



# Long-Term Levocarnitine Ameliorates Left Ventricular Diastolic as Well as Systolic Dysfunction in Hemodialysis Patients

## — Multi-Center Study —

Junko Naito, MD; Hiroshige Ohashi, MD, PhD; Michiya Ohno, MD, PhD;  
Masafumi Sugiyama, MD, PhD; Kazuyoshi Hayakawa, MD, PhD;  
Akihisa Kunishima, MD, PhD; Nobuyuki Takada, MD, PhD; Tatsuya Kariya, MD, PhD;  
Koshi Goto, MD, PhD; Hisato Takatsu, MD, PhD; Toshiki Ohira, MD, PhD;  
Koji Nakahara, MD, PhD; Ichijiro Murata, MD, PhD; Shingo Minatoguchi, MD, PhD;  
Gakuro Yoshida, MD, PhD; Hiroyuki Okura, MD, PhD; Shinya Minatoguchi, MD, PhD

**Background:** Levocarnitine has been reported to improve the left ventricular (LV) systolic function and decrease LV hypertrophy in hemodialysis (HD) patients. Its effect on LV diastolic dysfunction, however, has not yet been clarified.

**Methods and Results:** HD patients (n=88) were given levocarnitine i.v. 1,000 mg for 12 months at the end of every dialysis session through the dialysis circuit of the venous site. LV ejection fraction (EF), E/A, E/e', left atrial volume index (LAVI) and LV mass index (LVMI) were measured before and 3, 6, 9, and 12 months after the start of levocarnitine on echocardiography. We regarded  $E/A \leq 0.8$ ,  $E/e' > 14$  and  $LAVI > 34 \text{ mL/m}^2$  as LV diastolic dysfunction, and  $LVEF < 55\%$  as LV systolic dysfunction. We also investigated the effect of levocarnitine on HFpEF. Plasma brain natriuretic peptide, total carnitine, free carnitine, and acyl-carnitine and biochemistry parameters were measured. Levocarnitine significantly improved LV diastolic function in HD patients with LV diastolic dysfunction, but did not affect LV diastolic function in those with normal LV diastolic function. Levocarnitine significantly improved HFpEF. Levocarnitine significantly improved the LV systolic function in HD patients with LV systolic dysfunction but did not affect the LV systolic function in those with normal LV systolic function. Levocarnitine significantly decreased LVMI and increased plasma total, free, and acyl-carnitine.

**Conclusions:** Levocarnitine ameliorates LV diastolic as well as LV systolic dysfunction in HD patients.

**Key Words:** Hemodialysis; HFpEF; Levocarnitine; LV diastolic dysfunction; LV systolic dysfunction

It is widely accepted that carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) deficiency frequently occurs in patients with hemodialysis (HD).<sup>1,2</sup> Carnitine deficiency may contribute to clinical disorders such as cachexia, erythropoiesis-stimulating agent-resistant anemia, glucose intolerance and insulin resistance, muscle weakness, muscle cramp, and endothelial dysfunction.<sup>3,4</sup> Carnitine deficiency has been known to cause cardiac dysfunction.<sup>5</sup> The incidence of cardiovascular disease increases in HD patients, and the prognosis of HD patients with left ventricular (LV)

systolic dysfunction is poor.<sup>6</sup> LV hypertrophy (LVH) is an independent predictor of cardiac death in HD patients.<sup>7</sup> There have been many reports demonstrating the effect of levocarnitine therapy on LV systolic function and LV hypertrophy in HD patients.<sup>8–12</sup> In some studies levocarnitine improved the LV systolic function<sup>8,9,11</sup> and decreased the LV mass index (LVMI),<sup>8,9,12</sup> but others did not show an improved LV systolic function<sup>10</sup> or a decrease in LVH.<sup>10</sup> The effects of levocarnitine on cardiac function and LV hypertrophy were controversial, probably because the number

Received August 27, 2019; revised manuscript received August 27, 2019; accepted August 29, 2019; J-STAGE Advance Publication released online October 11, 2019 Time for primary review: 1 day

Department of Cardiology (J.N., Shingo M., G.Y., H. Okura), Department of Circulatory and Respiratory Advanced Medicine (Shinya M.), Gifu University Graduate School of Medicine, Gifu; Asahi University Hospital, Gifu (H. Ohashi, M.O.); Hashima Municipal Hospital, Hashima (M.S.); Gihoku Kosei Hospital, Yamagata (K.H.); Hirano Hospital, Gifu (A.K., N.T.); Sawada Hospital, Gifu (T.K., K.G.); Chuno Kosei Hospital, Seki (H.T.); Gifu Prefectural Gero Hospital, Gero (T.O.); Yamauchi Hospital, Gifu (K.N.); Gifu Prefectural General Medical Center, Gifu (I.M.); and Gifu Municipal Hospital, Gifu (Shinya M.), Japan  
Shinya M. is a member of *Circulation Reports*' Editorial Team.

Mailing address: Shinya Minatoguchi, MD, PhD, Department of Circulatory and Respiratory Advanced Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. E-mail: minatos@gifu-u.ac.jp

ISSN-2434-0790 All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cr@j-circ.or.jp

of patients was small. A recent randomized controlled study with a large number of HD patients and 12-month treatment with levocarnitine demonstrated that levocarnitine improved the LV ejection fraction (LVEF; LV systolic function) and decreased LVH.<sup>13</sup> The long-term effect of levocarnitine on LV diastolic dysfunction in HD patients, however, has not yet been fully elucidated. Hence, in the present study, we investigated the effects of long-term levocarnitine, focusing on LV diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF) in HD patients.

## Methods

Ninety-six patients who were undergoing HD at 8 hospitals: Asahi University Hospital, Chuno Kosei Hospital, Hashima Municipal Hospital, Sawada Hospital, Yamauchi Hospital, Gihoku Kosei Hospital, Hirano Hospital, and Gifu Prefectural Gero Hospital, were included in this study. The patients were enrolled consecutively according to the following inclusion criteria: (1) HD > 6 months; and (2) no history of carnitine use. The exclusion criteria were: (1) hypersensitivity to levocarnitine; (2) pregnancy; or (3) inclusion judged as inappropriate by the attending physician.

Of the 96 HD patients enrolled, 88 patients could be followed up for 12 months after the start of this study. Eight patients were excluded from the data analysis (3 died of complications and 5 patients did not have data available at 12 months). Of the 88 patients, 61 were male and 27 were female. Mean patient age was  $65.1 \pm 11.4$  years old, and the mean duration of HD was  $4.5 \pm 5.7$  years.

The protocol was approved by the Ethics Committee of Gifu University Graduate School of Medicine (approval number: 26-83). All patients provided written informed consent before the study commenced. The investigation conformed to the principles outlined in the Declaration of Helsinki. The public and trial registry number was R000040056.

