) or Ergo group (n = 10) by Junko Yano, and the allocation was concealed by finishing the randomization (Figure 1). The study was prospectively followed up for 3 months. At baseline and after 3 months of treatment, patients provided a complete history and underwent physical examination and blood chemistries just before the HD session. Patients were dialyzed for 4 to 5 hours with high-flux dialyzers three times a week. LC-treated patients received 1000 mg of LC intravenously just after the HD session. The remaining patients engaged in Ergo exercise using variable-load ergometer exercise equipment (TE2-70; Showa Denki, Osaka, Japan) under the guidance of the same physical therapists for 20 minutes every HD session for the first 2 hours of dialysis, with the intensity set at the 40% to 55% of the maximal work capacity as recommended for chronic kidney disease patients.[11] The primary endpoint was the comparable efficacy between Ergo and LC treatment on exercise capacity. Additional analyses were done on the changes of myokine levels before and after the treatment. Informed consent was obtained from all patients as specified in the International Committee of Medical Journal Editors Recommendations, and the study protocol was approved by the institutional ethics committees of Kurume University School of Medicine (Approval Number; 13282). This work was conducted in accordance with the Declaration of Helsinki and was registered with the University Hospital Medical Information Network clinical trials database (UMIN000033833).

2.2 | Data collection

The patients' medical histories were ascertained by a questionnaire. Vigorous physical activity and smoking were avoided for at least 30 minutes before the measurement of the exercise capacity and the HD session. Blood was drawn from an arteriovenous shunt just before starting the HD sessions to determine hemoglobin, serum albumin, blood urea nitrogen, creatinine (Cr), uric acid, calcium, phosphate, lipids (high- and low-density lipoprotein cholesterol, and triglycerides), and C-reactive protein; values were analyzed at commercially available

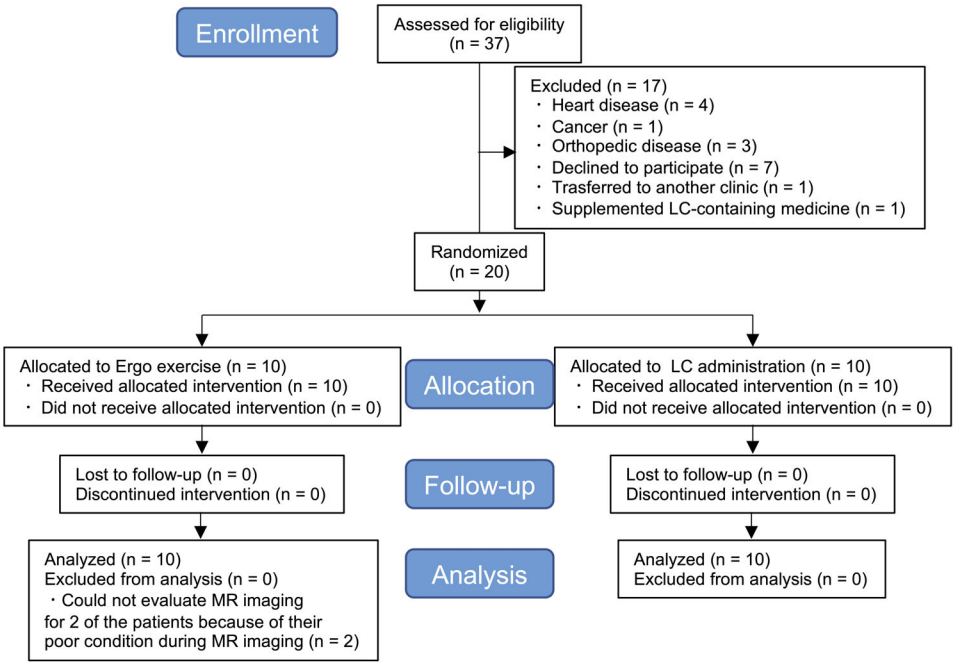

FIGURE 1 Trial flow diagram. Ergo, ergometer exercise, LC, L-carnitine, MR, magnetic resonance [Color figure can be viewed at wileyonlinelibrary.com]

