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Effects of L-carnitine supplementation on nutritional, immunological, and cardiac parameters in hemodialysis patients: a pilot study

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Abstract

Background: L-carnitine is an essential compound that facilitates the transport of long-chain fatty acid across the inner mitochondrial membrane for β -oxidation. However, the effect of L-carnitine supplementation remains to be fully explored in patients with chronic kidney disease. We aimed in this study to determine the multidirectional effects of L-carnitine supplementation on clinical parameters in more detail.

Methods: We orally administered L-carnitine to maintain serum-free carnitine levels within the normal range (30 to 70 μ mol/L) for 6 months in 21 hemodialysis (HD) patients (age, 74 ± 11 years; time on HD, 60 ± 84 months).

Results: L-carnitine supplementation significantly increased serum transferrin from 155 ± 41 to 175 ± 48 mg/dL ($p < 0.01$) and retinol-binding protein from 8.9 ± 2.5 to 10.0 ± 3.4 mg/dL ($p < 0.05$). The triceps skinfold thickness was also significantly increased ($p < 0.05$), while the skeletal muscle mass in limbs and handgrip strength was not. L-carnitine enhanced natural killer (NK) cell activity at the E (effector cell)/T (target cell) ratio of 20:1 from 17.3 ± 14.1 to 20.8 ± 17.7 % ($p < 0.01$). In addition, L-carnitine improved left ventricular functional shortening ($p < 0.01$) with a significant decrease of brain natriuretic peptide (BNP) from 621.4 ± 666.8 to 412.0 ± 426.0 pg/mL ($p < 0.05$).

Conclusions: These findings suggest that oral L-carnitine treatment improves immunological and cardiac markers in HD patients. In contrast, L-carnitine did not change the skeletal mass-related parameters during the 6-month follow-up.

Keywords: Hemodialysis, L-carnitine, Sarcopenia, Natural killer cell activity, Left ventricular function

Background

Protein-energy wasting (PEW) comprises excessive losses of body protein mass and energy reserves, and it is associated with high morbidity and mortality in patients with chronic kidney disease (CKD) [1]. There are many causes of PEW such as inadequate nutrient intake, increased nutrient losses from the dialysate, inflammation, oxidant stress, carbonyl stress, disorders of anabolic or catabolic hormones, and acidemia [1].

L-carnitine is a natural compound that is synthesized from lysine and methionine. L-carnitine is essential in

the transfer of long-chain fatty acids into the mitochondria for β -oxidation. When carnitine deficiency develops, long-chain fatty acids are not available for β -oxidation or energy production, which causes excessive lipid accumulation in organs such as the muscle. Carnitine is also necessary to maintain the function and morphology of the brown adipose tissue [2]. Approximately 95 % of the carnitine pool is present in the skeletal and cardiac muscle, 2–3 % in the liver and kidneys, and only 0.5–1 % in extracellular fluid [3].

Dialysis patients are especially at high risk for carnitine deficiency due to poor intake of sources of dietary carnitine such as red meat, reduced biosynthesis in the kidneys, and diffusive loss from the dialysate. In incident hemodialysis (HD) patients, the plasma carnitine level decreases within the first week of the treatment, with a

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continual decrease over the first 12 months of treatment [4]. The carnitine content of the skeletal muscle also decreases in hemodialysis patients and is inversely correlated with time on HD [4, 5].

Metabolism of skeletal muscle is carnitine-dependent because fatty acid oxidation is the main source of energy in the muscular cells. However, it remains to be fully determined whether or not L-carnitine supplementation improves muscle power, physical capacity, and muscular symptoms.

The aim of the present study is to further clarify the effect of L-carnitine supplementation on clinical parameters in HD patients. We prospectively administered L-carnitine orally to maintain free carnitine levels within the normal range for 6 months and examined the impact of L-carnitine supplementation on nutritional, immunological, inflammatory, and cardiac markers in more detail.

