

Re-evaluation of L-carnitine in Chronic Hemodialysis

Yasuo Kudoh

Yasuo Kudoh, Kidney Center, Sapporo South One Hospital, S1W13, Chuou-ku, Sapporo, 060-0061, Japan

Correspondence to: Yasuo Kudoh M.D, Kidney Center, Sapporo South One Hospital, S1W13, Chuou-ku, Sapporo, 060-0061, Japan.

Email: yasuo0302@view.ocn.ne.jp

Telephone: +81-11-271-3711 Fax: +81-11-281-0275

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ABSTRACT

L-carnitine has been administered in chronic hemodialysis patients worldwide for the past decade. Although it has been shown that L-carnitine deficiency can be improved by supplementation, evidence of the beneficial effects has not been fully confirmed clinically. The reason why there are so many controversies among the clinical trials is likely to be due to the heterogeneity of the study protocols, such as the difference of dosage, administration route, duration, sample size, characteristics of subjects, reliable methods, qualified outcomes, but mainly confidence in the study itself whether it is an RCT or not. Even meta-analysis has been influenced strongly by these biases. In order to overcome these uncertainties, a highly sophisticated, large scale RCT has been suggested. However, it might be quite difficult to achieve such a trial at the moment, as Wasserstein pointed out. In this review article, we attempt to reconcile the complicated strands concerning the efficacy of L-carnitine in chronic hemodialysis patients one at a time.

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Key words: L-carnitine; Hemodialysis; Anemia; Hypotension; Physical capacity

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CARNITINE KINETICS

In order to investigate the pharmacokinetics of L-carnitine, one compartment open model^[1] for oral intake, two^[2] and three^[3,4] compartment models for intravenous administration have been utilized respectively. These analyses are quite useful not only to understand the physiological and pathological dynamics of L-carnitine but also to clarify an optimal carnitine supplementation method in terms of tissue carnitine concentration.

1. Absorption

Carnitine intake is reported to be 23-135 mg/day in adults^[5] or 1-15 μ mol/kg/day in human^[6]. Red meat contains rich carnitine such as mutton (209mg/100g), pork loin (70mg/100g) and beef fillet (60mg/100g). On the other hand, vegetables contains little carnitine such as broccoli (0.3mg/100g) and wheat (0.4mg/100g). Therefore, vegans take less than 1 μ mol/kg/day from food^[6]. Plasma L-carnitine levels in strict vegetarians are approximately 10-20 % lower than in adults on a mixed diet^[7]. Carnitine in an omnivorous diet is absorbed from the intestinal mucosa by passive diffusion and active transport (OCTN2: carnitine organic cation transporter) mechanisms^[5]. However, the absorption speed is relatively slow (absorption time constant 0.025 /hr) comparing to the excretion (excretion time constant 0.3 /hr in normal subject)^[1]. Absorption is characterized by slow mucosal uptake, prolonged mucosal retention, and a very slow mucosal exit process^[8]. After oral L-carnitine, maximum increase in plasma carnitine was observed after several hours^[1,9,10].

In addition, it has been suggested that bioavailability is quite small, such as 16% after 2g^[11], 5% after 6g^[11], 5-18% after 1-6g^[5] and 5-25% after 600mg-7g^[6] administration, respectively. Because there is a threshold of intestinal absorption, a large amount of oral administration is not necessarily effective. It is suggested that the mucosal absorption of carnitine is already saturated at the 2g dose^[11]. Unabsorbed carnitine is excreted in feces and a part of it is converted into butyrobetaine^[6], trimethylamine^[12] and acyl-carnitine^[13] by microbiota in the large intestine.

2. Distribution and tissue uptake

Absorbed carnitine is diffused to extracellular fluid space^[2,14] and taken into tissues by means of active transport^[6] (Figure. 1). The half-life of the distribution phase is about half-hour, while that of the terminal beta phase is 2-6.5 hours after intravenous administration showing dose-related elimination^[2,11]. Because the main energy source is supposed to be lipid in muscle^[15], it is not surprising that the Michaelis Menten (Km) constant (concentration at which membrane transport is half maximally saturated) in muscle is thousand times lower than those in kidney, liver and brain^[16]. Total content of l-carnitine is estimated to be 120 mmol in healthy adult^[17,18], and 98% of body carnitine is found in skeletal and cardiac muscle^[4].

Cultured muscle cells demonstrated that a high affinity component with a Km of 2-5 μM , as well as a low affinity component with a Km of 80 μM which was changed during maturation^[19,20]. Carnitine transport in muscle strips was with a Km of 60 μM ^[19]. Rebouche summarized^[21] that skeletal muscle carnitine content was only dependent on plasma carnitine concentration at low concentrations (<70 μM). In our recent study, it is demonstrated that the active transport might be mainly depend on high affinity component (5-20 μM) in chronic hemodialysis patients^[22]. Therefore, the normal plasma carnitine concentration of 40 $\mu\text{M/L}$ is sufficient to yield near maximal rates of muscle carnitine uptake^[18].

Although passive diffusion is suggested in the human culture muscle under extremely high concentrations^[20], it is not proved *in vivo*^[18] and the effect of passive diffusion could be too small to be considered in clinical condition.

3. Synthesis and metabolism

L-carnitine is a low molecular amine (molecular weight 162) which is produced in liver, kidney and epididymis^[23] (Figure 2). Carnitine is synthesized from only synthetic substrate, trimethyl-lysine, which is made by the degradation of nuclear proteins such as histone and myosin. Because there is no methylase in mammals, lysine, monomethyl-lysine and dimethyl-lysine are not counted as synthetic substrates of carnitine. Trimethyl-lysine is changed to butyrobetaine in each tissue. Because the urinary excretion of trimethyl-lysine is quite large^[24], it is speculated that there is an enough supply of synthetic substrate^[25].