laboratories (Daiichi Pure Chemicals, Tokyo, Japan and Wako Pure Chemical Industries, Osaka, Japan). Serum carnitine fraction levels were determined as described previously.[12] Serum interleukin-6 (R&D Systems, Minneapolis, MN, USA), fibroblast growth factor-21 (FGF-21) (R&D Systems), myostatin (Immundiagnostik AG, Bensheim, Germany), and decorin (Abcam plc, Cambridge, UK) were determined by enzyme-linked immunosorbent assay according to the manufacturer's instruction. Changes of all data both before and after treatment were calculated using the following formula: (post data − pre data)/pre data × 100 (%).

2.3 | Evaluation of physical activities, muscle mass, and fat mass composition

Physical activity was evaluated via the functional reach (FR) test, the 10-m walk test (10mWT), thigh circumferences at a position of 10 cm above the knee (Thigh Cir), the time-up-and-go (TUG) test, the hand grip (HG) test, the 10 times chair stand-up (CS) test, and the Borg scale as described previously.[13] The total body muscle and fat mass were estimated by the bioelectrical impedance analysis (BIA) (Inbody 720; Biospace, Tokyo, Japan), a commonly used noninvasive method for estimating body composition. All exercise capacities as well as muscle and fat mass were independently measured once before treatments and once after 3 months of treatment at a day between dialysis session by the same expert physical therapists at the Division of Rehabilitation, Kurume University Hospital.

2.4 | MR imaging techniques and analysis

Magnetic resonance (MR) imaging was performed at a field strength of 3.0 T (Discovery MR750W; GE Medical Systems, Milwaukee, WI, USA) with two radiofrequency coils in combination (GEM 16-element anterior array and GEM 40-element posterior array, IL, GE Healthcare). The proton density fat fraction (PFF) image was evaluated by fat fraction mapping, which was obtained from the iterative decomposition of water and fat with echo asymmetry and least-squares estimation quantitation (IDEAL-IQ) sequence. Imaging parameters of the axial IDEAL-IQ sequence were as follows: TR, 8.2 ms; minimum TE, 1.0 ms; flip angle, 4°; echo train length, 3; slice thickness, 8 mm; FOV, 360 mm × 288 mm; matrix, 160 × 160; scan time, 22 seconds; and NEX, 0.5 times. The IDEAL-IQ images were analyzed using an imaging workstation (READY View; GE Healthcare). The PFF image was performed before and after the exercise or LC treatment. The whole area, the muscle area, and the intramuscular fat content were measured on a cross section of the femoral region 10 cm above the knee. The muscle area was defined as the area excluding the subcutaneous fat, femoral bone, and neurovascular bundle from the whole area (Figure 2A). For the measurements of the intramuscular fat content, three separate regions of interest (ROIs) were placed in the vastus medialis muscle, the vastus lateralis muscle, and the long head of biceps femoris muscle (each ROI area sampled was 100 mm^2) on the PFF image (Figure 2B). The intramuscular fat content was recorded as the mean values

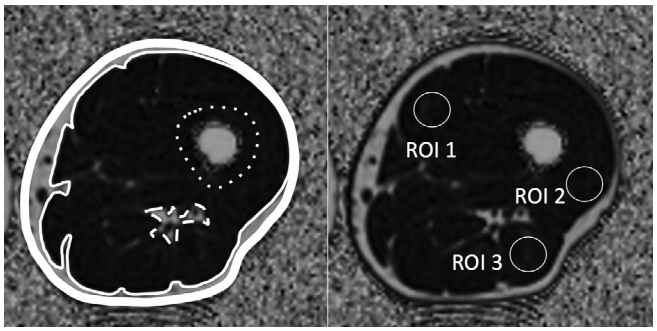

FIGURE 2 Representative MR imaging for evaluating muscle area, fat area, and fat fraction in the thigh muscle of HD patients. A, Muscle area excluding the subcutaneous fat, the femoral bone, and the neurovascular bundle from the whole area. B, For the measurements of intramuscular fat content, three separate regions of interest are placed in the vastus medialis muscle, the vastus lateralis muscle, and the long head of biceps femoris muscle. MR, magnetic resonance; HD, hemodialysis

generated from the three measurements; we could not evaluate the MR images of two of the patients in the Ergo group due to their poor condition during imaging. All MR imaging analyses were made by the consensus of two experienced board-certified radiologists (T.T., with 18 years of experience in abdominal imaging, and S.N., with 15 years of experience in musculoskeletal imaging).