### Protocol

The HD patients were given 1,000 mg i.v. levocarnitine at the end of HD through a dialysis circuit of the venous site at the end of every HD session and followed up for 12 months.

### LV Diastolic and Systolic Function

Echocardiography was performed at 24 h after HD to measure LV diastolic and systolic function. With regard to LV diastolic function,  $E/A \leq 0.8$ ,  $E/e' > 14$  (average of septal and lateral site measurement) or left atrial volume index (LAVI)  $> 34 \text{ mL/m}^2$  was defined as LV diastolic dysfunction according to the guidelines of the American Society of Echocardiography and European Association of Cardiovascular Imaging.<sup>14</sup>  $E/A$ ,  $E/e'$  and LAVI were measured before and 3, 6, 9, and 12 months after the start of levocarnitine. To measure LV systolic function, LVEF was measured before and 3, 6, 9, and 12 months after the start of levocarnitine. LVEF  $< 55\%$  was defined as LV systolic dysfunction. LVEF could be followed up in 88 patients.  $E/A$  could be followed up in 87 patients because 1 patient with atrial fibrillation (AF) was excluded.  $E/e'$  could be followed up in 86 patients because  $E/e'$  was not measured in one patient. LAVI could be followed up in 85 patients because 2 patients who could not obtain the data at 12 months were excluded.

### Effect of Levocarnitine on HFpEF

The effect of levocarnitine on HFpEF was investigated. HFpEF was defined as LVEF  $> 50\%$  and LAVI  $> 34 \text{ mL/m}^2$ , LVEF  $> 50\%$  and  $E/e' > 14$ , and LVEF  $> 50\%$  and  $e' < 7 \text{ cm/s}$  (measured at septal site) according to the guidelines for diagnosis and treatment of acute and chronic heart failure (JCS 2017/JHFS 2017).

### LVMI

Echocardiography was performed to measure LVMI before and, 3, 6, 9, and 12 months after the start of levocarnitine.  $\text{LVM (g)} = 0.8 \times \{1.04 \times [(\text{LVDD} + \text{IVSth} + \text{PWth})^3 - (\text{LVDD})^3]\} + 0.6$ , where LVDD is LV end-diastolic dimension, IVSth is interventricular septum thickness, and PWth is posterior wall thickness. Body height and weight were measured to calculate the body surface area; LVM was indexed per square area (LVMI  $\text{g/m}^2$ ).

### Plasma Carnitine

Blood samples were taken from the antecubital veins at 24 h after HD. Plasma carnitine (total carnitine, free carnitine, and acyl-carnitine) were measured using LC/MS/MS before and, 3, 6, and 12 months after the start of levocarnitine treatment.

### Brain Natriuretic Peptide (BNP)

Changes in BNP, an indicator of heart failure, were measured at 24 h after HD before and, 3, 6, 9, and 12 months after the start of levocarnitine.

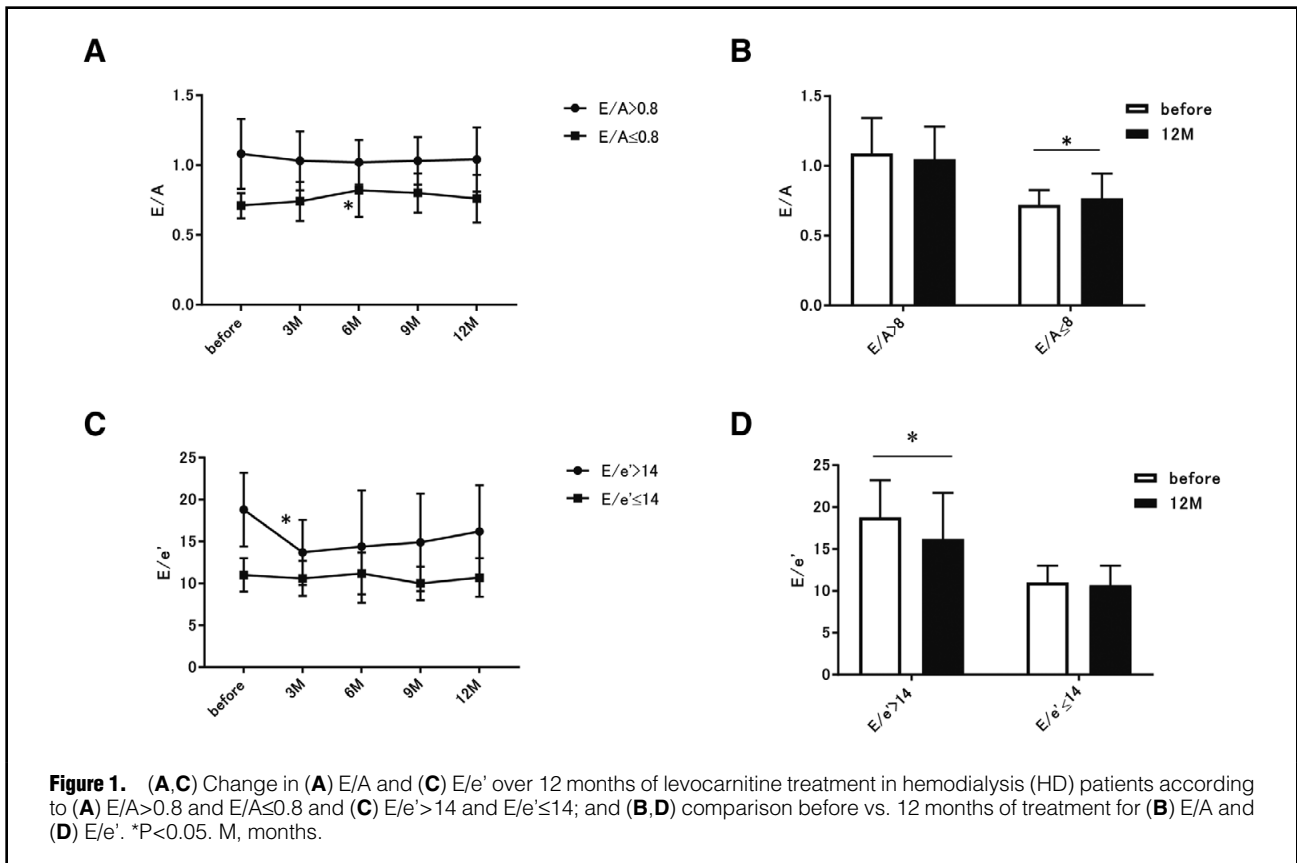
### Blood Biochemistry

Blood samples were taken from the antecubital veins at 24 h after HD. Hemoglobin (Hb), hematocrit (Ht), calcium (Ca), phosphorus (P), intact parathyroid hormone (iPTH), total bilirubin (T-Bil), aspartate transaminase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), blood urea nitrogen (BUN), creatinine (Cr), and BNP were measured before and 3, 6, 9, and 12 months after the start of levocarnitine.