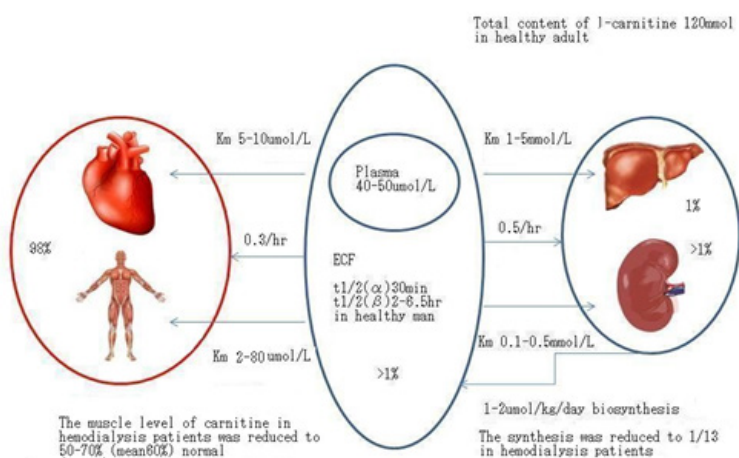


Figure 1 Distribution and tissue uptake of l-carnitine (ref. 22).

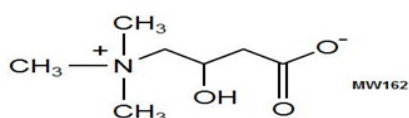


Figure 2 Structure of l-carnitine.

It has been reported^[26] that the limiting factors of carnitine synthesis are (1) amount of trimethyl-lysine as synthetic substrate; (2) transport of butyrobetaine to liver and kidney; (3) γ -butyrobetaine hydroxylase activity. It is reported^[27] that the transport constant of butyrobetaine to liver is extremely large. It is speculated that the γ -butyrobetaine hydroxylase activity is enough high by means of the studies of human liver and kidney biopsy^[28], and postmortem tissue^[29].

The rate of carnitine biosynthesis in humans has been estimated to be about 1 to 2 $\mu\text{mol/kg}$ body wt/day^[6]. In our previous study^[1], the carnitine synthesis in hemodialysis patients was reduced to less than one tenth of normal subjects. Although a minor degree of decarboxylation to β -methylcholine has been reported^[30], the metabolic clearance of l-carnitine is thought to be negligible^[5].

4. Excretion and tissue turnover

Renal carnitine clearance is reported^[14] to be 1 to 3 ml/min/kg, which is similar to that of creatinine (molecular weight 113). The extent of reabsorption of urinary tubules is about 98-99% under baseline conditions^[23]. On the other hand, the threshold of tubular reabsorption in healthy adults is similar in magnitude to the plasma l-carnitine concentrations^[5]. Therefore, this (threshold homeostatic mechanism) serves to maintain circulating carnitine concentration in a narrow normal range^[6]. Actually, it is reported that urinary recoveries during first 24 hours is 70% after 2g, and 82% after 6g intravenous administration, respectively^[11].

It is well known that some drugs, such as valproic acid^[5], zidovudine^[31], ibuprofen^[32] and so on^[5], interfere with the tubular transport of l-carnitine via OCTN2. In Fanconi's syndrome, the fractional excretion is markedly increased to 20-30%^[19].

Because carnitine is not combined with protein in blood, the extraction rate of dialyzer during single pass is reported to be 74%^[3]. It is estimated that weekly loss (3 session 4hr per week) is about 1000 μmol in hemodialysis patients^[13,33]. The daily excretion of carnitine is 100-300 μmol in humans^[16]. Thus, weekly total carnitine losses are similar in normal subjects and hemodialysis patients^[33,34], although the dialysis loss is irreversible.

Renal clearance of acetyl-l-carnitine was approximately fourfold greater than that of l-carnitine in healthy adult men under baseline condition^[13]. The extraction ratios of l-carnitine and acetyl-l-carnitine during hemodialysis were reported to be about 0.74 and 0.7, respectively^[35]. Therefore, the hemodialysis device lacks selectivity of carnitine derivatives.

The whole body turnover time is reported to be about 66 days^[4]. The rate constant for the exit of l-carnitine from muscle into the central compartment is estimated to be about 0.005/hr, meaning that the half-life of this process is about 5-6 days^[5]. Because skeletal muscle contains 3,000-5,000 nmol total carnitine/g, it has a turnover of several days^[4,18]. The slow turnover of muscle carnitine and the effectiveness of renal reabsorption result in a slow whole body turnover^[19].

PLASMA AND TISSUE CARNITINE CONCENTRATIONS IN CHRONIC HEMODIALYSIS PATIENTS

1. Plasma carnitine level

Plasma carnitine concentration is maintained within narrow range, 40-50 $\mu\text{mol/L}$ in normal subjects. Plasma and serum l-carnitine levels tend to be approximately 10-20% lower in women^[7,36,37]. The extremely high concentration of carnitine in the epididymis by the action of androgen hormones is observed^[16]. Because of the regulation

of steroid hormones^[38], plasma carnitine levels in normal male may be higher than in females. On the other hand, we reported^[37] that chronic hemodialysis results in a significant reduction of plasma carnitine levels and no significant difference is observed in plasma carnitine levels between male and female. (Figure 3 left) It is also demonstrated^[39] recently that the histogram of plasma l-carnitine concentration in hemodialysis patients shifted to left side (lower concentration area), comparing to that in normal subjects. (Figure 3 right).

However, there is still disagreement about pre-dialysis plasma level of carnitine. Our result is consistent with those in previous reports^[17,40-42], but not with those from others^[43-46], in which plasma carnitine levels recovered to almost equal or surpass normal levels until next dialysis. Firstly, considering the fact that meat or protein contains rich l-carnitine, the difference in eating habits may be related in part to the discrepancy in pre-dialysis plasma carnitine levels among countries. Secondly, it is interesting to say that there is a negative correlation between plasma carnitine concentration and dialysis duration^[47] (Figure 4 left). Recently, we re-confirmed an exponential correlation between plasma carnitine level and dialysis duration, using larger sample size (more than 100 patients) by means of enzyme cycling method^[39] (Figure 4 right).

Therefore, the reason of discrepancy about plasma carnitine concentration in chronic hemodialysis patients is suggested to be mainly due to the difference of dialysis duration in subjects. Talking about carnitine deficiency in hemodialysis patients, dialysis duration has to be considered, because the dialysis duration is thought to be relatively short in Europe and USA.