2.5 | Statistical analysis

We could analyze all datasets in 20 participants except for PFF (n = 18). Almost all of the datasets were small and not normally distributed; thus, nonparametric analyses were performed. Wilcoxon-Mann-Whitney was used to compare Ergo and LC groups. To compare the clinical variables before and after the treatments, the Wilcoxon signed-rank test was used. For exploratory data analysis, Spearman's rank correlation coefficient was obtained to determine the relation between changes in exercise capacity and baseline myokines. Data are presented as mean ± SD. Statistical significance was defined as $P < .05$. All statistical analyses were performed using JMP Pro ver.14 Software (SAS Institute Inc., NC).

3 | RESULTS

3.1 | Demographic data at baseline

All patients in this study completed the treatment (LC: n = 10, Ergo: n = 10) (Figure 1). Baseline free carnitine levels were below 36 μmoL/L, while acyl/free ratio was above 0.4 in all patients, suggesting that all patients were carnitine deficient. There were no significant differences in the baseline data between the two groups, including metabolic and anthropometric variables (Table 1). TUG was shorter in LC-treated patients compared with that in Ergo exercise patients (6.67 ± 1.27 vs 7.90 ± 0.86, $P = .017$) (Table 1).

3.2 | Effects of Ergo exercise or LC administration on clinical variables, physical activities, and muscle and fat composition

Total carnitine, free carnitine, acylcarnitine, and triglyceride levels were significantly increased by LC administration ($P = .002$, $P = .002$, $P = .002$, $P = .010$, respectively), whereas serum Cr levels and acyl/free ratio were significantly decreased ($P = .006$, $P = .010$, respectively) (Table 2); in contrast, Ergo exercise significantly increased acyl/free ratio ($P = .025$) (Table 2).

LC administration significantly improved the 10mWT ($P = .037$), Thigh Cir ($P = .027$), and CS test ($P = .002$), whereas Ergo exercise did not (Table 3). LC significantly increased the whole muscle mass ($P = .023$) and decreased the fat mass ($P = .007$), both of which were unaffected by Ergo exercise. The muscle area in the thigh by MR imaging tended to be increased by Ergo exercise ($P = .055$), but not by the LC treatment. However, the fat fraction was significantly decreased ($P = .047$) by the LC treatment evaluated by MR imaging (Table 3). Ergo exercise did not have any effect on physical activities or muscle and fat mass composition (Table 3).

3.3 | Relationship between changes in carnitine fraction levels and exercise activities in HD patients

Changes in free carnitine were associated with changes in 10mWT ($\rho = -0.498$, $P = .026$) and CS test ($\rho = -0.590$, $P = .006$) (Table 4). Changes in acyl/free ratio were associated with those in the Thigh Cir ($\rho = -0.508$, $P = .022$) and CS test ($\rho = 0.556$, $P = .011$) (Table 4).

3.4 | Comparison of the changes in physical activities and muscle and fat composition between Ergo exercise and LC supplementation

Physical activities, such as the 10mWT and the CS test, significantly improved in LC-treated patients compared with Ergo-treated patients ($P = .026$, $P = .014$,