### Statistical Analysis

Data are given as mean  $\pm$  SD. The normality of data distributions was tested using the Kolmogorov-Smirnov test. This was a prospective cohort study. Given that we divided the data into 2 groups: LV normal diastolic function and LV diastolic dysfunction, or LV normal systolic function and LV systolic dysfunction, respectively, there was a concurrent control in this study.

To assess the effect of 12-month treatment of levocarnitine on cardiac function and LVMI, we could not use one-way ANOVA with repeated measures, because the data at 3, 6, and 9 months were sometimes defective. Therefore, the effect of levocarnitine treatment for 12 months was assessed using the perfect paired data before and at 12 months with the paired Student's t-test. In addition, the difference between before and at 12 months was compared between patients with normal LV function and LV dysfunction using the unpaired Student's t-test. Assessment of unpaired data between groups before and at 3, 6, 9, and 12 months was performed using 1-way ANOVA followed by multiple comparison with Tukey method. This statistical analysis was performed using GraphPad Prism 7 (GraphPad Software). Univariate analysis was performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R



(R Foundation for Statistical Computing, Vienna, Austria).  $P<0.05$  was considered significant,  $P<0.01$  was considered highly significant, and  $P<0.001$  was considered very highly significant.

## Results

### Diastolic Function According to E/A, E/e' and LAVI

We divided HD patients into 2 groups before levocarnitine treatment according to E/A: E/A≤0.8 and E/A>0.8 (**Figure 1A**). In the E/A>0.8 group, E/A was  $1.08\pm 0.25$  (n=49),  $1.03\pm 0.21$  (n=46),  $1.02\pm 0.16$  (n=45),  $1.03\pm 0.17$  (n=44), and  $1.04\pm 0.23$  (n=49) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the E/A≤0.8 group, it was  $0.71\pm 0.09$  (n=38),  $0.74\pm 0.14$  (n=38),  $0.82\pm 0.19$  (n=31),  $0.80\pm 0.14$  (n=31), and  $0.76\pm 0.17$  (n=38) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the E/A≤0.8 (LV diastolic dysfunction) group, E/A was significantly increased at 6 months as compared with that before treatment (**Figure 1A**). Levocarnitine significantly increased E/A at 12 months as compared with before levocarnitine treatment when paired data were assessed (**Figure 1B**), but in patients with E/A>0.8 (LV normal diastolic function), levocarnitine did not affect E/A (**Figure 1A,B**).

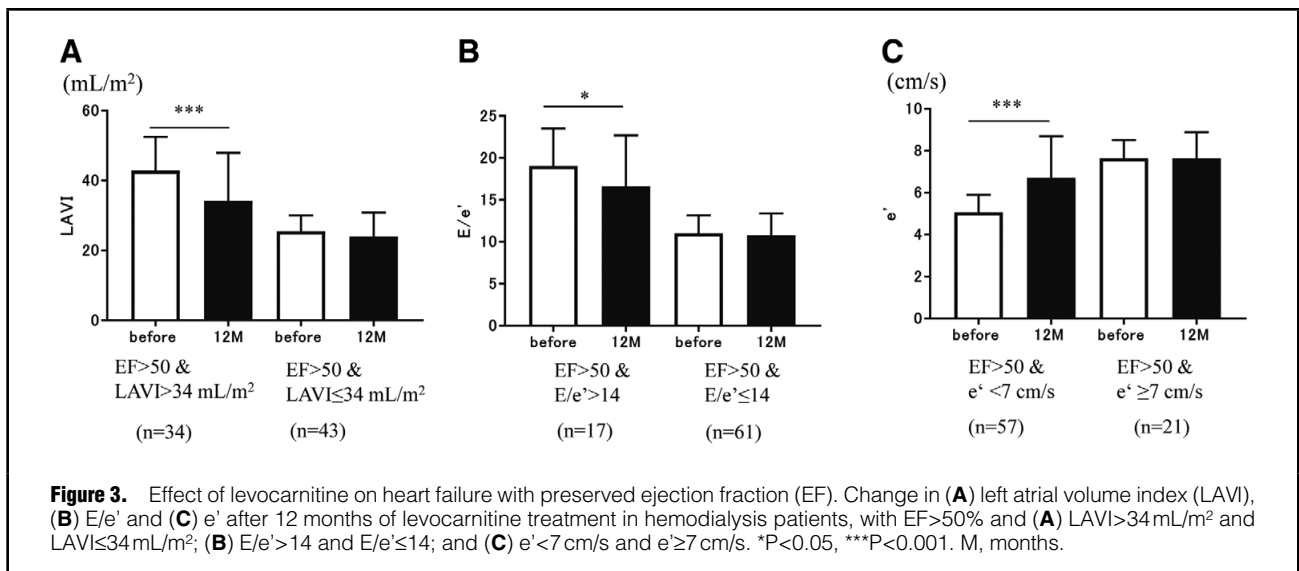
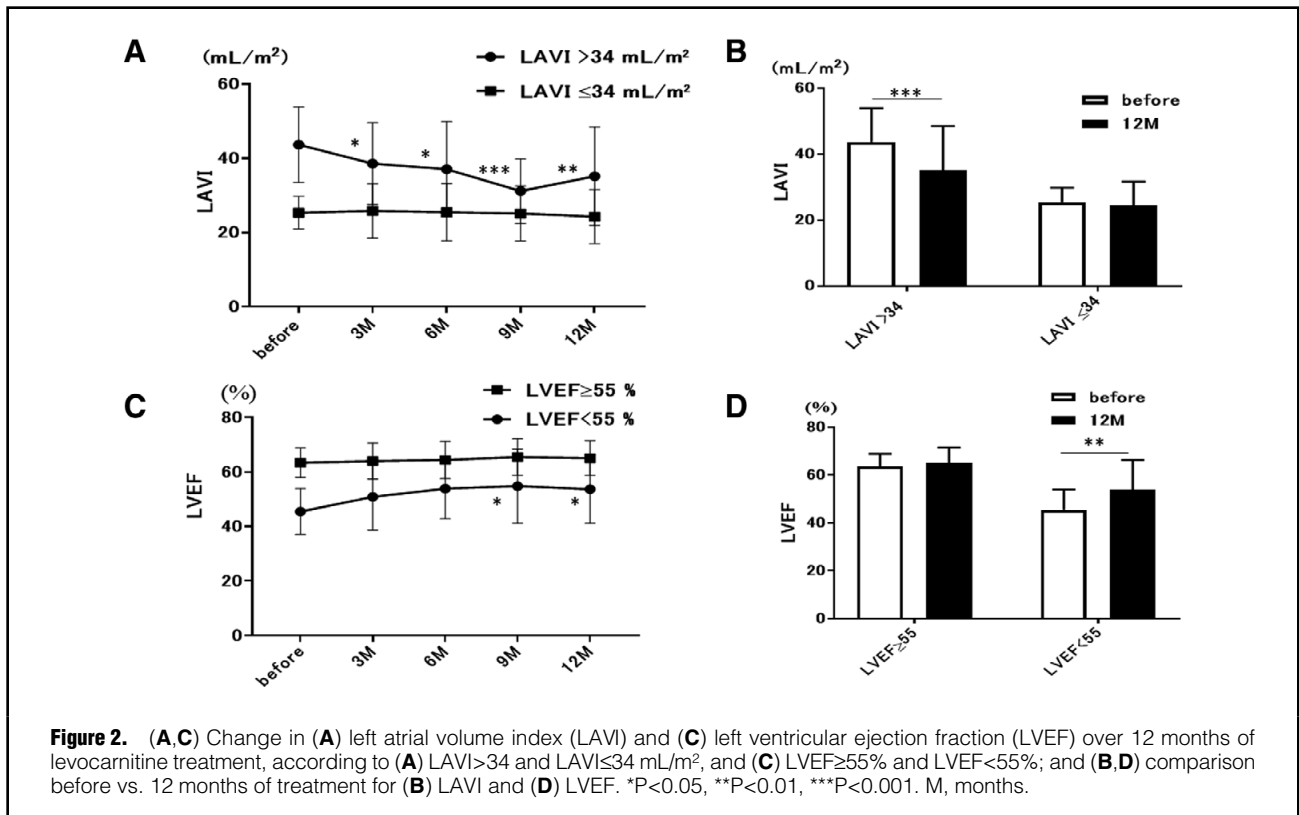
The HD patients were then divided into 2 groups according to E/e': E/e'>14 and E/e'≤14 (**Figure 1C**). In the E/e'>14 group, E/e' was  $18.8\pm 4.4$  (n=24),  $13.7\pm 3.9$  (n=23),  $14.4\pm 6.7$  (n=17),  $14.9\pm 5.8$  (n=19), and  $16.2\pm 5.5$  (n=24) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the E/e'≤14 group, it was  $11.0\pm 2.0$