Methods

Patients

This study was conducted in a single dialysis center (National Hospital Organization Yanai Medical Center, Yamaguchi, Japan). At first, 26 HD patients, who had given their consent, were enrolled in this study. There was no patient who had received any L-carnitine supplementation before the entry. The study design had been approved by the appropriate ethics review boards of the institution within which the study was undertaken, and it conformed to the provisions of the Declaration of Helsinki.

All patients had been subjected to regular HD using polysulfone dialyzer membranes with bicarbonate buffers for 3 to 4 h three times per week.

L-carnitine supplementation

We originally modified the initial dose of oral L-carnitine according to dry weight (DW) to maintain serum level within normal range without risk, since excess L-carnitine can be converted by intestinal microbiota into trimethylamine-N-oxide (TMAO), a potential promoter of coronary arteriosclerosis [6]. When DW was higher than 50 kg, we initially administered 400 mg/day once a day, while when DW ranged from 30 to 50 kg, we administered 300 mg/day. Also, if DW was less than 30 kg, we started with the dose of 200 mg/day.

We then measured predialysis serum carnitine levels every month for the initial 3 months. When the total carnitine concentration exceeded the normal upper limit (≥ 70 $\mu\text{mol/L}$), we decreased the dose by 100 mg daily until the next measurement. In contrast, when the serum level of total carnitine was less than the lower limit (< 30 $\mu\text{mol/L}$), we increased the dose by 100 mg/day until the next measurement.

Blood sampling and laboratory examinations

Blood samples were drawn from the arterial site of the arteriovenous fistula at each dialysis session after a 2-day interval at 0, 3, and 6 months after the enrollment. Blood cell counts, blood urea nitrogen, serum creatinine, total protein, albumin, C-reactive protein (CRP), lipids (low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides), hemoglobin, ammonia, and brain natriuretic peptide (BNP) were measured by standard laboratory techniques using an auto-analyzer. We calculated Kt/V for urea using a single pool kinetic model [7]. We also measured systolic and diastolic blood pressure just before the start of HD.

Immunoglobulin (Ig), transthyretin, transferrin, retinol-binding protein, free fatty acid, ceruloplasmin, β_2 -microglobulin, and intact parathyroid measurement were entrusted to Bio-Medical Laboratories, Inc. (Tokyo, Japan). Total, free, and acylcarnitine were also measured by enzyme cycling methods. Serum interleukin-6 (IL-6) was determined with a Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA).

Natural killer (NK) cell activity was measured using a standard ^{51}Cr release assay against K562 (a highly undifferentiated and granulocytic myelogenous leukemia cell line from blast crisis) as reference target cells with 6-h incubation. NK activity was expressed as the E (effector cell)/T (target cell) ratio (reference ranges; E:T ratio = 20:1: 17.1–48.7 % and E:T ratio = 10:1: 8.9–29.5 %). Cluster of differentiation (CD) 4/CD8 was implemented by flow cytometry with the use of FACSCalibur™ (BD Biosciences, San Jose, CA, USA) and KX-21 (Sysmex, Kobe, Japan). Serum soluble interleukin-2 receptor (IL-2R) was also measured using enzyme-linked immunoassay kits (reference, 122–496 U/mL).

The serum selenium, zinc, and copper levels were measured by atomic absorption spectrophotometry (reference ranges; selenium, 107–171 $\mu\text{g/dL}$; zinc, 59–135 $\mu\text{g/dL}$; and copper, 66–130 $\mu\text{g/dL}$).

Nutritional assessment

Ideal body weight (BW) was defined as that calculated from the patients' heights using a body mass index (BMI) of 22.0 kg/m^2 . BMI was calculated as dry weight in kilograms divided by the square of the height in meters. We calculated Onodera's prognostic nutrition index (PNI) using serum albumin, total lymphocytes (TLC), and dry BW as follows: $\text{PNI} = [10 \times \text{albumin (g/dL)}] + [0.005 \times \text{TLC}]$ [8]. We also determined geriatric nutritional risk index (GNRI) as follows: $\text{GNRI} = [14.89 \times \text{albumin (g/dL)}] + [41.7 \times \text{dry/ideal BW}]$. When DW was greater than the ideal weight, GNRI was calculated as a DW/ideal BW ratio identical to 1.0 [9].