TABLE 1 Clinical characteristics of the patients

Variables	Ergo group	LC group	*P*
Patients (n)	10	10	
Age (years old)	53.9 ± 16.5	57.1 ± 11.1	.520
Sex (n) (male/female)	6/4	4/6	
HD duration[a] (months) (range)	131 (12-256)	157 (8-215)	.623
Body mass index (kg/m^2)	22.8 ± 4.4	22.3 ± 3.14	.970
Hemoglobin (g/dL)	11.7 ± 1.2	12.1 ± 1.6	.596
Serum albumin (g/dL)	3.70 ± 0.28	3.55 ± 0.23	.343
BUN (mg/dL)	60.4 ± 10.9	66.5 ± 7.7	.257
Serum Cr (mg/dL)	11.4 ± 2.0	12.1 ± 1.9	.520
Uric acid (mg/dL)	8.19 ± 1.09	7.87 ± 1.31	.623
Corrected ca (mg/dL)	8.88 ± 0.81	8.96 ± 0.67	.970
Phosphate (mg/dL)	5.44 ± 1.95	5.29 ± 0.75	.470
LDL-cholesterol (mg/dL)	87.2 ± 40.8	98.1 ± 23.6	.273
HDL-cholesterol (mg/dL)	48.6 ± 16.2	52.6 ± 13.5	.623
Triglycerides[a] (mg/dL) (range)	156 (54-501)	115 (42-308)	.791
CRP (mg/dL)	0.11 ± 0.14	0.26 ± 0.29	.226
Total carnitine (μmol/L)	38.1 ± 6.4	41.7 ± 10.1	.520
Free carnitine (μmol/L)	22.4 ± 3.9	24.3 ± 6.4	.705
Acylcarnitine (μmol/L)	15.7 ± 4.0	17.4 ± 4.1	.344
Acy/free ratio	0.71 ± 0.19	0.73 ± 0.12	.705
BIA			
Muscle mass (kg)	22.4 ± 4.1	22.1 ± 5.3	.791
Fat mass (kg)	13.7 ± 7.2	15.2 ± 6.6	.473
Physical activities			
FR test (cm)	29.6 ± 5.2	35.2 ± 5.8	.064
10mWT (sec)	8.54 ± 0.86	7.78 ± 0.72	.064
Thigh Cir (cm)	41.9 ± 3.6	39.7 ± 3.1	.307
TUG (sec)	**7.90 ± 0.86**	**6.67 ± 1.27**	**.017**
HG test (kg)	19.9 ± 5.3	24.8 ± 9.8	.384
CS (sec)	26.8 ± 8.7	22.5 ± 8.4	.384
Borg scale	7.8 ± 1.9	7.8 ± 1.9	.999
MRI			
Whole area (cm^2)	134 ± 29	118 ± 23	.198
Muscle area (cm^2)	70.8 ± 17.5	63.7 ± 13.6	.351
Fat fraction (%)	2.5 ± 0.7	2.7 ± 1.2	.858
Diabetes (n) (−/+)	8/2	7/3	.651

Notes: Values are shown as mean ± SD or range. Bold values are expressed as a statistically significance.

Abbreviations: Acy/Free ratio, acylcarnitine free carnitine ratio; BIA, bioelectrical impedance analysis; BUN, blood urea nitrogen; Cr, creatinine; Ca, calcium; CRP, C-reactive protein; CS, chair stand; FR test, functional reach test; HD, hemodialysis; HG, hand grip; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance image; No., number; 10mWT, 10-m walk test; Thigh Cir, thigh circumferences; TUG, time-up-and-go.

[a]These variables are shown in the original scale after using log-transformed values.

TABLE 2 Effects of Ergo exercise or LC supplementation on clinical variables in HD patients