(n=62),  $10.6\pm 2.1$  (n=62),  $11.2\pm 2.5$  (n=60),  $10.0\pm 2.0$  (n=56), and  $10.7\pm 2.3$  (n=62) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the E/e'>14 (LV diastolic dysfunction) group, E/e' significantly decreased at 3 months as compared with that before treatment (**Figure 1C**). Levocarnitine significantly decreased E/e' at 12 months as compared with that before levocarnitine treatment when paired data were assessed (**Figure 1D**), but in patients with E/e'≤14 (LV normal diastolic function), levocarnitine did not affect E/e' (**Figure 1C,D**).

The HD patients were also divided into 2 groups according to LAVI: LAVI>34 mL/m<sup>2</sup> and LAVI≤34 mL/m<sup>2</sup> (**Figure 2A**). In the LAVI>34 mL/m<sup>2</sup> group, LAVI was  $43.7\pm 10.2$  (n=41),  $38.6\pm 11.0$  (n=40),  $37.1\pm 12.8$  (n=37),  $31.2\pm 8.7$  (n=31), and  $35.2\pm 13.3$  mL/m<sup>2</sup> (n=41) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the LAVI≤34 mL/m<sup>2</sup> group, LAVI was  $25.4\pm 4.4$  (n=44),  $25.9\pm 7.3$  (n=40),  $25.5\pm 7.7$  (n=40),  $25.2\pm 7.4$  (n=43), and  $24.3\pm 7.3$  mL/m<sup>2</sup> (n=44) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In patients with LAVI>34 mL/m<sup>2</sup> (LV diastolic dysfunction), LAVI significantly decreased at 3, 6, 9 and 12 months as compared with before treatment (**Figure 2A**). Levocarnitine significantly decreased LAVI at 12 months as compared with that before levocarnitine treatment when paired data were assessed (**Figure 2B**). In the LAVI≤34 mL/m<sup>2</sup> (LV normal diastolic function) group, however, levocarnitine did not affect LAVI (**Figure 2A,B**).

### LV Systolic Function According to LVEF

We divided patients into 2 groups: LVEF≥55% and

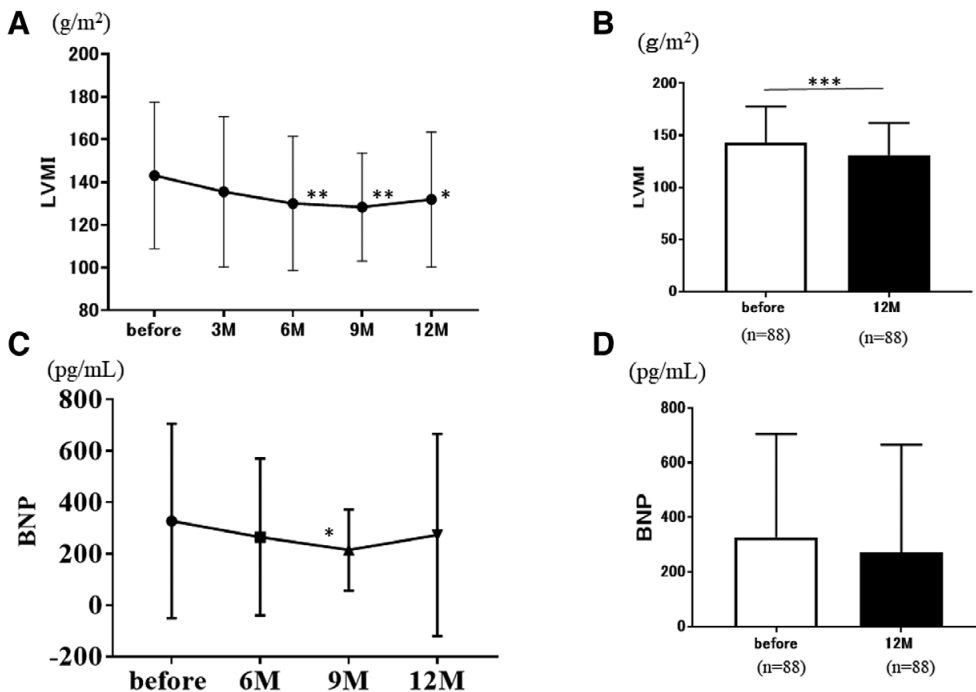


LVEF<55% (Figure 2C). LVEF in patients with LVEF≥55% was 63.4±5.4 (n=72), 64.0±6.6 (n=71), 64.4±6.8 (n=64), 65.5±6.7 (n=65), and 65.1±6.3% (n=72), before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the LVEF<55% group, it was 45.5±8.4 (n=16), 50.9±12.2 (n=15), 53.9±11.0 (n=13), 54.8±13.6 (n=11), and 53.7±12.5% (n=16) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively. In the LVEF<55% (LV systolic dysfunction) group, LVEF significantly increased at 9 and 12 months as compared with that before treatment

