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

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Short title: L-carnitine vs cycle ergometer in HD patients

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-meter walking time ($p = .037$). The fat fraction was improved by the L-carnitine ($p = .047$). Compared with the exercise group, L-carnitine improved the changes in 10-meter 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.

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 Torres (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.

Materials and methods

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 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 (1 pacemaker implantation, 2 CVD, 1 cyanosis renal disease), one had hepatocellular carcinoma, three had orthopedic problems (2 osteoarthritis of the knee, 1 toe amputation), seven could not participate in daytime research, one transferred to another clinic, and one supplemented diet medicine, including LC (Fig 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 (Fig 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 4–5 h 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

95 (TE2-70, Showa Denki, Osaka, Japan) under the guidance of the same physical therapists
96 for 20 min every HD session for the first 2 hours of dialysis, with the intensity set at the
97 40–55% of the maximal work capacity as recommended for chronic kidney disease
98 patients (11). The primary endpoint was the comparable efficacy between Ergo and LC
99 treatment on exercise capacity. Additional analyses were done on the changes of
100 myokines levels before and after the treatment. Informed consent was obtained from all
101 patients as specified in the International Committee of Medical Journal Editors
102 Recommendations, and the study protocol was approved by the institutional ethics
103 committees of Kurume University School of Medicine (Approval Number; 13282). This
104 work was conducted in accordance with the Declaration of Helsinki and was registered
105 with the University Hospital Medical Information Network clinical trials database
106 (UMIN000033833).
107
108 **Data collection**
109 The patients' medical histories were ascertained by a questionnaire. Vigorous
110 physical activity and smoking were avoided for at least 30 min before the measurement
111 of the exercise capacity and the HD session. Blood was drawn from an arteriovenous
112 shunt just before starting the HD sessions to determine hemoglobin, serum albumin, blood

113 urea nitrogen, creatinine (Cr), uric acid, calcium, phosphate, lipids (high- and low-density
114 lipoprotein cholesterol, and triglycerides), and C-reactive protein; values were analyzed
115 at commercially available laboratories (Daiichi Pure Chemicals, Tokyo, Japan and Wako
116 Pure Chemical Industries, Osaka, Japan). Serum carnitine fraction levels were determined
117 as described previously (12). Serum interleukin-6 (R&D Systems, MN, USA), fibroblast
118 growth factor-21 (FGF-21) (R&D Systems), myostatin (Immundiagnostik AG, Bensheim,
119 Germany), and decorin (Abcam plc, Cambridge, UK) were determined by enzyme-linked
120 immunosorbent assay according to the manufacturer's instruction. Changes of all data
121 both before and after treatment, were calculated using the following formula: (post data
122 – pre data) / pre data × 100 (%).
123
124 **Evaluation of physical activities, muscle mass, and fat mass composition**
125 Physical activity was evaluated via the functional reach (FR) test, the 10-meter
126 walk test (10mWT), thigh circumferences at a position of 10 cm above the knee (Thigh
127 Cir), the time-up-and-go (TUG) test, the hand grip (HG) test, the 10 times chair stand-up
128 (CS) test, and the Borg scale as described previously (13). The total body muscle and fat
129 mass were estimated by the Bioelectrical Impedance Analysis (BIA) (Inbody 720,
130 Biospace, Tokyo, Japan), a commonly used non-invasive method for estimating body

131 composition. All exercise capacities as well as muscle and fat mass were independently
132 measured once before treatments and once after 3 months of treatment at a day between
133 dialysis session by the same expert physical therapists at the Division of Rehabilitation,
134 Kurume University Hospital.
135
136 **MR imaging techniques and analysis**
137 Magnetic resonance (MR) imaging was performed at a field strength of 3.0 T
138 (Discovery MR750W; GE Medical Systems, Milwaukee, WI, USA) with two
139 radiofrequency coils in combination (GEM 16-element anterior array and GEM 40-
140 element posterior array, Illinois, GE Healthcare). The proton density fat fraction (PFF)
141 image was evaluated by fat fraction mapping, which was obtained from the iterative
142 decomposition of water and fat with echo asymmetry and least-squares estimation
143 quantitation (IDEAL-IQ) sequence. Imaging parameters of the axial IDEAL-IQ sequence
144 were as follows: TR, 8.2 ms; minimum TE, 1.0 ms; flip angle, 4°; echo train length, 3;
145 slice thickness, 8 mm; FOV, 360 mm × 288 mm; matrix, 160 × 160; scan time, 22 s; and
146 NEX, 0.5 times. The IDEAL-IQ images were analyzed using an imaging workstation
147 (READY View; GE Healthcare). The PFF image was performed before and after the
148 exercise or LC treatment. The whole area, the muscle area, and the intramuscular fat