	Ergo group			LC group		
Variables	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***
Hemoglobin (g/dL)	11.7 ± 1.2	11.7 ± 0.6	.984	12.1 ± 1.6	11.6 ± 1.4	.289
Serum albumin (g/dL)	3.70 ± 0.28	3.67 ± 0.26	.391	3.55 ± 0.23	3.62 ± 0.16	.344
BUN (mg/dL)	60.4 ± 10.9	62.0 ± 13.6	.770	65.5 ± 7.7	67.3 ± 8.6	.625
Serum Cr (mg/dL)	11.4 ± 2.0	10.8 ± 1.9	.068	**12.1 ± 1.9**	**11.5 ± 1.6**	**.006**
Uric acid (mg/dL)	8.19 ± 1.09	8.16 ± 1.16	1.000	7.87 ± 1.31	7.56 ± 1.20	.344
Corrected ca (mg/dL)	8.88 ± 0.81	8.83 ± 0.69	.445	8.96 ± 0.67	8.77 ± 0.42	.598
Phosphate (mg/dL)	5.44 ± 1.95	5.38 ± 1.90	.770	5.29 ± 0.75	5.39 ± 1.04	.676
LDL-cholesterol (mg/dl)	87.2 ± 40.8	91.5 ± 28.9	.820	98.1 ± 23.6	90.8 ± 32.0	.131
HDL-cholesterol (mg/dL)	48.6 ± 16.2	50.1 ± 16.6	.557	52.6 ± 13.5	52.2 ± 11.7	1.000
Triglycerides[a] (mg/dL)	156 (54-501)	105 (39-508)	.492	**115 (42-308)**	**131 (67-293)**	**.010**
CRP (mg/dL)	0.11 ± 0.14	0.17 ± 0.26	.625	0.26 ± 0.29	0.15 ± 0.17	.063
Total carnitine (μmol/L)	38.1 ± 6.4	36.1 ± 6.4	.188	**41.7 ± 10.1**	**420.7 ± 51.8**	**.002**
Free carnitine (μmol/L)	22.4 ± 3.9	20.2 ± 3.6	.131	**24.3 ± 6.4**	**257.5 ± 29.6**	**.002**
Acylcarnitine (μmol/L)	15.7 ± 4.0	15.9 ± 3.9	.577	**17.4 ± 4.1**	**163.2 ± 25.0**	**.002**
Acyl/free ratio	**0.71 ± 0.19**	**0.80 ± 0.18**	**.025**	**0.73 ± 0.12**	**0.63 ± 0.06**	**.010**

Notes: Values are shown as mean ± SD or range. Bold values are expressed as a statistically significance.
Abbreviations: Acyl/Free ratio, acylcarnitine free carnitine ratio; BUN, blood urea nitrogen; Cr, creatinine; Ca, calcium; CRP, C-reactive protein; Ergo, ergometer; HD, hemodialysis; HDL, high-density lipoprotein; LC, L-carnitine; LDL, low-density lipoprotein.
[a]This variable is shown in the original scale after using log-transformed values.

TABLE 3 Effects of Ergo exercise or LC administration on exercise activities and muscle status in HD patients

	Ergo group			LC group		
Variables	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***
Physical activity						
FR test (cm)	29.6 ± 5.2	29.5 ± 8.7	.945	35.2 ± 5.8	37.4 ± 6.6	.125
10mWT (sec)	8.54 ± 0.86	9.07 ± 1.74	.432	**7.78 ± 0.72**	**7.36 ± 0.97**	**.037**
Thigh Cir (cm)	41.9 ± 3.6	41.7 ± 3.5	.633	**39.7 ± 3.1**	**40.7 ± 3.0**	**.027**
TUG (sec)	7.90 ± 0.85	7.86 ± 1.16	.492	6.67 ± 1.27	6.67 ± 1.58	.828
HG test (kg)	19.9 ± 5.3	20.4 ± 4.3	.930	24.8 ± 9.8	24.0 ± 10.7	.740
CS test (sec)	26.8 ± 8.7	25.7 ± 7.5	.922	**22.5 ± 8.4**	**17.1 ± 5.9**	**.002**
Borg scale	7.8 ± 1.9	7.4 ± 1.3	.500	7.8 ± 1.9	7.4 ± 0.8	1.000
BIA						
Muscle mass (kg)	22.4 ± 4.1	22.6 ± 4.8	.664	**22.1 ± 5.3**	**22.8 ± 5.5**	**.023**
Fat mass (kg)	13.7 ± 7.2	14.8 ± 9.5	.707	**15.2 ± 6.6**	**14.1 ± 6.5**	**.007**
MRI						
Whole area (cm^2)	133 ± 27	143 ± 32	.109	118 ± 23	119 ± 21	.275
Muscle area (cm^2)	70.8 ± 17.5	74.9 ± 17.6	.055	63.7 ± 13.6	65.6 ± 14.1	.322
Fat fraction (%)	2.5 ± 0.7	2.4 ± 0.9	.719	**2.7 ± 1.2**	**2.4 ± 1.2**	**.047**

Notes: Values are shown as mean ± SD. Bold values are expressed as a statistically significance.
Abbreviations: BIA, bioelectrical impedance analysis; CS, chair stand; Ergo, ergometer; FR, functional reach; HD, hemodialysis; HG, hand grip; LC, L-carnitine; MRI, magnetic resonance imaging; 10mWT, 10-m walk test; Thigh Cir, thigh circumferences; TUG, time-up-and-go.