We determined normalized protein catabolic rate (nPCR) and percentile creatinine generation rate (%CGR), which can estimate dietary protein intake and skeletal muscle mass, respectively [7, 10].

Anthropometric parameters

At the start and end of the study, we measured the mid-arm circumference (MAC) and triceps skinfold thickness (TSF) using skinfold calipers on the limb not used for vascular access after a HD session. We calculated the midarm muscle circumference (MAMC) using MAC and TSF as follows: $MAMC = MAC - (TSF \times 3.14)$. All measurements were performed by the same trained researcher.

We also determined the thigh muscle area by computed tomography (CT; Aquilion ONE TSX-301A, Toshiba, Tokyo, Japan). Each patient was examined in the supine position with the thigh muscle relaxed. An axial CT image was obtained at the midpoint of a line extending from the superior border of the patella to the greater trochanter of the femora. The thickness of the slice was 10 mm. The thigh muscle area and bone area in the thigh were determined using SCFM-001A software (Toshiba, Tokyo, Japan). We used the standardized thigh muscle area (TMA) divided by the thigh bone area (TBA) to avoid the influence of body size, a surrogate marker of thigh sarcopenia [11].

Handgrip strength

We measured handgrip strength on the upper part of the arm not used for vascular access just before a HD session using a Smedley handgrip dynamometer. Each patient made two maximum force attempts, with an interval of at least 1 min between attempts. The highest recording was taken as the handgrip strength reading.

M-mode echocardiography

M-mode echocardiography was conducted on a non-dialysis day at study entry and at 6 months. All measurements were conducted by the same trained physician.

Left ventricular systolic function was assessed as ejection fraction (EF) and fractional shortening (FS). EF and FS were calculated as follows: $EF (\%) = (\text{left ventricular end-diastolic volume (LVEDV)} - \text{left ventricular end-systolic volume (LVESV)}) / \text{LVEDV} \times 100$ and $FS (\%) = (\text{left ventricular end-diastolic dimension (LVDd)} - \text{left ventricular end-systolic dimension (LVDs)}) / \text{LVDd} \times 100$.

Left ventricular mass (LVM) was calculated as follows: $LVM (g) = 1.04 \times [(\text{LVDd} + \text{interventricular septum (IVS)} + \text{left ventricular posterior wall end-diastolic thickness (LVPW)})^3 - \text{LVDd}^3] - 14$. LVM was adjusted as to the

body surface area in square meters and expressed as the left ventricular mass index (LVMI).

Chest X-ray

The cardiothoracic ratio (CTR) was measured on a chest X-ray just before HD therapy by a trained researcher.

Statistical analysis

For statistical analysis, JMP (Version 11.1.1, SAS Institute, Cary, NC, USA) was used. Descriptive results were expressed as the means \pm standard deviation (SD). Parametric analyses were performed using Student's *t*-test, and the Wilcoxon *t* test was used for non-parametric analyses. A value of $p < 0.05$ was considered statistically significant different.

Results

Basal characteristics

During the follow-up, since we had lost five patients due to death ($n = 4$) and acute infection ($n = 1$), we finally analyzed a total of 21 patients.

Table 1 presents the basal characteristics. The mean age was 73.5 ± 11.6 years old, with an average time on HD of 60 ± 84 months. The causes of end-stage kidney disease were as follows: diabetic nephropathy in nine, hypertensive nephrosclerosis in four, chronic glomerulonephritis in two, others in three, and unknown in three.