149 content were measured on a cross section of the femoral region 10 cm above the knee.
150 The muscle area was defined as the area excluding the subcutaneous fat, femoral bone,
151 and neurovascular bundle from the whole area (Fig 2a). For the measurements of the
152 intramuscular fat content, three separate regions of interest (ROIs) were placed in the
153 vastus medialis muscle, the vastus lateralis muscle, and the long head of biceps femoris
154 muscle (each ROI area sampled was 100 mm^2) on the PFF image (Fig 2b). The
155 intramuscular fat content was recorded as the mean values generated from the three
156 measurements; we could not evaluate the MR images of two of the patients in the Ergo
157 group due to their poor condition during imaging. All MR imaging analyses were made
158 by the consensus of two experienced board-certified radiologists (T.T., with 18 years of
159 experience in abdominal imaging, and S.N., with 15 years of experience in
160 musculoskeletal imaging).
161
162 **Statistical analysis**
163 We could analyze all datasets in 20 participants except for PFF (n = 18). Almost
164 all of the datasets were small and not normally distributed; thus, non-parametric analyses
165 were performed. Wilcoxon-Mann-Whitney was used to compare Ergo and LC groups. To
166 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 ± standard deviation. Statistical significance was defined as $p < .05$. All statistical analyses were performed using JMP Pro ver.14 Software (SAS Institute Inc., NC, USA).

Results

Demographic data at baseline

All patients in this study completed the treatment (LC: n = 10, Ergo: n = 10) (Fig. 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).

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

Total carnitine, free carnitine, acylcarnitine, and triglycerides levels were

185 significantly increased by LC administration (p = .002, p = .002, p = .002, p = .010,
186 respectively), whereas serum Cr levels and Acyl/Free ratio were significantly decreased
187 (p = .006, p = .010, respectively) (Table 2); by contrast, Ergo exercise significantly
188 increased Acyl/Free ratio (p = .025) (Table 2).

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

197

198 **Relationship between changes in carnitine fraction levels and exercise activities in**
199 **HD patients**

200 Changes in free carnitine were associated with changes in 10mWT (ρ = –0.498,
201 p = .026) and CS test (ρ = –0.590, p = .006) (Table 4). Changes in Acyl/Free ratio were
202 associated with those in the Thigh Cir (ρ = –0.508, p = .022) and CS test (ρ = 0.556, p

= .011) (Table 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 ($p = .026$, $p = .014$, 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.

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.

Discussion

In this study, we demonstrated that (1) LC administration significantly improves physical activities, such as the 10mWT, CS test, and Thigh Cir; (2) LC administration, rather than Ergo exercise, increases muscle mass and decreases fat mass and fraction; (3) 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 (4) 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.

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

239 of exercise activities, such as the TUG test, the FR test, and the 10mWT, in HD patients
240 (15); furthermore, in this study we demonstrated that LC administration significantly
241 improved the 10mWT, Thigh Cir, and CS test. Decreased serum free carnitine and
242 increased Acyl/Free ratio are known to reflect the disruption of intracellular
243 mitochondrial TCA cycle activation. Since LC administration not only increases free
244 carnitine levels and decreases Acyl/Free ratio but also decreases the fat fraction in the
245 thigh muscle of HD patients, LC treatment beneficially influences muscle quality through
246 carnitine-elicited mitochondrial energy metabolism. In this study, although the serum
247 carnitine fraction levels had increased almost 10-fold following LC administration, the
248 changes in free carnitine and Acyl/Free ratio were associated with the 10mWT, CS test,
249 and Thigh Cir (Table 4). While this may not necessarily reflect muscle levels, it has been
250 reported that circulating carnitine fraction levels are positively correlated with muscle
251 carnitine levels in both LC-treated and non-treated HD patients (16, 17), suggesting that
252 changes in circulating carnitine levels may reflect the muscle carnitine status. Since LC
253 administration significantly improved the 10mWT, CS test, and Thigh Cir, changes in the
254 carnitine fraction may predict further improvement of exercise capacity by LC treatment
255 in HD patients. It is thought that LC supplementation is capable of eliminating
256 dysfunctional mitochondria by the induction of autophagy in the skeletal muscle of high-