The measurement of plasma l-carnitine has been achieved by means of DTNB method or tandem mass spectrometer. However, it becomes easier recently to measure plasma l-carnitine concentration by enzyme cycling method^[39], which enables automation and Kit for the l-carnitine measurement. The studies concerning plasma carnitine level could be done more easily in near future.

2. Muscle carnitine concentration

It is well known that the transport of carnitine from muscle to plasma is quite slow phase^[3,4]. Therefore, muscle carnitine concentration is thought to decrease with apparent time span after the cessation of carnitine supply. Actually, it is reported^[48] that the plasma and muscle carnitine concentration gradually decreased until it reached stable around 14-16 weeks after the end of carnitine treatment.

Therefore, it is reasonable to think that both plasma and muscle carnitine contents might be depleted in chronic hemodialysis patients, if the dialysis duration is enough long^[17,49,50]. In fact, it has been reported that the muscle carnitine content in hemodialysis patients decreased to 50-70% normal^[17,48,51,52] (Figure 5), although a few suggested normal muscle carnitine concentration in hemodialysis patients^[45,46].

PATHOPHYSIOLOGICAL ROLE OF L-CARNITINE IN CHRONIC HEMODIALYSIS PATIENTS

1. Physiological role of l-carnitine

Firstly, l-carnitine is an essential factor for the membrane transport of acyl-CoA compounds, especially across the mitochondrial membrane and β-oxidation of lipid (Figure 6). Therefore, carnitine is a key regulator of mitochondrial energy metabolism. Secondly, l-carnitine also regulates the acetyl-CoA/CoA ratio, thereby affecting the pyruvate dehydrogenase activity and the oxidation metabolism of glucose. Thirdly, carnitine plays an additional role in the cell by

removing potentially toxic organic acids by esterification forming acyl carnitine. Fourthly, several actions of l-carnitine on circulating red blood cell were reported^[53]. L-carnitine and carnitine palmitoyl transferase in red blood cell play a role in terms of membrane phospholipid fatty acid turnover. L-carnitine may improve the viscoelastic properties of red blood cell by intervening on both the outer and inner side of the erythrocyte membrane. Fifthly, the anti-inflammatory activity of l-carnitine^[54] and the prevention of apoptosis^[55] were also suggested.

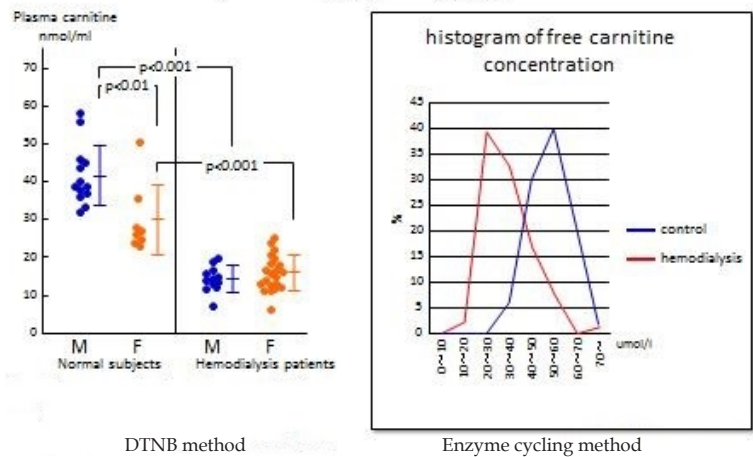


Figure 3 Plasma carnitine level in hemodialysis patients (ref.37, 39).

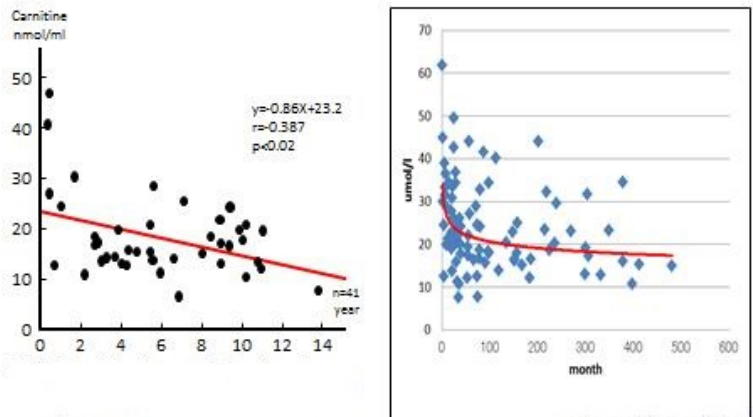


Figure 4 The relationship between plasma carnitine level and dialysis duration (ref. 47, 39).

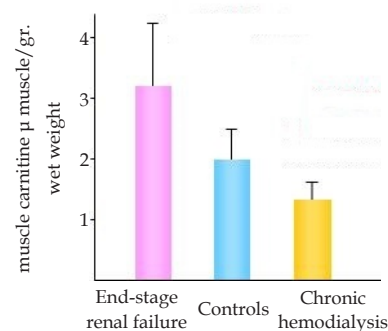


Figure 5 Muscle carnitine concentration in the patients with chronic kidney disease (ref. 17).

2. Pathophysiological role of carnitine deficiency (animal model)

In 1988, Koizumi et al reported^[56] a strain of CH3.OH mouse with visceral fatty infiltration inherited in an autosomal recessive manner. This mouse was later named the juvenile visceral steatosis (JVS) mouse. They subsequently found a marked decrease of the serum, liver, and muscle carnitine levels in JVS mice and established that this mouse was a model of systemic carnitine deficiency.

JVS mice show cardiac hypertrophy which is caused by enlargement of the cardiac muscle cell associated with increases of non-collagen protein and DNA content^[57] (Figure 7). Cardiac and skeletal muscle cells of these mice exhibited a ragged-red appearance after trichrome staining, which is observed in the patients with mitochondrial myopathy^[58]. Electron microscopy demonstrated compression or distortion of the myofibril bundles, the increased number of mitochondria and lipid droplets in the cells.