TABLE 4 Relationship between changes in serum carnitine fractions and exercise activities before and after the treatments in HD patients

	ΔFR test	Δ10mWT	Δthigh Cir	Δtug	ΔHG test	ΔCS test	ΔBorg scale
ΔTotal carnitine	0.134 (0.574)	−0.361 (0.118)	−0.002 (0.995)	−0.289 (0.217)	−0.150 (0.529)	−0.221 (0.349)	0.300 (0.199)
Δ Free carnitine	0.129 (0.587)	**−0.498 (0.026)**	0.350 (0.131)	−0.218 (0.356)	−0.017 (0.622)	**−0.590 (0.006)**	0.278 (0.236)
Δ Acylcarnitine	0.020 (0.935)	−0.164 (0.490)	−0.246 (0.300)	−0.194 (0.413)	−0.105 (0.661)	−0.071 (0.767)	0.237 (0.315)
Δ Acyl/free ratio	−0.035 (0.885)	0.376 (0.102)	**−0.508 (0.022)**	0.032 (0.895)	0.118 (0.620)	**0.556 (0.011)**	−0.072 (0.762)

Note: Bold values are expressed as a statistically significance.
Abbreviations: Acyl/Free ratio, acylcarnitine free carnitine ratio; CS, chair stand; HG, hand grip; HD, hemodialysis; FR, functional reach; 10mWT, 10-m walk test; Thigh Cir, thigh circumferences; TUG, time-up-and-go.

respectively) (Table 5). There was no significant difference regarding the other physical activities, muscle and fat composition, or muscle status, including fat fraction between the two groups (Table 5). These findings suggest that LC treatment, rather than Ergo exercise, may be more effective for improving exercise activity in HD patients.

3.5 | Relationship between baseline myokines and exercise activities in LC-treated patients

There was no significant difference of myokines before and after the LC treatment (data not shown). However, baseline FGF-21 was positively associated with changes in Thigh Cir ($\rho = 0.673$, $P = .033$) (Table 6). Baseline myostatin was positively associated with the changes in the FR test ($\rho = -0.636$, $P = .048$) and inversely associated with the changes in the 10mWT ($\rho = -0.733$, $P = .016$) (Table 6). There was no side effect related to the Ergo exercise and LC administration during the study period.

4 | DISCUSSION

In this study, we demonstrated that (a) LC administration significantly improves physical activities, such as the 10mWT, CS test, and Thigh Cir; (b) LC administration, rather than Ergo exercise, increases muscle mass and decreases fat mass and fraction; (c) changes in serum carnitine fractions before and after the treatments correlated with the changes in the 10mWT, CS test, and Thigh Cir, and these exercise activities were significantly improved by the LC treatment compared with Ergo exercise; and (d) although neither treatment affected serum myokine levels, baseline FGF-21 and myostatin levels, known as markers of insulin resistance, were associated with changes in the FR test, 10mWT, and Thigh Cir. To our knowledge, this is the first report to demonstrate the beneficial efficacy of LC administration on exercise activities, muscle mass, and muscle status in HD patients.