257 fat diet mice (18). Further, LC supplementation decreased serum malondialdehyde,
258 intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, which are
259 oxidative stress and vascular injury markers in HD patients (19). These findings suggest
260 that the antioxidant action of LC in the mitochondria seems to be protective against HD-
261 related decelerating physical activity and muscle status.

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

273 In our patients, Acyl/Free ratio was increased by Ergo exercise. Free carnitine
274 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 one-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.

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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Conflicts of interest

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

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Figure legends

Fig 1. Trial flow diagram

Ergo: ergometer exercise, LC: L-carnitine, MR: magnetic resonance.

Fig 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

417 **Table 1. Clinical characteristics of the patients.**

418

Variables	Ergo group	LC group	P
No. of patients	10	10	
Age (years old)	53.9 ± 16.5	57.1 ± 11.1	.520
Sex (No.) (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 (No.) (–/+)	8/2	7/3	.651

Values are shown as mean ± SD or range. No.=number. HD=hemodialysis; BUN=blood urea nitrogen; Cr=creatinine; Ca=calcium; LDL=low-density lipoprotein; HDL=high-density lipoprotein; CRP=C-reactive protein; Acy/Free ratio=acylcarnitine free carnitine ratio; BIA= bioelectrical impedance analysis; FR test=functional reach test; 10mWT=10-meter walk test; Thigh Cir=thigh circumferences; TUG=time-up-and-go; HG=hand grip; CS=chair stand; MRI=magnetic resonance image. [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**

Values are shown as mean ± SD or range. Ergo=ergometer; LC=L-carnitine; HD=hemodialysis; BUN=blood urea nitrogen; Cr=creatinine; Ca=calcium; LDL=low-density lipoprotein; HDL=high-density lipoprotein; CRP=C-reactive protein; Acyl/Free ratio=acylcarnitine free carnitine ratio.

[a]This variable is shown in the original scale after using log-transformed values.

441

442 **Table 3. Effects of Ergo exercise or LC administration on exercise activities and**
443 **muscle status in HD patients.**

444

	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**

445

446 Values are shown as mean ± SD.

447 Ergo=ergometer; LC=L-carnitine; HD=hemodialysis; FR=functional reach; 10mWT=10-
448 meter walk test; Thigh Cir=thigh circumferences; TUG=time-up-and-go; HG=hand grip;
449 CS=chair stand; BIA=bioelectrical impedance analysis; MRI=magnetic resonance
450 imaging
451

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)

HD=hemodialysis; FR=functional reach; 10mWT=10-meter walk test; Thigh Cir=thigh circumferences; TUG=time-up-and-go; HG=hand grip; CS=chair stand; Acyl/Free ratio=acylcarnitine free carnitine ratio.

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
ΔHGT	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

Values are shown as mean ± SD.

Ergo=ergometer exercise; LC=L-carnitine; HD=hemodialysis; FR=functional reach; 10mWT=10-meter walk test; Thigh Cir=thigh circumferences; TUG=time-up-and-go; CS=chair stand; BIA= bioelectrical impedance analysis; MRI=magnetic resonance imaging

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)