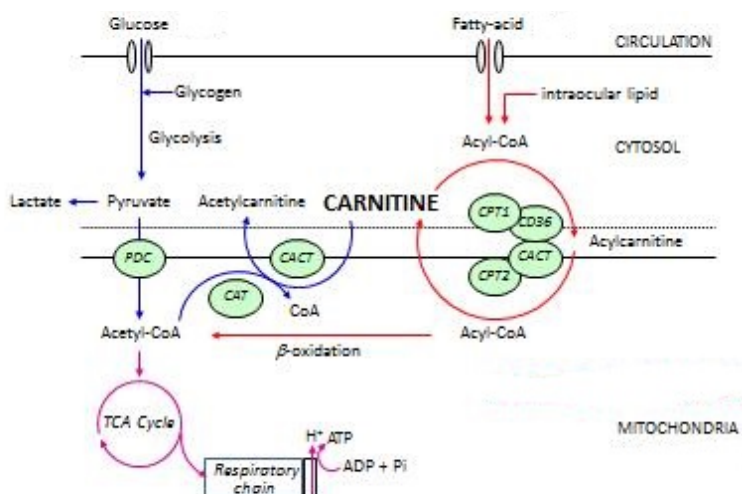


Figure 6 Physiological role of L-carnitine.

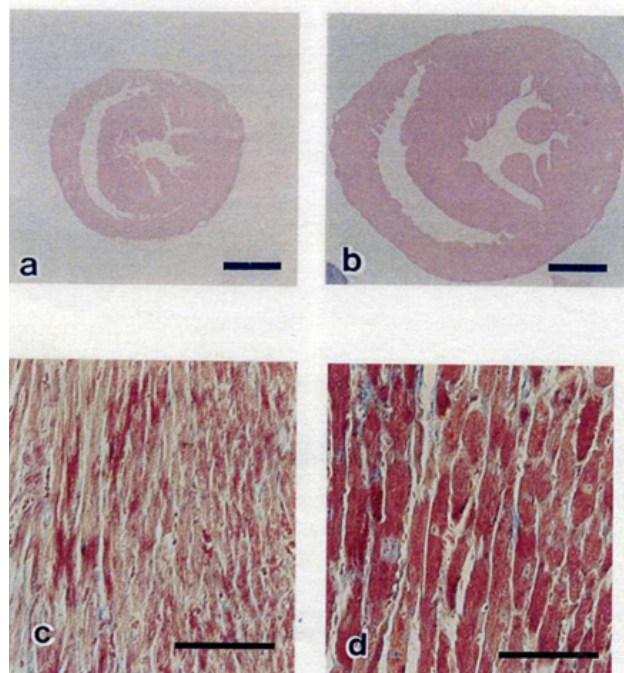


Figure 7 Cardiomyopathy in mice with systemic carnitine deficiency (ref. 57). Hematoxylin-eosin staining of cross-sections of heart (a: control, b: JVS). Compared to the control heart, the JVS mouse heart is much larger. Azan-Mallory staining (c: control, d: JVS). Compared to the control heart, myocyte diameter in JVS heart is longer. Fibrosis in JVS heart is not observed.

In the cardiac muscle cells, the bundles of myofibrils were buried under numerous mitochondria, suggesting the existence of disturbed contractile function in the heart of JVS mice. In fact, most of JVS mice die of a cardiac insufficiency by 3-4 weeks after the birth. It is reported that the content of skeletal muscle carnitine in these mice decreased to 20% normal^[59] and the mechanism of systemic carnitine deficiency might be due to the carnitine transport defect^[60,61].

3. Pathophysiological role of carnitine deficiency (human finding)

It has been reported that inherited systemic carnitine deficiency might be one of the causes of familial cardiomyopathy in human^[62,63]. Therefore, it is reasonable to suppose that acquired carnitine deficiency may be one of the causes of dialysis resistant cardiomegaly in patients with chronic hemodialysis. In our previous study^[37], the CTR (cardio thoracic ratio) was inversely correlated with plasma carnitine levels. This result strongly suggests the above-mentioned hypothesis.

Bertoli et al reported^[17] the presence of numerous lipid droplets within muscle fiber in hemodialysis patients by histological examination. It is also reported^[64] that hemodialysis patients with muscle symptoms exhibit a significantly lower level of free carnitine and higher acyl/free carnitine ratio than those without symptoms. Taking account of a positive correlation between endogenous muscle carnitine content and maximal exercise capacity in hemodialysis patients^[49], systemic carnitine deficiency may influence the morphological and functional damage of muscle in hemodialysis patients.

Despite the absence of mitochondria in mammalian red blood cell (RBC), evidence for a role of carnitine in RBC metabolism is suggested by the presence of carnitine in RBC^[53]. There are some studies that found a significant negative correlation of plasma total carnitine levels and rHuEPO (recombinant human erythropoietin) requirement in hemodialysis patients^[65,66]. Carnitine deficiency might contribute to renal anemia in hemodialysis patients.

OPTIMAL SUPPLEMENTATION METHOD IN CHRONIC HEMODIALYSIS PATIENTS

1. Oral and intravenous administration

L-carnitine has been supplied for the secondary deficiency in chronic hemodialysis patients worldwide as an oral or intravenous use^[22]. However, the direct comparison of oral to intravenous administration has not been reported in these patients^[67].

It has been thought that it might be difficult to maintain high plasma carnitine level and increase the muscle content in healthy control after oral administration^[18,68], because of the low bioavailability and kidney adjustment. However, oral L-carnitine supplementation might be effective in hemodialysis patients, because renal excretion is completely diminished in these patients. Accelerated recovery of post-dialysis plasma carnitine fall by oral carnitine is reported^[10]. It is also reported^[69] that muscle levels of carnitine increased to normal levels after 60 days of oral L-carnitine (2g/day) treatment. Considering about lower bioavailability after larger dose of oral intake, it might be useful that small oral dosage of carnitine is administered frequently.

It is known that there are no serious side effects after oral administration, although minor gastrointestinal symptoms such as transient nausea, vomiting and diarrhea have been reported.

On the other hand, there is no doubt that the intravenous administration of L-carnitine just after hemodialysis procedure is effective. It has not been reported that the supra-physiological level

of plasma carnitine observed after intravenous administration induced any adverse effects.

Fagher *et al* reported^[70] that the muscle carnitine level increased about 60% after 6 weeks of 2g intravenous carnitine administration. It is also reported^[71] that the muscle carnitine level increased 200% after 6 months of 20mg/kg intravenous administration.