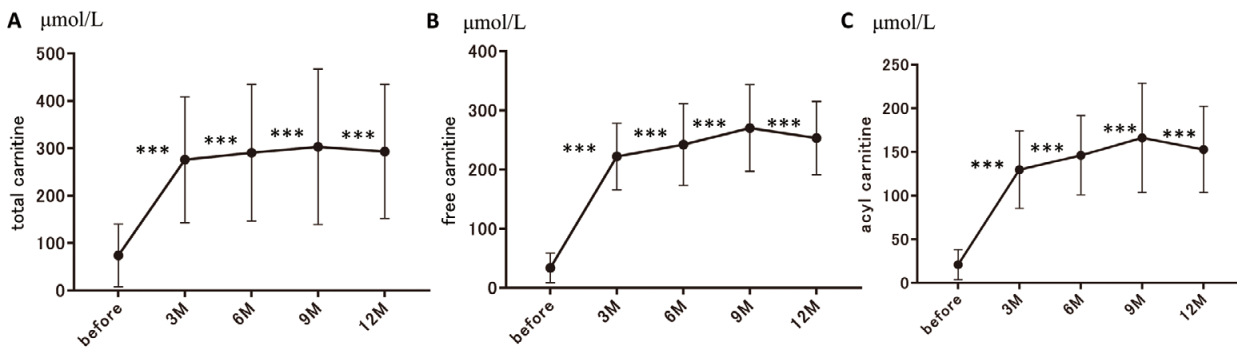
(Figure 2C). Levocarnitine treatment significantly increased LVEF at 12 months as compared with before, when paired data were assessed (Figure 2D). In patients with LVEF≥55% (LV normal systolic function), however, levocarnitine did not affect LVEF (Figure 2C,D).

**Effect of Levocarnitine on HFpEF**

Levocarnitine treatment significantly improved the deteriorated E/e', LAVI and e' at 12 months as compared with before treatment in HFpEF patients (Figure 3).



**Figure 4.** (A,C) Change in (A) left ventricular mass index (LVMI) and (C) brain natriuretic peptide (BNP) over 12 months of levocarnitine treatment in hemodialysis patients; and (B,D) comparison of before vs. after 12 months of treatment for (B) LVMI and (D) BNP. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. M, months.



**D**

	before	3 months	6 months	9 months	12 months
Hb (g/dL)	11.1±0.8 (n=87)	12.2±1.0 (n=87)	11.1±1.2 (n=87)	11.0±1.0 (n=77)	12.0±1.0 (n=87)
Ht (%)	33.4±2.6 (n=87)	33.6±3.1 (n=87)	33.5±3.5 (n=87)	33.3±3.0 (n=77)	36.9±3.2 (n=87)
Ca (mg/dL)	8.5±0.6 (n=87)	8.5±0.7 (n=87)	8.5±0.6 (n=87)	8.4±0.5 (n=77)	8.5±0.5 (n=87)
P (mg/dL)	5.6±1.3 (n=87)	5.4±1.0 (n=87)	5.4±1.0 (n=87)	5.5±0.9 (n=77)	5.5±1.0 (n=87)
PTH (pg/mL)	154.8±103.3 (n=87)	148.1±93.6 (n=87)	147.4±89.1 (n=85)	145.8±89.7 (n=76)	138.8±79.7 (n=87)
T-Bil (mg/dL)	0.29±0.10 (n=87)	0.29±0.09 (n=87)	0.29±0.08 (n=87)	0.27±0.07 (n=73)	0.27±0.07 (n=87)
AST (U/L 37°C)	10.7±3.6 (n=87)	10.4±3.2 (n=87)	10.6±3.7 (n=87)	11.0±3.2 (n=77)	10.2±3.2 (n=87)
ALT (U/L 37°C)	10.1±3.5 (n=87)	9.44±3.0 (n=87)	9.68±3.4 (n=87)	9.5±2.5 (n=77)	9.7±3.0 (n=87)
LDH (U/L 37°C)	209.7±48.3 (n=87)	205.7±45.5 (n=87)	201.3±40.5 (n=87)	208.8±48.3 (n=77)	201.5±42.3 (n=87)
CK (U/L 37°C)	87.0±42.1 (n=87)	92.4±51.9 (n=85)	85.6±41.9 (n=87)	95.2±49.1 (n=75)	88.1±43.8 (n=87)
BUN (mg/dL)	61.0±10.5 (n=87)	60.3±12.6 (n=87)	67.3±61.3 (n=87)	60.3±10.7 (n=77)	61.0±12.3 (n=87)
Cr (mg/dL)	10.6±10.9 (n=87)	9.3±1.7 (n=87)	9.1±1.5 (n=87)	8.9±1.4 (n=77)	9.9±8.5 (n=87)

**Figure 5.** Change in (A) total carnitine, (B) free carnitine and (C) acyl-carnitine over 12 months of levocarnitine treatment in hemodialysis patients. (D) Change in biochemistry data over 12 months of levocarnitine treatment in hemodialysis patients. ALT, alanine aminotransferase; AST, aspartate transaminase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; Ca, calcium; CK, creatine kinase; Cr, creatinine; Hb, hemoglobin; Ht, hematocrit; iPTH, intact parathyroid hormone; LDH, lactate dehydrogenase; P, phosphorus; T-Bil, total bilirubin. \*\*\*P<0.001. M, months.