TABLE 5 Compared effects of ergo exercise or LC supplementation on changes in exercise activities and muscle status in HD patients

Variables	Ergo group	LC group	*P*
Physical activity (%)			
ΔFR test	−1.48 ± 16.4	6.65 ± 12.5	.345
Δ10mWT	**6.16 ± 15.9**	**−5.54 ± 6.65**	**.026**
ΔThigh Cir	−0.44 ± 4.07	2.65 ± 3.21	.096
ΔTUG	−0.44 ± 9.96	−0.31 ± 9.04	.910
ΔHG test	6.93 ± 23.84	−3.50 ± 13.00	.650
ΔCS test	**−2.16 ± 14.5**	**−22.8 ± 14.6**	**.014**
ΔBorg scale	−3.76 ± 8.09	−1.12 ± 21.72	.576
BIA (%)			
ΔMuscle mass	0.26 ± 3.16	3.17 ± 3.77	.112
ΔFat mass	9.64 ± 33.96	−8.50 ± 6.32	.212
MRI (%)			
ΔWhole area	7.59 ± 10.74	1.53 ± 5.80	.307
ΔMuscle area	6.43 ± 8.45	3.29 ± 6.57	.505
ΔFat fraction	−3.52 ± 24.29	−13.39 ± 16.99	.562

Notes: Values are shown as mean ± SD. Bold values are expressed as a statistically significance.
Abbreviations: BIA, bioelectrical impedance analysis; CS, chair stand; Ergo, ergometer; HD, hemodialysis; LC, L-carnitine; FR, functional reach; MRI, magnetic resonance imaging; 10mWT, 10-m walk test; Thigh Cir, thigh circumferences; TUG, time-up-and-go.

Long-chain free fatty acids in the carnitine shuttle play a central role in energy production via β-oxidation followed by activation of the TCA cycle in the mitochondria[14]; thus, carnitine deficiency in myocytes induces muscle weakness and atrophy. We recently found that decreased free carnitine levels were associated with the impairment of exercise activities, such as the TUG test,

TABLE 6 Relationship between baseline myokines and changes in exercise activities in LC-treated HD patients

	ΔFR test	Δ10mWT	Δthigh Cir	Δtug	ΔHG test	ΔCS test	ΔBorg scale
IL-6 (pg/mL)	−0.103 (0.777)	0.006 (0.987)	0.273 (0.446)	0.122 (0.738)	−0.474 (0.166)	0.042 (0.907)	0.356 (0.312)
FGF-21 (pg/mL)	0.588 (0.074)	0.164 (0.652)	**0.673 (0.033)**	0.146 (0.688)	−0.037 (0.920)	0.527 (0.117)	0.096 (0.792)
Myostatin (ng/mL)	**0.636 (0.048)**	**−0.733 (0.016)**	0.333 (0.347)	0.000 (1.000)	−0.420 (0.228)	0.297 (0.405)	0.192 (0.595)
Decorin (pg/mL)	−0.406 (0.244)	0.297 (0.405)	−0.394 (0.260)	0.286 (0.424)	0.225 (0.532)	−0.055 (0.881)	0.103 (0.778)

Note: Bold values are expressed as a statistically significance.

Abbreviations: CS, chair stand; FR, functional reach; HD, hemodialysis; HG, hand grip; FGF-21, fibroblast growth factor-21; IL-6, interleukin-6; LC, L-carnitine; 10mWT, 10-m walk test; Thigh Cir, thigh circumferences; TUG, time-up-and-go.

the FR test, and the 10mWT, in HD patients[15]; furthermore, in this study, we demonstrated that LC administration significantly improved the 10mWT, Thigh Cir, and CS test. Decreased serum free carnitine and increased acyl/free ratio are known to reflect the disruption of intracellular mitochondrial TCA cycle activation. Since LC administration not only increases free carnitine levels and decreases acyl/free ratio but also decreases the fat fraction in the thigh muscle of HD patients, LC treatment beneficially influences muscle quality through carnitine-elicited mitochondrial energy metabolism. In this study, although the serum carnitine fraction levels had increased almost 10-fold following LC administration, the changes in free carnitine and acyl/free ratio were associated with the 10mWT, CS test, and Thigh Cir (Table 4). While this may not necessarily reflect muscle levels, it has been reported that circulating carnitine fraction levels are positively correlated with muscle carnitine levels in both LC-treated and nontreated HD patients,[16,17] suggesting that changes in circulating carnitine levels may reflect the muscle carnitine status. Since LC administration significantly improved the 10mWT, CS test, and Thigh Cir, changes in the carnitine fraction may predict further improvement of exercise capacity by LC treatment in HD patients. It is thought that LC supplementation is capable of eliminating dysfunctional mitochondria by the induction of autophagy in the skeletal muscle of high-fat diet mice.[18] Further, LC supplementation decreased serum malondialdehyde, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, which are oxidative stress and vascular injury markers in HD patients.[19] These findings suggest that the antioxidant action of LC in the mitochondria seems to be protective against HD-related decelerating physical activity and muscle status.