If the bioavailability is supposed to be 20%, total amount of L-carnitine absorbed into body is 1,400 mg/week (1,000mg \times 0.2 \times 7) after oral 1,000mg daily supplementation. This amount might be equal to 1,500mg/week (500mg \times 3times) after 500mg intravenous administration (after each dialysis session). Therefore, it is suggested that oral and intravenous supplementation might have equal efficacy from the view point of total carnitine supply, although the plasma carnitine concentration curves after administration were quite different^[22] (Figure 8).

2. Dosage and duration

The dosage of L-carnitine has not been established. L-carnitine treatment regimens used up to now in hemodialysis derive from the empirical evidence of efficacy inferred from previous studies^[48,72]. Weekly necessary dose of L-carnitine in hemodialysis patients would be calculated like below. 1. ordinary oral intake 160mg/w; 2. synthesis 160mg/w; 3. excretion to dialysate 160mg/w (please refer each chapter). Almost 500mg L-carnitine supply weekly (1+2+3) might be enough dosage. Although 20mg/kg of L-carnitine after each dialysis procedure has become the most widely used intravenous dosage in clinical studies^[53], smaller dosage might be sufficient.

The dosages of carnitine supplemented in children on chronic dialysis have been reported to be oral 20mg/kg/day^[73,74] or intravenous 20-40mg/kg three times weekly at the end of each dialysis session^[75], which is almost same as those in adult patients.

Early recovery of muscle carnitine content has been reported 6 weeks after the supplementation^[70]. In our recent studies, improvement of clinical outcomes such as anemia^[76], physical capacity^[77], cardiac function^[78], intradialytic hypotension^[78], has been observed almost 3 months after the administration. Therefore, it is suggested that the duration of administration should be more than 3months and L-carnitine therapy should be discontinued if no clinical improvement has occurred within 9-12 months^[79].

In summary, oral administration of L-carnitine has been recommended to be 1-2 g after hemodialysis session or divided dosage in UK and Germany, and 1.8-3.6 g/day (three times a day) in Japan. However, much smaller dosage such as 500mg/day (three times a day) might be sufficient. Intravenous dosage has been recommended to be 10-20 mg/kg after each hemodialysis session,

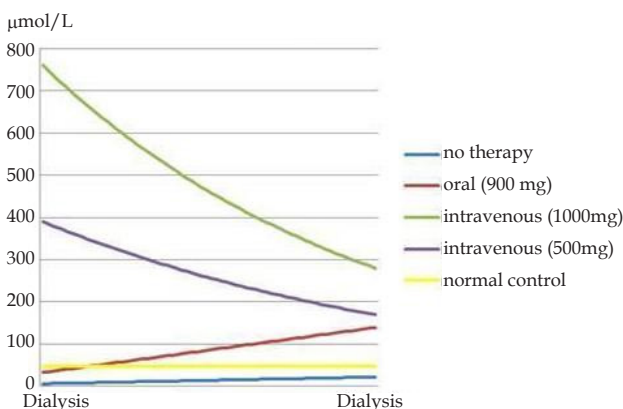


Figure 8 Changes of plasma free carnitine level from the end of dialysis to the next dialysis after each supplementation method (ref. 22).

since 1990th. However, much smaller dosage such as 3-5 mg/kg after each hemodialysis session might be possible. Further examination concerning the dosage of L-carnitine should be necessary.

3. Peritoneal dialysis

Studies concerning the use of L-carnitine for peritoneal dialysis patients are far less than those of hemodialysis patients. Because the plasma free carnitine levels in the peritoneal dialysis patients have been reported to be lower^[80,81], oral supplementation has to be useful. Actually, the beneficial effects of carnitine supplementation on renal anemia^[82] and lipid profile^[83] have been reported in these patients. Oral dosages in those studies were 2g/day^[82] and 50mg/kg^[83] respectively, although the peritoneal administration (2g/bag) has been highlighted recently^[84].

CLINICAL TRIAL OF CARNITINE SUPPLEMENTATION IN CHRONIC HEMODIALYSIS PATIENTS

Carnitine has been given to hemodialysis patients for the first time in 1978 by Bizzi *et al*^[10] in Italy, because of the clinical benefit, as suggested by a report in a patient with systemic carnitine deficiency^[85]. Since that time, many clinical trials using exogenous carnitine administration have been done in hemodialysis patients.

In 1994, AAKP (American Association of Kidney Patients) carnitine renal dialysis consensus group recommended^[72] carnitine treatment for certain conditions in dialysis patients who do not adequately respond to standard therapy, by means of analyzing the results of 42 published studies in approximately 600 hemodialysis patients on the effects of L-carnitine supplementation. Those conditions are 1. muscle cramps and hypotension, 2. lack of energy, 3. skeletal muscle weakness and/or myopathy, 4. cardiomyopathy, 5. anemia of uremia unresponsive to or requiring large doses of EPO. In 1999, the U.S. Food and Drug Administration (FDA) granted approval for the use of the injectable levocarnitine for the prevention and treatment of carnitine deficiency in patients with end-stage renal disease who are undergoing dialysis.

In 2002, Centers for Medicare & Medicaid Services (CMS) referred the matter concerning about a national coverage decision on the use of levocarnitine for end-stage renal disease patients to the Medicare Coverage Advisory Committee (MCAC).

According to the decision memo, a literature search was conducted using the following terms: carnitine, kidney failure, chronic or renal dialysis, or dialysis. Multiple searches resulted in 186 articles. 44 were initially excluded for the following reasons: non-English, non-human subjects, acute renal failure. The following inclusion criteria were applied to these 142 articles: clinical trials, human ESRD subjects, minimum of 10 subjects, published after 1980, clinically relevant outcome measures.

After applying these inclusion criteria, there were 36 articles, including the 16 RCTs, 19 non-randomized clinical trials (primarily crossover design), and one case series. The overall subject population from the 36 studies was approximately 1,100 subjects. Twenty-four studies investigated intravenous administration of carnitine, 12 studies investigated oral administration, and 4 examined carnitine in the dialysate solution. In general, the number of subjects in each study was small. Only 9 of the 36 studies enrolled more than 30 subjects. The study duration varied from as little as four weeks to greater than one year, with a mean follow-up of 23.3 weeks. A majority of these studies utilized double blinded methodology with a placebo control group.