Table 1. Factors That May Affect HD Patient LV Diastolic Function vs. E/A and E/e'			
	E/A>0.8 (n=49)	E/A≤0.8 (n=38)	P-value
<b>Characteristics</b>			
Age (years)	63.3±9.6	67.4±13.1	0.084
Sex (M/F)	33 (67) / 16 (33)	27 (71) / 11 (29)	0.715
DM	26 (53)	17 (45)	0.447
HTN	41 (84)	68 (48)	0.095
HL	10 (20)	9 (24)	0.718
LVMl decrease	39 (80)	23 (61)	0.052
<b>Drugs used</b>			
ACEI	1 (2)	1 (3)	0.857
ARB	31 (32)	20 (53)	0.324
CCB	34 (69)	21 (55)	0.179
BB	8 (16)	3 (8)	0.245
Diuretics	7 (14)	4 (11)	0.606
Statins	4 (8)	5 (13)	0.454
Anti-DM drugs	8 (16)	6 (16)	0.947
	E/e'>14 (n=24)	E/e'≤14 (n=62)	P-value
<b>Characteristics</b>			
Age (years)	65.0±12.6	65.1±11.1	0.947
Sex M/F	17 (71) / 7 (29)	43 (69) / 19 (31)	0.895
DM	13 (54)	28 (45)	0.459
HTN	15 (63)	51 (82)	0.052
HL	6 (25)	14 (23)	0.814
LVMl decrease	19 (79)	43 (69)	0.369
<b>Drugs used</b>			
ACEI	2 (8)	1 (1)	0.131
ARB	10 (42)	21 (34)	0.505
CCB	14 (58)	19 (30)	0.054
BB	4 (17)	8 (13)	0.856
Diuretics	2 (8)	4 (6)	0.762
Statins	2 (8)	1 (2)	0.131
Anti-DM drugs	4 (15)	6 (10)	0.37

Data given as mean±SD or n (%). A, atrial systolic wave; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta-blockers; CCB, calcium channel blockers; DM, diabetes mellitus; E, early diastolic wave; e', early diastolic wall motion velocity; HD, hemodialysis; HL, hyperlipidemia; HTN, hypertension; LVMl, left ventricular mass index.

### Effect on LVMl

LVMl was 143.1±34.3 (n=88), 135.5±35.2 (n=87), 130.0±31.4 (n=78), 128.3±25.2 (n=79), and 131.9±31.6 g/m<sup>2</sup> (n=88) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively (**Figure 4A**). LVMl significantly decreased at 6, 9, and 12 months as compared with before levocarnitine treatment. Levocarnitine treatment significantly decreased LVMl at 12 months as compared with that before levocarnitine treatment, when paired data were assessed (**Figure 4B**).

### Effect on BNP

BNP was 327.4±377.8 (n=88), 264.4±158.1 (n=81), 214.2±158.1 (n=71), and 273.1±393.3 pg/mL (n=88) before and 6, 9, and 12 months after the start of levocarnitine, respectively. BNP significantly decreased at 9 months as compared with before treatment (**Figure 4C**). Levocarnitine treatment did not affect LVMl at 12 months as compared with that before treatment, when paired data were assessed (**Figure 4D**).

### Plasma Carnitine

Plasma total carnitine was 73.9±66.1 (n=76), 276.0±132.7 (n=87), 290.8±143.8 (n=79), 303.3±163.8 (n=79), and 293.3±141.3 ng/mL (n=87) before and 3, 6, 9, and 12 months after the start of levocarnitine, respectively (**Figure 5A**). Plasma total carnitine significantly increased at 3, 6, 9, and 12 months after the start of levocarnitine treatment as compared with that before treatment. Plasma free carnitine was 33.8±24.9 (n=53), 222.0±56.1 (n=54), 242.1±68.9 (n=46), 270.3±73.3 (n=44), and 253.0±62.0 ng/mL (n=54) before and 6, 9, and 12 months after the start of levocarnitine, respectively (**Figure 5B**). Plasma free carnitine significantly increased at 3, 6, 9, and 12 months after the start of levocarnitine as compared with that before treatment. Plasma acyl-carnitine was 20.0±17.3 (n=54), 129.7±44.4 (n=54), 146.2±45.5 (n=46), 166.2±62.5 (n=44), and 152.9±49.2 ng/mL (n=54) before and 3, 6, 9, and 12 months after the start of levocarnitine treatment, respectively, and significantly increased at 3, 6, 9 and 12 months after the start of levocarnitine as compared with that

<b>Table 2. Factors That May Affect HD Patient LV Diastolic Function vs. LAVI and LVEF</b>			
	<b>LAVI&gt;34 (n=41)</b>	<b>LAVI≤34 (n=44)</b>	<b>P-value</b>
<b>Characteristics</b>			
Age(years)	64.4±12.3	65.4±10.8	0.655
Sex (M/F)	26 (63) / 15 (37)	34 (77) / 10 (23)	0.727
DM	18 (44)	21 (47)	0.727
HTN	27 (66)	41 (93)	0.004
HL	9 (22)	10 (22)	0.933
LVMl decrease	28(68)	32 (72)	0.779
<b>Drugs used</b>			
ACEI	1 (2)	1 (2)	0.96
ARB	22 (54)	31 (70)	0.113
CCB	24 (59)	32 (72)	0.172
BB	2 (5)	8 (18)	0.101
Diuretics	5 (12)	4 (9)	0.101
Statins	3 (7)	7 (16)	0.224
Anti-DM drugs	8 (20)	10 (22)	0.721
<b>Characteristics</b>			
	<b>LVEF≥55 (n=72)</b>	<b>LVEF&lt;55 (n=16)</b>	<b>P-value</b>
Age (years)	65.8±10.6	62.1±14.7	0.215
Sex (M/F)	47 (65) / 25 (35)	14 (88) / 2 (12)	0.112
DM	35 (49)	7 (44)	0.74
HTN	58 (81)	11 (69)	0.586
HL	15 (21)	2 (13)	0.592
LVMl decrease	50 (69)	12 (75)	0.678
<b>Drugs used</b>			
ACEI	2 (3)	0 (0)	0.503
ARB	46 (64)	7 (44)	0.337
CCB	51 (71)	8 (50)	0.276
BB	8 (11)	3 (19)	0.262
Diuretics	7 (10)	4 (25)	0.262
Statins	7 (10)	1 (6)	0.966
Anti-DM drugs	14 (19)	2 (13)	0.679

Data given as mean±SD or n (%). Abbreviations as in Table 1.

before treatment (Figure 5C).

### Blood Biochemistry

The levels of Hb, Ht, Ca, P, PTH, T-Bil, AST, ALT, LDH, CK, BUN and Cr before and 3, 6, 9, and 12 months after levocarnitine treatment are listed in Figure 5D. There was no significant change in any parameter during the 12 months.

### Factors That May Affect LV Diastolic and Systolic Function

On univariate analysis, some factors that may be associated with LV diastolic function were compared according to  $E/A \leq 0.8$  and  $E/A > 0.8$ , and according to  $E/e' > 14$  and  $E/e' \leq 14$  groups (Table 1), and between  $LAVI > 34$  and  $LAVI \leq 34$  mL/m<sup>2</sup> (Table 2). There was no difference in these factors between  $E/A \leq 0.8$  and  $E/A > 0.8$ , or between  $E/e' > 14$  and  $E/e' \leq 14$ . There was also no difference in these factors between  $LAVI > 34$  and  $LAVI \leq 34$  mL/m<sup>2</sup>, except for hypertension, which was more frequent in the  $LAVI \leq 34$  mL/m<sup>2</sup> group, which might not have contributed to the improvement of LAVI in the  $LAVI > 34$  mL/m<sup>2</sup> group (Table 2). There was no difference in these factors between the  $LVEF \geq 55\%$  and  $LVEF < 55\%$  groups (Table 2).