Sarcopenia and frailty are the strong predictors of disabilities and high mortality rates in patients with HD.[20] Recently, it has become a widely accepted fact that intradialytic Ergo exercise avoids the progression of sarcopenia and frailty.[21] Intradialytic Ergo exercise and pedometer programming for 12 months improved aspects of physical function in HD patients.[22] However, in this study, there was no benefit on physical activity and muscle and fat composition in Ergo exercise-treated patients. These findings might be due to the shortened duration of exercise, inadequate exercise tolerance, and the small number of the patients. Since it might be difficult to achieve enough exercise tolerance for Ergo exercise in HD patients with sarcopenia and frailty, intravenous LC administration could be a promising therapeutic approach in these patients.

In our patients, acyl/free ratio was increased by Ergo exercise. Free carnitine also tended to be decreased. The alteration in skeletal muscle metabolism during exercise causes changes in circulating carnitine levels.[23] In normal healthy subjects, after high-intensity exercise, free and acylcarnitine levels are increased.[24] In low-intensity exercise, long-chain acylcarnitine concentration increases; however, there are no changes in the plasma concentrations of free carnitine, short-chain acylcarnitine, and total acylcarnitine levels.[24] The discrepancy between HD patients and healthy subjects may be explained by the condition of muscle mass. Exercise in HD patients with carnitine deficiency may consume excess mitochondrial energy, which could lead to further carnitine wasting.

Myokines may potentially be predictive markers for exercise activity.[25] In this study, we examined myokines associated with inflammation and insulin resistance. Although LC administration did not affect any of the serum myokine levels, FGF-21 and myostatin levels at baseline were associated with changes in the FR test, the 10mWT, and the Thigh Cir in LC-treated patients, suggesting that these myokines might become predictive markers for LC-treated improvement of exercise activities in LC-treated HD patients. Although FGF-21 is recognized as one of the adipokines, a high FGF-21 level has been reported to be an independent predictor of all-cause mortality in HD patients,[26] suggesting that circulating FGF-21 levels may serve as a predictive marker for mortality in HD patients. In this study, since baseline FGF-21

level was positively associated with changes in the Thigh Cir of LC-treated patients, patients with higher baseline FGF-21 level may be more responsive to LC treatment. In contrast, myostatin is released from the skeletal muscle and is responsible for muscle degradation and atrophy. Serum myostatin is higher in HD patients compared with healthy subjects,[27] and the association between muscle mass and concentrations of myostatin has been established.[28] Further, myostatin levels are associated with 1-year mortality, suggesting the utility of myostatin as a biomarker for muscle status and mortality.[28] In this study, higher baseline myostatin levels were associated with improvements in the FR test and the 10mWT in LC-treated HD patients. High myostatin-induced muscular derangement might be ameliorated by LC treatment.

There are several limitations in this study. First, the sample size of the patients was too small; thus, the statistical power was weak. Second, the short study duration might affect the efficacy of Ergo exercise on physical activities; therefore, further large and longitudinal clinical studies with stronger exercise tolerance are therefore warranted to verify the efficacy of Ergo exercise and LC treatment on sarcopenia, frailty, and QOL in HD patients.

5 | CONCLUSION

In conclusion, LC administration, rather than Ergo exercise for 3 months, significantly improved exercise activities and muscle status in HD patients. These findings suggest the effectiveness of LC treatment as a novel therapeutic strategy for sarcopenia and frailty in HD patients.

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CONFLICT OF INTEREST

Kei Fukami has received honoraria, including lecture fees, from Otsuka Pharmaceutical Co., Ltd.

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