Outcome measures were grouped into following five general categories based on specific outcome measures that are similar to categories used in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI): 1. anemia, 2. intra and interdialytic complications or symptoms, 3. cardiac function, 4. exercise capacity and muscle strength, 5. lipid metabolism.

As a result, intravenous levocarnitine was only covered in ESRD patients who have been on dialysis for a minimum of three months for one of the following indications. Patients must have documented carnitine deficiency, as noted by a pre-dialysis plasma free carnitine level <40 micromol/L, along with signs and symptoms of erythropoietin-resistant anemia or hypotension on hemodialysis.

Soon after this decision, critical comment was published and it was suggested that new randomized prospective controlled trial should be conducted to determine the utility of iv L-carnitine supplementation for anemia management and refractory dialysis associated hypotension^[86].

In 2003, National Kidney Foundation Carnitine Consensus Conference recommended^[79] the administration of L-carnitine for selected dialysis patients with clinical manifestations of dialysis – related carnitine disorder such as anemia that is hyporesponsive to erythropoietin therapy, intradialytic hypotension, cardiomyopathy, and skeletal muscle dysfunction manifested as generalized fatigability.

Although it has been shown that L-carnitine deficiency can be improved by supplementation, evidence of the beneficial effects has not been fully confirmed clinically. The reason why there are so many controversies among the clinical trials is likely to be due to the heterogeneity of the study protocols, such as the difference of dosage, administration route, duration, sample size, characteristics of subjects, reliable methods, qualified outcomes, but mainly confidence in the study itself whether it is an RCT or not. Even meta-analysis has been influenced strongly by these biases. In order to overcome these uncertainties, a highly sophisticated, large scale RCT has been suggested. However, it might be quite difficult to achieve such a trial at the moment, as Wasserstein pointed out^[87]. In this review article, we attempt to reconcile the complicated strands concerning the efficacy of L-carnitine in chronic hemodialysis patients one at a time.

1. Renal anemia

In 2002, the Medicare Coverage Advisory Committee (MCAC) reported that the use of levocarnitine to treat ESRD patients with erythropoietin-resistant anemia is clinically effective, and therefore, reasonable and necessary. A total of eleven studies were reviewed^[69,88-97], eight of which were randomized controlled trials (RCTs)^[88-91,93-96], concerning the effect of carnitine on anemia parameters. All but two studies included less than 30 patients. Eight studies involved IV carnitine, two involved oral carnitine, and one delivered carnitine via dialysate.

Of the eleven studies reviewed, 5 studies reported that carnitine caused an increase or prevented a decrease in hemoglobin or hematocrit compared to placebo^[69,89,91,96,97]. Of the 5 studies that examined erythropoietin requirement, 4 studies showed a decrease in erythropoietin use after treatment with carnitine or an increase in erythropoietin requirements in the control group^[89-92].

However, the therapeutic role of L-carnitine in the anemia of ESRD has been debated, especially in 2005^[98,99], and 2006^[100,101].

In KDOQI clinical practice guideline and clinical practice recommendations for anemia in chronic kidney disease 2006, the Work Group reported that there is insufficient evidence of efficacy to recommend use of L-carnitine in the management of anemia in

patients with CKD^[67]. The statement is supported by results from 6 available RCTs of IV L-carnitine administration to ESR-treated HD patients^[89-91,94,102,103].

Five of the 6 RCTs were judged to be of low quality. The RCTs were characterized by small numbers of enrolled patients, short duration of observation, concomitant use of IV and oral iron, adjustments in ESA dosage, high dropout rates, and uncertainty about specific ESA and/or iron dosing during the study. A probability of bias rendered the overall quality of evidence very low^[67].

In KDIGO clinical practice guideline for anemia in chronic kidney disease 2012, adjuvant therapy including L-carnitine is not suggested, because there is no convincing evidence for recommendation^[104]. However, the quality of the supporting evidence is shown as very low (2D), which means that the estimate of effect is very uncertain, and often will be far from the truth.

In 2011, Bonomini et al reviewed^[53], concerning pharmacological use of L-carnitine in uremic anemia. They suggested that in two studies^[93,103], L-carnitine administration did not show any favorable action on anemia, whereas in two other studies^[89,90] L-carnitine administration seemed to be efficacious in improving the anemic condition only in a sub-group of HD patients.

Labonia WD reported^[91] that L-carnitine treatment promoted a 38.1% reduction in rHuEPO requirement in the active group (1g intravenously after every dialysis session for 6months, $p<0.02$). This active group (responder) was 7 out of 13 patients. Kletzmayer *et al* revealed^[90] that after four months of coadministration of intravenous iron and L-carnitine, the rHuEPO requirement decreased in 8 of 19 evaluable HD patients. In these responders, the weekly rHuEPO dose was decreased significantly by $36.9\pm 23.3\%$ ($p<0.001$). However, the rHuEPO requirement was unchanged when all carnitine-treated patients were compared between at study entry and after four months treatment.

This result was consistent with those of Labonia WD^[91] and the recent our study^[76]. Although the beneficial effect of L-carnitine supplementation on renal anemia was not observed in all patients, at least 40% patients (responder) showed significant improvement of ERI (almost 40% reduction of rHuEPO dose) in our study^[76] (Figure 9). Reuter et al suggested^[66] that the apparent dichotomy of patients