Table 1 Characteristics of the study population

No.	21
Duration of hemodialysis (months)	60 ± 84
Age (years)	73.5 ± 11.6
Gender (male/female)	12/9
Dry weight (kg)	48.0 ± 12.5
Body height (cm)	158.0 ± 10.5
BMI (kg/m^2)	19.0 ± 3.2
Comorbidity	
Cardiovascular disease (%)	23.8
Cerebral vascular disease (%)	19.0
HCV Ab-positivity (%)	19.0
Laboratory parameters	
Blood urea nitrogen (mg/dL)	47.5 ± 15.5
Creatinine (mg/dL)	6.9 ± 2.8
β_2 -MG (mg/L)	25.0 ± 8.1
Intact PTH (pg/mL)	90 ± 88

Data, means \pm SD

BMI body mass index, HCV Ab anti-hepatitis C antibody, β_2 -MG β_2 -microglobulin, PTH parathyroid hormone, SD standard deviation

Carnitine levels

L-carnitine supplementation had normalized the serum total, free, and acylcarnitine levels at 3 months. The total carnitine levels were increased from 31.1 ± 10.0 to 98.6 ± 27.9 (normal, 45.0–91.0) ($p < 0.001$), the free carnitine levels from 19.8 ± 6.6 to 63.3 ± 14.3 (normal, 36.0–76.0) ($p < 0.001$), and the acylcarnitine levels from 11.4 ± 4.9 to 35.3 ± 15.9 (normal, 6.0–23.0) $\mu\text{mol/L}$ ($p < 0.001$) at 6 months (Fig. 1). However, there was no difference in free to acylcarnitine ratio between before (1.92 ± 0.14) and after the treatment (1.98 ± 0.10).

The final dosage of L-carnitine was 191 ± 114 mg/day (4.0 ± 2.1 mg/kg DW/day). No difference was found in L-carnitine dosage between HD patients with preserved residual kidney function (3.8 mg/kg DW/day, $n = 7$) and those without (4.5 mg/kg DW/day, $n = 14$).

Nutritional parameters

Despite the identical DW, significant increases were found in the serum transferrin ($p < 0.01$), retinol-binding protein ($p < 0.05$), and triglyceride levels ($p < 0.05$) (Table 2). In contrast, GNRI, PNI, nPCR, and %CGR did not change during the follow-up.

Anthropometric parameters

TSF was significantly increased from 0.4 ± 0.3 to 0.6 ± 0.3 cm ($p < 0.05$), while MAC and MAMC were not. There was no difference in the TMA/TBA ratio or handgrip strength during the treatment (Table 2). L-carnitine did not change as to the TMA/TBA ratio in the males (10.1 ± 3.3 vs. 9.7 ± 3.3) or females (8.0 ± 2.2 vs. 7.8 ± 2.6). Handgrip strength was also identical between males (20 ± 6 vs. 21 ± 6 kg) and females (9 ± 6 vs. 8 ± 6 kg).