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

Figure.1

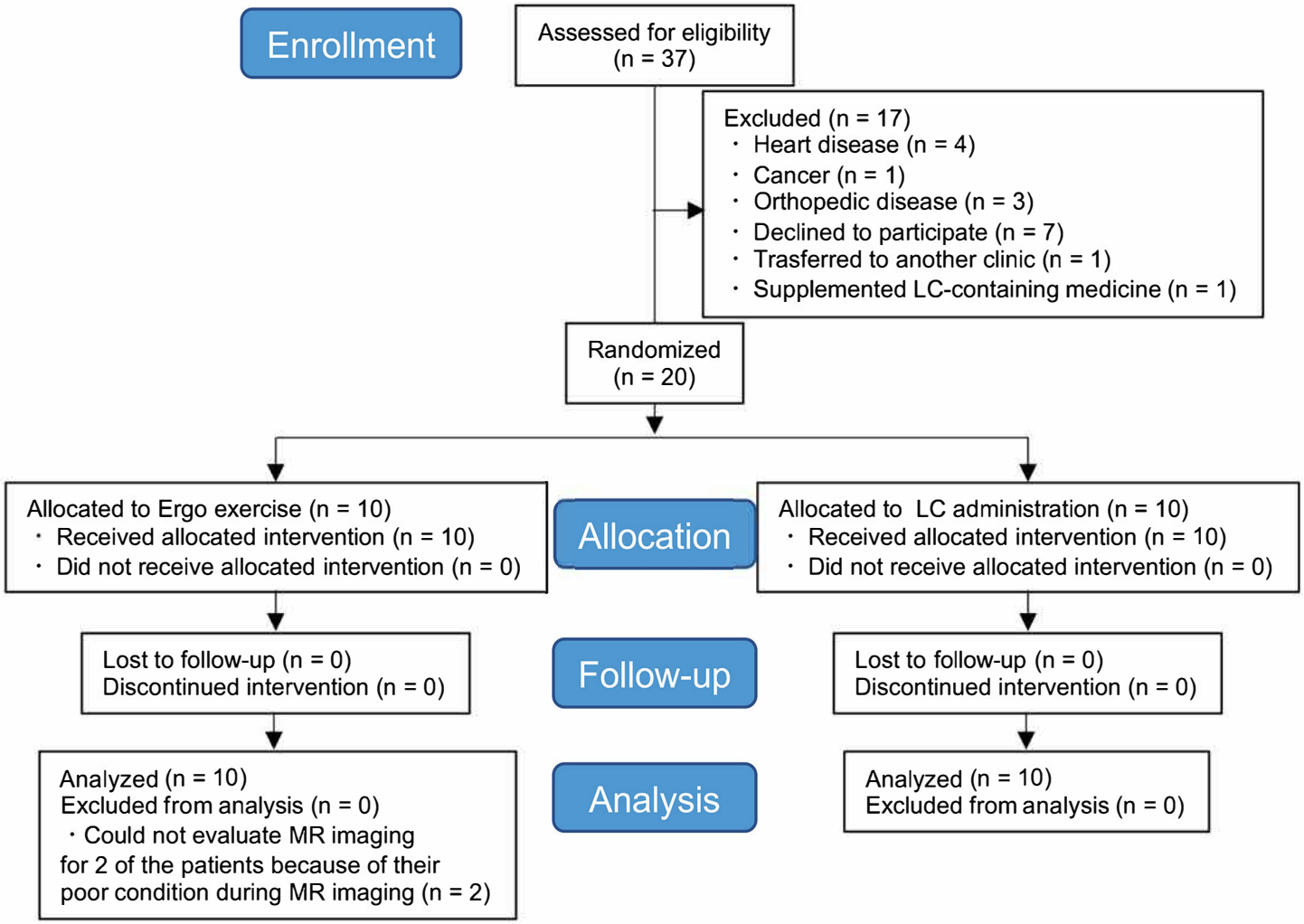
Enrollment
Assessed for eligibility
(n = 37)
Excluded (n = 17)
・Heart disease (n = 4)
・Cancer (n = 1)
・Orthopedic disease (n = 3)
・Declined to participate (n = 7)
・Trasferred to another clinic (n = 1)
・Supplemented LC-containing medicine (n = 1)
Randomized
(n = 20)
Allocated to Ergo exercise (n = 10)
・Received allocated intervention (n = 10)
・Did not receive allocated intervention (n = 0)
Allocation
Allocated to LC administration (n = 10)
・Received allocated intervention (n = 10)
・Did not receive allocated intervention (n = 0)
Lost to follow-up (n = 0)
Discontinued intervention (n = 0)
Follow-up
Lost to follow-up (n = 0)
Discontinued intervention (n = 0)
Analyzed (n = 10)
Excluded from analysis (n = 0)
・Could not evaluate MR imaging
for 2 of the patients because of their
poor condition during MR imaging (n = 2)
Analysis
Analyzed (n = 10)
Excluded from analysis (n = 0)

Figure.2