### Discussion

Carnitine is a natural compound mainly derived from dietary sources such as red meat, fish, and dairy products. In humans, most carnitine is absorbed from the small intestine,<sup>15</sup> although a small amount is synthesized in the liver, kidney, and brain from the amino acids lysine and methionine. Most total body carnitine is found in skeletal muscle, and a small amount is found in the liver and kidney.<sup>16</sup> The myocardium and skeletal muscle totally depend on carnitine uptake from the blood. Carnitine is present in free and esterified forms. In the myocardium, carnitine plays a crucial role in energy metabolism of both fatty acids and carbohydrates,<sup>17</sup> and in transporting activated long chain fatty acids (acyl-CoAs) from the cytosol into the mitochondrial matrix where  $\beta$ -oxidation and the subsequent production of ATP occur.<sup>18-20</sup>

Carnitine deficiency in HD patients is caused by the loss of carnitine during HD,<sup>21</sup> and contributes to the pathogenesis of cardiomegaly in HD patients.<sup>22</sup> As noted, carnitine is involved in myocardial fatty acid metabolism by transporting long-chain fatty acids from the cytoplasm to

the mitochondrial matrix for  $\beta$ -oxidation,<sup>23</sup> and the normal fatty acid metabolism requires adequate carnitine concentration in intracellular compartments, especially in myocardial cells, which preferentially use fatty acids rather than glucose.<sup>24</sup> Therefore, theoretically, treatment with levocarnitine would improve cardiac function through the improvement of impaired myocardial fatty acid metabolism in HD patients. This has also been demonstrated in many reports, which found that levocarnitine treatment improved LVEF, an indicator of LV systolic function, in HD patients.<sup>8,9,11,12</sup>

It has also been reported that LV diastolic dysfunction commonly occurs in HD patients.<sup>25</sup> Furthermore, LV diastolic dysfunction is a significant risk factor for cardiovascular events in HD patients.<sup>26,27</sup> Therefore, if LV diastolic dysfunction in HD patients is improved, the prognosis of HD patients with LV dysfunction may be improved. Reports on whether levocarnitine treatment improves LV diastolic dysfunction in HD patients, however, are very few. One report stated that levocarnitine improves LVEF but not E/A or E/e' in HD patients.<sup>28</sup> In another report, levocarnitine did not improve LA dilation or E/A, but improved only E/e' at 12 months in HD patients.<sup>13</sup> In these reports, however, the LV function was not divided into 2 groups: normal LV diastolic function and LV diastolic dysfunction.<sup>13,28</sup> In the present study, however, we have shown for the first time that levocarnitine treatment for 12 months improved the LV diastolic function in HD patients with LV diastolic dysfunction on E/A, E/e' and LAVI. Levocarnitine treatment, however, did not change the LV diastolic function in patients with normal LV diastolic function as assessed on the same parameters. Furthermore, LV diastolic dysfunction was assessed in patients with HFpEF, with EF>50% using LAVI, E/e' and e' according to the guidelines for diagnosis and treatment of acute and chronic heart failure (JCS 2017/JHFS 2017). As a result, LV diastolic dysfunction as assessed on those parameters was improved in HFpEF patients (Figure 3). This suggests that levocarnitine improves LV diastolic dysfunction with preserved LV systolic function.

In the present study, levocarnitine treatment improved the LV systolic function in HD patients with LV systolic dysfunction assessed on LVEF, which is consistent with previous reports.<sup>8,9,11,13</sup> Levocarnitine treatment, however, did not change the LV systolic function in patients with normal LV systolic function as assessed on LVEF.

Therefore, according to the present data, levocarnitine treatment is beneficial for improving the LV diastolic function or LV systolic function only when these functions have deteriorated.

Cardiac myocyte energy metabolism primarily depends on the oxidation of fatty acids. Fatty acid metabolism needs carnitine. Although the precise mechanism by which levocarnitine improves LV systolic and diastolic dysfunction is unclear, 1 candidate mechanism may involve the transportation of activated long chain fatty acids (acyl-CoAs) from the cytosol into mitochondrial matrix with  $\beta$ -oxidation and ATP production, and regulation of carbohydrate metabolism by modulating the intra-mitochondrial acyl-CoA to CoA ratio.<sup>18-20</sup> In the present study, levocarnitine treatment significantly increased plasma total and free carnitine and acyl-carnitine, which might improve LV diastolic dysfunction (Figure 5A–C). Levocarnitine treatment did not affect the blood biochemistry in the present study (Figure 5D), suggesting that levocarnitine did not

cause side-effects.

LV diastolic dysfunction may be involved in the decrease in LVMI, an indicator of LV hypertrophy, because LV hypertrophy has been reported to be associated with LV diastolic dysfunction.<sup>29</sup> In the present study, however, LVMI decrease was not associated with improvement of LV dysfunction (Tables 1,2).

BNP significantly decreased at 9 months after the start of levocarnitine treatment, as compared with that beforehand (Figure 4C), but there was no significant difference in plasma BNP between before and after 12 months of levocarnitine treatment, when paired data were assessed (Figure 4D). BNP has generally been considered an indicator of valve disease, LVH, AF, LV diastolic dysfunction, and LV systolic dysfunction.<sup>30</sup> BNP is a sensitive indicator of acute changes in the volume status of HD patients.<sup>31</sup> This may explain why changes in the LV diastolic function or LV systolic function before and after levocarnitine were not necessarily associated with changes in BNP in the present study.

Of the many factors such as age, sex, diabetes mellitus, hypertension, hyperlipidemia, smoking and drugs used, none of them affected the improvement of LV diastolic or LV systolic dysfunction (Tables 1,2).

### Study Limitations

First, it was difficult to clarify the precise mechanism by which levocarnitine improved LV diastolic dysfunction in HD patients in the clinical setting of this study. Second, although this was a prospective cohort study with a concurrent control, a placebo-controlled study is warranted. Third, because the number of HD patients examined was relatively small, a clinical study with a larger number of HD patients is required.

### Conclusions

Levocarnitine treatment ameliorates LV diastolic dysfunction and HFpEF as well as LV systolic dysfunction in HD patients.

### Author Contributions

Shinya M. & H. Ohashi designed the experiment, J.N., H. Ohashi, M.O., M.S., K.H., A.K., N.T., T.K., H.T., T.O., K.N., K.G., I.M., and G.Y. collected data, Shingo M. performed statistical analysis, H. Okura performed data interpretation, and J.N. and Shinya M. wrote the manuscript.