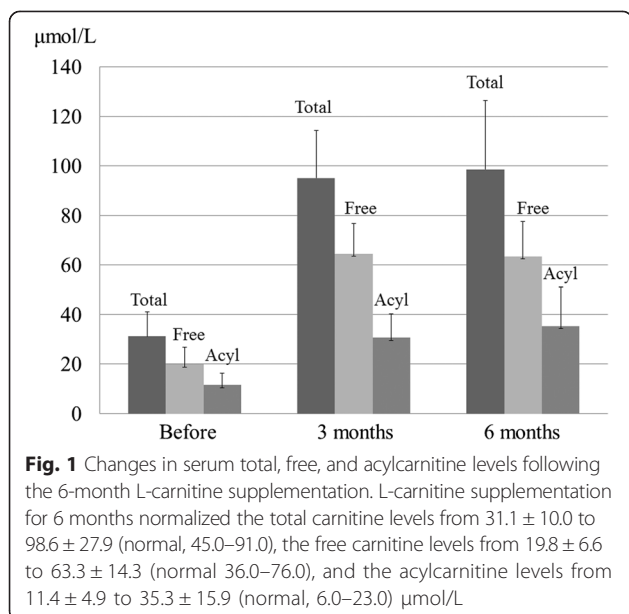


Table 2 Effects of L-carnitine supplementation on nutritional parameters

Variables	Before	3 months	6 months
Laboratory parameters			
Blood urea nitrogen (mg/dL)	47.5 ± 15.5	42.9 ± 12.0	45.4 ± 12.7
Creatinine (mg/dL)	6.9 ± 2.8	6.7 ± 2.2	6.6 ± 2.3
Albumin (g/dL)	3.2 ± 0.3	3.1 ± 0.4	3.2 ± 0.4
Transthyretin (mg/dL)	22.4 ± 5.9	22.5 ± 6.5	24.7 ± 9.7
Transferrin (mg/dL)	154.9 ± 40.5	$175.5 \pm 42.6^{**}$	$174.9 \pm 47.8^{**}$
Retinol-binding protein (mg/dL)	8.9 ± 2.5	9.2 ± 2.8	$10.0 \pm 3.4^*$
Total lymphocyte count (/ μL)	1190 ± 440	1250 ± 610	1150 ± 430
HDL cholesterol (mg/dL)	41 ± 13	44 ± 13	43 ± 14
LDL cholesterol (mg/dL)	73 ± 21	77 ± 27	$81 \pm 29^*$
Triglyceride (mg/dL)	91 ± 56	107 ± 81	$103 \pm 58^*$
Free fatty acid (mEq/L)	0.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.1
Ceruloplasmin (mg/dL)	22.5 ± 4.9	23.2 ± 10.3	22.6 ± 4.7
Hemoglobin (g/dL)	10.0 ± 1.2	10.2 ± 2.0	10.2 ± 1.7
Ammonia ($\mu\text{g/dL}$)	21 ± 10	$32 \pm 10^{**}$	$36 \pm 13^{**}$
Nutritional assessment index			
GNRI	83.9 ± 7.9	82.3 ± 7.8	82.7 ± 8.8
PNI	38.2 ± 3.7	37.5 ± 4.3	37.3 ± 4.8
nPCR	0.73 ± 0.04	0.66 ± 0.03	0.70 ± 0.04
Kt/V for urea	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
%CGR	69.5 ± 6.8	65.7 ± 5.5	63.9 ± 5.4
Anthropometric parameters			
Dry weight (kg)	48.0 ± 12.5	47.8 ± 12.1	47.7 ± 11.7
BMI (kg/m ²)	19.0 ± 3.2	18.9 ± 3.0	18.8 ± 2.9
TMA/TBA ratio ^a	9.1 ± 3.0	ND	8.8 ± 3.1
Handgrip strength (kg) ^b	16.0 ± 8.1	ND	16.1 ± 8.6
MAC (cm)	21.8 ± 3.1	ND	23.1 ± 3.8
TSF (cm)	0.4 ± 0.3	ND	$0.6 \pm 0.3^*$
MAMC (cm)	20.5 ± 3.1	ND	21.3 ± 3.6

BMI body mass index, GNRI geriatric nutritional risk index, PNI prognostic nutrition index, nPCR normalized protein catabolic rate, Kt/V for urea clearance of urea normalized to total body water using a single pool model, %CGR creatinine generation rate, HDL high-density lipoprotein, LDL low-density lipoprotein, TMA thigh muscle area, TBA thigh bone area, MAC midarm circumference, TSF triceps skinfold thickness, MAMC midarm muscle circumference, ND not done, SD standard deviation

* $p < 0.05$, ** $p < 0.01$ vs. before treatment. Data, means \pm SD

^aData were available for 20 out of 21 patients

^bData were available for 16 out of 21 patients

Immunological and inflammatory markers

A significant increase in NK cell activity was found at E/T ratio of both 10:1 (10.2 ± 8.8 vs. 12.6 ± 12.4 %; normal, 8.9–29.5 %) ($p < 0.02$) and 20:1 (17.2 ± 14.1 vs. 20.8 ± 17.7 %; normal, 17.1–48.7 %) ($p < 0.01$). In contrast, the CD4/CD8 ratio, serum CRP, and soluble interleukin-2 receptor (sIL-2R) did not change during the 6-month follow-up. The mean serum IL-6 level had rather increased from 5.2 ± 2.5 to 7.8 ± 5.1 at

3 months ($p < 0.05$), and to 7.7 ± 5.0 pg/mL at 6 months ($p = 0.051$) (Table 3).