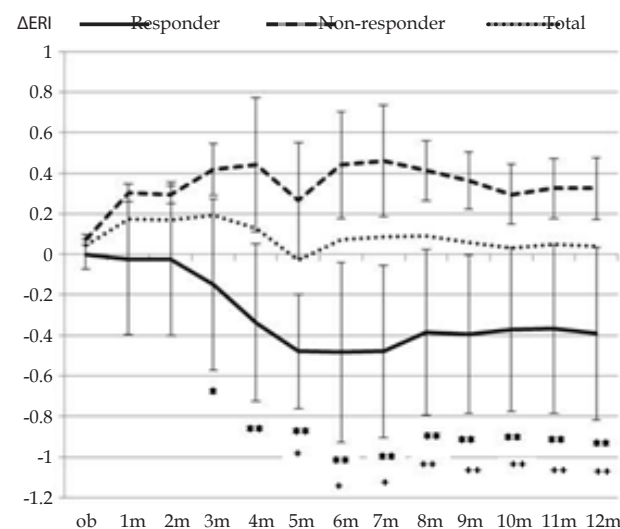


Figure 9 Changes of ERI in responder and non-responder (ref. 76). ERI: erythropoietin resistance index (weekly rHuEPO dose per gram of hemoglobin adjusted by dry weight); ob: observation period; ** $p<0.01$, * $p<0.05$ responder vs non-responder; ++ $p<0.01$, + $p<0.05$ baseline vs at each period.

between responder and non-responder may depend on the proportion of non-acetyl acylcarnitines within the total carnitine pool. This might be coincident with recent our study^[76], because acyl/free carnitine ratio is a strong predicting factor distinguishing responder from non-responder.

Although it might be speculated that a low acyl/free carnitine ratio indicates a better tendency to erythropoietin responsiveness after carnitine supplementation, further examination must be waited to answer the question of which patients would benefit from and should receive l-carnitine.

2. Intradialytic hypotension and other complications

(1) Intradialytic hypotension

According to the comment of the Medicare Coverage Advisory Committee (MCAC) in 2002, the three studies reviewed, Ahmad^[105], Casciani^[106] and Fujita^[107], all showed reduction in hypotensive episodes with carnitine. Although the studies were relatively small and contained methodological flaws, there was consistency and reproducibility of results, the flaws were not fatal, and the magnitude of the benefit was of the moderate size. As a result, the evidence is adequate to conclude that the use of levocarnitine to treat hypotension on hemodialysis that is unresponsive to usual management and interfere with dialysis is clinically effective, and, therefore, reasonable and necessary. The use of l-carnitine for intradialytic hypotension has been described in European Best Practice Guidelines on Haemodialysis (EBPG) 2007^[108] and K/DOQI clinical practice guidelines for cardiovascular disease in dialysis patients 2005^[109].

In 2008, Lynch *et al* reported a meta-analysis concerning the effects of l-carnitine on dialysis-related hypotension^[110]. All published English-language reports of randomized placebo controlled trial of l-carnitine supplementation in adult long-term hemodialysis patients were assessed. Analysis of the 5 studies^[94,103,105,106,111] examining the response of intradialytic hypotension to l-carnitine supplementation yielded a pooled odds ratio of 0.28 (95% confidence interval 0.04-2.23, $p=0.2$). They concluded that the available evidence does not confirm a beneficial effect of l-carnitine supplementation on intradialytic hypotension.

However, the small number of available studies yielded limited statistical power. In addition, there was considerable inter-study heterogeneity. For example, treatment length was short such as 8 weeks in two studies^[106,111]. Mean age of the subjects in two studies

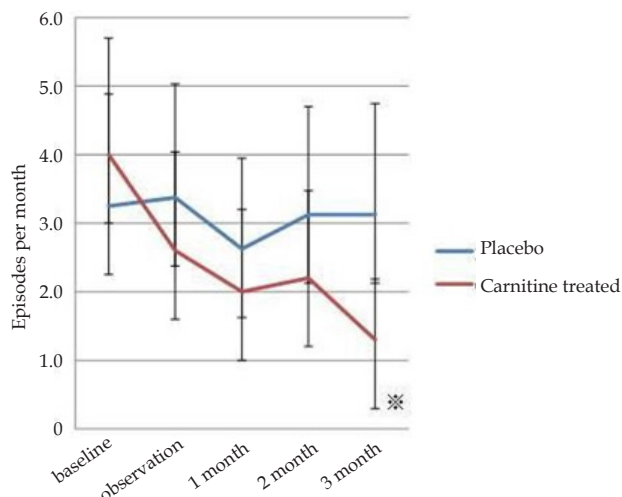


Figure 10 The changes of hypotensive episodes after l-carnitine supplementation (ref. 78).

was relatively young, 47.8 and 43.8 years old, respectively^[105,111]. Outcome considered in two studies was all or nothing assessment, carnitine 1/10 vs placebo 1/10^[111] and carnitine 0/13 vs placebo 0/13^[103], respectively. Therefore, more quantitative analysis should be required.

Recently we reported^[78] that hypotensive episodes significantly decreased from 4 to 1.3 times per month ($p<0.05$) 3 months after the l-carnitine supplementation comparing to placebo, although patient's body weight did not change significantly (Figure 10). The findings might indicate that l-carnitine has great potential for use in the treatment of dialysis-related hypotension.

(2) intradialytic other complications

In 2002, MCAC reported that the evidence is not adequate to conclude that the use of levocarnitine to treat other intra and inter dialytic complications or symptoms, such as muscle cramp, fatigue, asthenia, and quality of the measurements is clinically effective. The studies reported conflicting results, the outcomes were heterogeneous and non-specific, and some studies had important methodological flaws, although 6 out of 9 studies reviewed had positive results^[64,69,70,88,94,95,105,106,112].

In 2008, a meta-analysis revealed^[110] that the available evidence does not confirm a beneficial effect of l-carnitine supplementation on dialysis-related muscle cramping, although suggestive [a pooled odds ratio of 0.30 (95% confidence interval 0.09-1.00, $p=0.05$)].

3. Cardiac function

Although several studies^[113-115] did show significant improvement in ejection fraction ((EF) with the use of levocarnitine, these studies were not adequately controlled. Therefore, it is not possible to determine if the purported increase of EF were caused by levocarnitine or other factors. The patient populations were not the same in the various studies and therefore, conclusion about the clinical effectiveness of levocarnitine cannot be drawn across these studies. Only two randomized double blind control study about the relationship of l-carnitine and cardiac function have been reported so far^[45,78]. Fagher *et al* reported in 1985^[45] that carnitine depletion is not responsible for cardiac dysfunction in patients on hemodialysis and there was no improvement in EF after carnitine supplementation. However, their patients had normal levels of skeletal and blood carnitine. Hemodialysis durations in their patients were not described. Echocardiographic measurements were performed 16-18 h prior to dialysis. The duration of l-carnitine administration in their study was relatively short (6 weeks).