### Acknowledgments

We thank Mrs Kaori Osawa for technical assistance.

### Source of Funding

This study was supported by funding from Gifu University Graduate School of Medicine (to Shinya M.).

### Disclosures

Shinya M. is a member of *Circulation Reports*' Editorial Team. The other authors declare no conflicts of interest.

### References

1. Ahmad S. L-Carnitine in dialysis patients. *Semin Dial* 2001; **14**: 209–217.
2. Evans AM, Faul RJ, Nation RL, Prasad S, Elias T, Reuter SE, et al. Impact of hemodialysis on endogenous plasma and muscle carnitine levels in patients with end-stage renal disease. *Kidney Int* 2004; **66**: 1527–1534.



3. Shreiber B. Levocarnitine and dialysis: A review. *Nutr Clin Pract* 2005; **20**: 218–243.
4. Matera M, Bellinghieri G, Costantino G, Santoro G, Calvani M, Savica V. History of L-carnitine: Implication for renal disease. *J Ren Nutr* 2003; **13**: 2–14.
5. Hart ZH, Chang CH, Di Mauro S, Farooki Q, Ayyar R. Muscle carnitine deficiency and fatal cardiomyopathy. *Neurology* 1978; **28**: 147–151.
6. Zoccali C, Benedetto FA, Tripepi G, Mallamaci F, Rapisarda F, Seminara G, et al. Left ventricular systolic function monitoring in asymptomatic dialysis patients: A prospective cohort study. *J Am Soc Nephrol* 2006; **17**: 1460–1465.
7. Silberberg JS, Barre PE, Prichard SS, Sniderman AD. Impact of left ventricular hypertrophy on survival in end-stage renal disease. *Kidney Int* 1989; **36**: 286–290.
8. Van Es A, Henny FC, Kooistra MP, Lobatto S, Scholte HR. Amelioration of cardiac function by L-carnitine administration in patients on haemodialysis. *Contrib Nephrol* 1992; **98**: 28–35.
9. Fagher B, Cederblad G, Monti M, Olsson L, Rasmussen B, Thysell H. Carnitine and left ventricular function in hemodialysis patients. *Scand J Clin Lab Invest* 2008; **45**: 193–198.
10. Sakurabayashi T, Miyazaki S, Yuasa Y, Sakai S, Suzuki M, Takahashi S, et al. L-Carnitine supplementation decreases the left ventricular mass in patients undergoing hemodialysis. *Circ J* 2008; **72**: 926–931.
11. Romaqnoli GF, Naso A, Carraro G, Lidestri V. Beneficial effects of L-carnitine in dialysis patients with impaired left ventricular function: An observational study. *Curr Med Res Opin* 2002; **18**: 172–175.
12. Maruyama T, Abe M. Effects of levocarnitine treatment on the cardiac function in hemodialysis patients. *Intern Med* 2018; **57**: 3503–3505.
13. Higuchi T, Abe M, Yamazaki T, Okawa E, Ando H, Hotta S, et al. Levocarnitine improves cardiac function in hemodialysis patients with left ventricular hypertrophy: A randomized controlled trial. *Am J Kidney Dis* 2016; **67**: 260–270.
14. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016; **29**: 277–314.
15. Rebouche CJ, Chenard CA. Metabolic fate of dietary carnitine in human adults: Identification and quantification of urinary and faecal metabolites. *J Nutr* 1991; **121**: 539–546.
16. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schönekeess BO. Regulation of fatty acid oxidation in the mammalian heart in health and disease. *Biochem Biophys Acta* 1994; **1213**: 263–276.
17. Schönekeess BO, Lopaschuk GD. The effects of carnitine on myocardial carbohydrate metabolism. In: de Jong JW, Ferrari R, editors. *The carnitine system: A new therapeutical approach to cardiovascular diseases*. Dordrecht: Kluwer Academic Publishers, 1995; 39–52.
18. Engel AG, Rebouche CJ. Carnitine metabolism and inborn errors. *J Inher Metab Dis* 1984; **7**(Suppl 1): 38–43.
19. McGarry JD, Brown NF. Mitochondrial carnitine palmitoyl transferase system: From concept to molecular analysis. *Eur J Biochem* 1997; **244**: 1–14.
20. Flanagan JL, Simmons PA, Vehige J, Willcox M, Garrett Q. Role of carnitine in disease. *Nutr Metab* 2010; **7**: 30.
21. Bohmer T, Bergrem H, Eiklid K. Carnitine deficiency induced during intermittent haemodialysis for renal failure. *Lancet* 1978; **1**: 126–128.
22. Kudoh Y, Shoji T, Oimatsu H, Yoshida S, Kikuchi K, Iimura O. The role of L-carnitine in the pathogenesis of cardiomegaly in patients with chronic hemodialysis. *Jpn Circ J* 1983; **47**: 1391–1397.
23. Fritz IB. Carnitine and its role in fatty acid metabolism. *Adv Lipid Res* 1963; **1**: 285–334.
24. Neely JR, Rovetto MJ, Oram JF. Myocardial utilization of carbohydrate and lipids. *Prog Cardiovasc Dis* 1972; **15**: 289–329.
25. Selby NM, McIntyre CW. The vicious cycle of dialysis-induced cardiac injury: Are dynamic changes in diastolic function involved? *Am J Kidney Dis* 2013; **62**: 442–444.
26. Iwabuchi Y, Ogawa T, Inoue T, Otsuka K, Nitta K. Elevated E/E' predict cardiovascular events in hemodialysis patients with preserved systolic function. *Intern Med* 2012; **51**: 155–160.
27. Han JH, Han JS, Kim EJ, Doh FM, Koo HM, Kim CH, et al. Diastolic dysfunction is an independent predictor of cardiovascular events in incident dialysis patients with preserved systolic function. *Plos One* 2015; **10**: e0118694.
28. Kudoh Y, Aoyama S, Torii T, Chen Q, Nagahara D, Sakata H, et al. Hemodynamic stabilizing effects of L-carnitine in chronic hemodialysis patients. *Cardiorenal Med* 2013; **3**: 200–207.
29. Lazzeroni D, Rimoldi O, Camici PG. From left ventricular hypertrophy to dysfunction and failure. *Circ J* 2016; **80**: 555–564.
30. Struthers AD. Introducing a new role for BNP: As a general indicator of cardiac structural disease rather than a specific indicator of systolic dysfunction only. *Heart* 2002; **87**: 97–98.
31. Sivalingam M, Vilar E, Mathavakkannan S, Farrington K. The role of natriuretic peptides in volume assessment and mortality prediction in haemodialysis patients. *BMC Nephrol* 2015; **16**: 218.