Trace elements

Serum selenium significantly decreased from 60.4 ± 13.9 to 52.4 ± 12.2 $\mu\text{g/L}$ during the follow-up ($p = 0.01$). The serum zinc and copper levels did not change (Table 3).

Cardiac parameters

L-carnitine increased as to FS from 42.2 ± 11.4 to 46.1 ± 9.8 % ($p < 0.01$). There was no difference in the CTR, EF, and LVMI values between the two measurements. However, predialysis BNP had significantly decreased from 621.4 ± 666.8 to 412.0 ± 426.0 pg/mL after the 6-month supplementation ($p < 0.02$) (Table 4).

Discussion

In this study, we orally administered L-carnitine to maintain the serum levels of free carnitine within normal range for 6 months by carefully monitoring and modifying the dosage during the first 3 months. As a result, we kept the free carnitine levels within normal range despite a relatively lower dose (3.8 to 4.5 mg/kg BW/day) compared to the recommended dosage (5 to 10 mg/kg BW/day).

We also showed that the regimen of L-carnitine supplementation had several beneficial effects on nutritional, immunological, and cardiac parameters, as follows.

Effects on nutrition

L-carnitine supplementation is reported to improve nutritional status in HD patients. Oral L-carnitine

Table 3 Effects of L-carnitine supplementation on immunological, inflammatory parameters, and trace elements

Variables	Before	3 months	6 months
Immunological and inflammatory parameters			
NK cell activity; E/T ratio 10:1 (%)	10.2 ± 8.8	10.5 ± 9.9	$12.6 \pm 12.4^*$
E/T ratio 20:1 (%)	17.3 ± 14.1	17.4 ± 15.3	$20.8 \pm 17.7^{**}$
CD4/CD8 ratio	2.2 ± 3.3	2.0 ± 2.2	2.1 ± 2.3
IgG (mg/dL)	1460 ± 410	1430 ± 350	1490 ± 400
IL-6 (pg/mL)	5.2 ± 2.5	$7.8 \pm 5.1^*$	7.7 ± 5.0
sIL-2R (U/mL)	1311 ± 1020	1103 ± 448	1233 ± 498
C-reactive protein (mg/dL)	0.6 ± 1.0	0.6 ± 0.9	1.0 ± 1.8
Trace elements			
Selenium ($\mu\text{g/L}$)	60.4 ± 13.9	63.5 ± 17.0	$52.4 \pm 12.2^*$
Zinc ($\mu\text{g/dL}$)	48.5 ± 8.6	52.3 ± 10.1	51.7 ± 9.6
Copper ($\mu\text{g/dL}$)	94.0 ± 19.1	90.8 ± 21.9	91.4 ± 23.0

NK cell natural killer cell, E/T ratio effector cells/target cells ratio, CD cluster of differentiation, IgG immunoglobulin G, IL-6 interleukin-6, sIL-2R soluble interleukin-2 receptor, SD standard deviation

* $p < 0.05$, ** $p < 0.01$ vs. before treatment. Data, means \pm SD

Table 4 Effects of L-carnitine supplementation on cardiac parameters

Variables	Before	6 months
Systolic blood pressure (mmHg)	156.6 ± 3.4	155.0 ± 2.9
Diastolic blood pressure (mmHg)	76.6 ± 3.2	73.4 ± 2.9
Antihypertensive medication		
Diuretics (%)	23.8	19.0
α -blocker (%)	4.8	4.8
β -blocker (%)	23.8	23.8
Calcium antagonist (%)	42.9	42.9
Angiotensin II type 1 receptor blocker (%)	47.6	52.4
M-mode echocardiography		
EF (%)	69.1 ± 10.8	69.8 ± 10.5
FS (%)	42.2 ± 11.4	$46.1 \pm 9.8^{**}$
LVDd (cm)	4.5 ± 0.9	4.6 ± 0.8
LVDs (cm)	2.6 ± 0.9	2.5 ± 0.8
IVS (mm)	14 ± 3	15 ± 3
LVPW (cm)	1.4 ± 0.2	1.3 ± 0.3
LVM (g)	308.2 ± 131.1	303.5 ± 104.4
LVMI (g/m^2)	217.8 ± 100.2	212.3 ± 77.3
Chest X-ray		
CTR (%)	52.8 ± 5.3	52.4 ± 5.0
Laboratory data		
BNP (pg/mL)	621.4 ± 666.8	$412.0 \pm 426.0^*$

EF ejection fraction, FS fractional shortening, LVDd left ventricular end-diastolic dimension, LVDs left ventricular end-systolic dimension, IVS interventricular septal thickness, LVPW left ventricular posterior wall end-diastolic thickness, LVFS left ventricular fractional shortening, LVM left ventricular mass, LVMI left ventricular mass index, CTR cardiothoracic ratio, BNP: brain natriuretic peptide, SD standard deviation

* $p < 0.05$, ** $p < 0.01$ vs. before treatment. Data, means \pm SD

administration increased nPCR independently of dialysis dose [12]. L-carnitine also improved nutritional parameters such as serum transferrin and albumin in HD patients [13, 14]. Intravenous administration of L-carnitine (2 g) at the end of a HD session also increases the morphological diameters of skeletal muscle fibers [15]. L-carnitine treatment prevented deterioration of the peak rate of oxygen consumption (VO_2 max) [16].

In this study, L-carnitine significantly increased serum retinol-binding protein and transferrin 6 months later. In addition, the treatment increased serum ammonia despite within the normal range, indicating a possibility that L-carnitine may enhance ammonia production via urea cycle by increased food intake. However, nPCR, an indirect marker of dietary protein intake, remained lower during the study. L-carnitine also did not improve other nutritional parameters such as GNRI and PNI. There was no difference in muscle mass area, handgrip power, and %CGR. These findings convincingly suggest that oral L-carnitine treatment for 6 months to maintain

serum-free carnitine within normal range did not alleviate poor nutritional status.

In contrast, TSF was significantly increased after the treatment. However, since TMA/TBA ratio, a marker of thigh skeletal mass area, was rather decreased from 9.1 ± 3.0 to 8.8 ± 3.1 despite no gain of body weight, it might be explained by the changes in regional body composition due to progressive sarcopenia at the upper arm.

Effects on immune response

NK cells activity plays an important role in the defense mechanism against infections and tumors. In HD patients, the activity of NK cells is either lowered or unchanged [17]. A higher CD4/CD8 ratio, a marker of Ig production by B cells, is also associated with a better serological response to hepatitis B vaccination [18].

L-carnitine can restore the age-related changes in neutrophil function such as chemotactic and phagocytic activity in vitro [19]. L-carnitine treatment also prevented agent-induced experimental colitis by abrogation of both innate and adaptive immune responses in mice [20]. In addition, L-carnitine can reduce the apoptotic levels of CD4⁺ and CD8⁺ cells [21].

In the present study, L-carnitine supplementation significantly increased NK cell activity at E/T ratios of 10:1 and 20:1, but it first became evident at 6 months. So, at least more than 3 months will be needed to restore NK cell activity following L-carnitine therapy. Selenium deficiency is potentially associated with cellular immunity in HD patients [17]. However, serum selenium was rather decreased from 60.4 ± 13.9 to 52.4 ± 12.2 $\mu\text{g/L}$ after the supplementation, suggesting no association of selenium deficiency with restored NK cell activity.

Effects on cardiac parameters

L-carnitine prevents the development of ventricular fibrosis and heart failure with preserved EF in hypertensive heart disease [22]. L-carnitine also blocks dietary vitamin B deficiency-induced left ventricular dysfunction and attenuates the diet-caused increase in serum BNP [23]. In patients with chronic heart failure, the plasma carnitine levels are correlated with %FS, EF, and LVMI [24]. A meta-analysis also revealed that L-carnitine is associated with a 27 % reduction in all-cause mortality and a 40 % reduction in angina symptoms in patients experiencing acute myocardial infarction [25].

It has been reported that oral L-carnitine treatment significantly improved left ventricular function [26, 27], while it reduced LVMI [28]. In this study, we confirmed that L-carnitine supplementation caused a significant increase in %FS concomitantly with a reduction of BNP. In contrast, L-carnitine did not change LVMI. The reason for this discrepancy remains unknown, but it may

be partly due to the shorter period of supplementation than in the previous study (12 months) [28].

Effects on inflammation

A link between inflammation and carnitine deficiency has been well suggested in HD patients. However, the results of the recent two meta-analysis studies involving randomized controlled trials did not coincide; one study [29] showed that L-carnitine significantly decreased serum CRP, whereas the other disclosed no effect [30].

In this study, oral L-carnitine did not change the serum CRP level, while it rather increased the serum IL-6 level after 6 months. Because it is reported that oral administration of 1000 mg/kg BW of L-carnitine decreased serum CRP and IL-6 [31, 32], a higher dose may be required to alleviate the uremia-related inflammatory response.

Study limitations

There are several limitations to this study. Firstly, this study was not carried out in a randomized controlled manner, and the number of patients was clearly small. So, future studies involving more samples are needed to confirm the association of L-carnitine treatment and clinical parameters. Secondly, we simply compared a lot of clinical markers before and after L-carnitine treatment. So, it remains unknown whether or not these immunological and echocardiographic alterations may lead to beneficial effects on clinical outcomes. Thirdly, since we administered L-carnitine orally at a relatively low dose, its effect may be limited compared to intravenous administration. Fourthly, we did not measure the appendicular skeletal mass index using bioelectrical impedance (BIA) or dual-energy X-ray absorptiometry, so we could not precisely confirm the presence of sarcopenia. Finally, muscle atrophy progresses over time, so it will be necessary to evaluate the effect of L-carnitine in the long term.

Conclusions

We showed that L-carnitine supplementation had several effects on clinical parameters. L-carnitine increased NK cell activity following the 6-month treatment. There was a significant increase in %FS with a decrease in BNP. The findings suggested that L-carnitine can improve immunological and LV-related systolic functional parameters. In contrast, L-carnitine did not ameliorate sarcopenic markers and did not reduce the inflammatory response. So, future well-designed RCT is obviously needed to confirm the multidirectional actions of L-carnitine supplementation in patients on regular HD.

Abbreviations

%CGR: creatinine generation rate; BIA: bioelectrical impedance; BNP: brain natriuretic peptide; BW: body weight; CD: cluster of differentiation; CKD: chronic kidney disease; CRP: C-reactive protein; CT: computed tomography; CTR: cardiothoracic ratio; DW: dry weight; E/T ratio: effector cells/target cells ratio; EF: ejection fraction; FS: fractional shortening; GNRI: geriatric nutritional risk index; HD: hemodialysis; HDL: high-density lipoprotein; Ig: immunoglobulin; IL-6: interleukin-6; IVS: interventricular septal thickness; LDL: low-density lipoprotein; LVDd: left ventricular end-diastolic dimension; LVDs: left ventricular end-systolic dimension; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVM: left ventricular mass; LVMI: left ventricular mass index; MAC: midarm circumference; MAMC: midarm muscle circumference; NK: natural killer; nPCR: normalized protein catabolic rate; PEW: protein-energy wasting; PNI: prognostic nutrition index; RRFC: rate of reabsorption of free carnitine; sIL-2R: soluble interleukin-2 receptor; TBA: thigh bone area; TLC: total lymphocyte count; TMA: thigh muscle area; TSF: triceps skinfold thickness.

Competing interests

None of the authors have any conflict of interest to disclose.

Authors' contributions

HS, conception and design, data collection, data analysis and interpretation, and manuscript writing; AK, conception and design, data analysis and interpretation, and final approval of the manuscript; HO, conception and design, support of preparation, and drafting of the manuscript; RS and HO, general management and critical reading and discussion of the manuscript. All authors discussed the results and commented on the manuscript. All authors read and approve the final manuscript.

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