In our current study^[78], it is not emphasized that l-carnitine has a positive inotropic effect, because the increase of ejection fraction in treated group is only 3% and statistical significance is marginal. However, taking account of the significant reduction of hypotensive episodes during hemodialysis procedure, l-carnitine treatment might positively affect cardiac function even for the patients with normal cardiac function.

4. Physical capacity

The benefit of l-carnitine treatment on muscle function and weakness is not established^[116-121]. Although clinical assessment of symptomatology always presents serious problems of interpretation and reliability, studies that used more objective measures to evaluate exercise capacity also revealed conflicting results^[70,88,103,105,122-125]. Most of these trials used heterogeneous design or assessment methods that have been poorly validated in dialysis patients.

Ahmad *et al* reported^[105] that VO₂max (maximal oxygen

consumption) measured during a progressive work exercise test improved significantly and calculated mid-arm muscle area increased in the carnitine treated group (20mg/kg intravenously, 6month) (Figure 11 left). On the other hand, Brass *et al* reported^[88] that L-carnitine supplementation (20mg/kg intravenously, 6month) increased plasma carnitine concentrations, but did not affect VO₂max (Figure 11 right).

After carnitine administration, an increase of total electromyography power was noted, suggesting that carnitine has a prevalent myotrophic effect^[125]. Increased amplitude of the motor action potential, and improved motor unit characteristics were reported^[122]. Conflicting results were reported in another study, in which no significant changes in sensory and motor nerve conduction velocities or in vibration threshold were noted after carnitine treatment^[70].

Carnitine supplementation lowered serum myoglobin^[123] and increased about 7% in the diameter of type I and type IIa fibers, which can utilize carnitine for fatty acid oxidation to produce energy^[124]. However, Vaux *et al* reported that muscle bioenergetics failed to improve after carnitine administration (20mg/kg intravenously, 16week) by the use of magnetic resonance spectroscopy and near-infrared spectroscopy^[103].

In order to clarify the effects of L-carnitine on physical capacity and lipid metabolism, CPX (cardio-pulmonary exercise test using bicycle ergometer) was performed before and after 3 month oral L-carnitine supplementation under double blind conditions, recently^[77]. The exercise time decreased and HR (AT) (heart rate at anaerobic threshold) increased in control group 3 months after study period, but there was no such change observed in treated group. It was indicated that the shift of energy source from carbohydrate to lipid makes oxygen demand increase.

It is reported^[126] that L-carnitine supplementation resulted in RER (respiratory exchange rate) reduction, suggesting an increase in lipid oxidation and possible glycogen sparing. The L-carnitine supplementation might have some beneficial effects on physical capacity in chronic hemodialysis patients due to the improvement of lipid metabolism in muscle.

5. Lipid profile

There is little data to support the use of levocarnitine for the treatment of any type of dyslipidemia^[118,127]. The majority of studies showed no significant changes in lipid parameters, although a few recent meta-analyses suggest a promising effect of L-carnitine on LDL-cholesterol^[128,129]. Moreover, there are well-established effective lipid lowering therapies that already exist. There were no studies that directly compared carnitine therapy to conventional lipid lowering therapy.

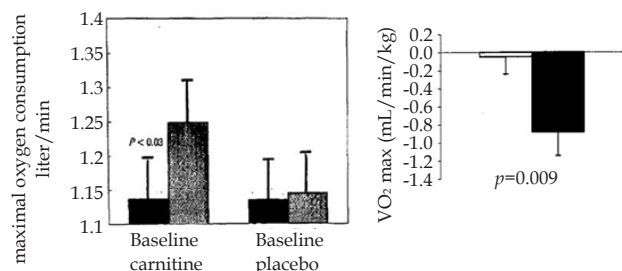


Figure 11 Effect of L-carnitine on VO₂ max in hemodialysis patients (ref. 105, 88). Left: maximal oxygen consumption, measured during a progressive work exercise test, improved significantly in the carnitine group (ref. 105); Right: L-carnitine therapy was associated with a statistically significant smaller deterioration in VO₂ max (□ carnitine ■ placebo) (ref88).

TRIMETHYLAMINE-N-OXIDE (TMO) FORMATION AFTER ORAL L-CARNITINE ADMINISTRATION

Recently it is highlighted that TMO from intestinal microbial metabolism of L-carnitine may enhance cardiovascular risk via promoting atherosclerosis lesion development^[130]. However, it is still premature to conclude whether oral L-carnitine supplementation is really harmful at the moment^[131].

Tang *et al* reported^[132] that participants who had major adverse cardiovascular events had higher baseline TMO levels (3.0-8.8 μM) compared with those who did not have cardiovascular events (2.4-5.9 μM). On the other hand, Bain *et al* demonstrated^[12] that plasma concentrations of TMO were continually rising and approximately doubled from 289±236.1 to 529±237.9 μM in a two-week period after oral administration of L-carnitine at a dose of 1g daily. It is interesting to say that the plasma levels of TMO in hemodialysis patients already increased more than 40 times higher than that in risky group reported by Tang *et al*.

Recently, it has been reported that there was no correlation between TMO and atherosclerosis in hemodialysis patients^[133,134]. Fukami *et al* reported^[133] that although oral L-carnitine supplementation was associated with increased TMO levels, it might be beneficial on vascular injury in patients on hemodialysis because of decreasing markers of vascular injury and oxidative stress such as soluble forms of intracellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and malondialdehyde (MDA) levels.

It is also reported^[134] that serum TMO concentrations were markedly elevated and correlated directly with biochemical markers of nutritional status and inversely with markers of inflammation in patients receiving hemodialysis. There was no significant association between serum TMO concentrations and all-cause mortality, cardiovascular death, or hospitalizations. For now, there is no specific rationale to restrict from oral L-carnitine supplementation in hemodialysis patients.

CONFLICT OF INTERESTS

There is no conflict of interest.

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Peer reviewer: Riham Eid El-Sayed Abo-Sayed Ahmed, Pediatric Nephrology and Dialysis Unit, Mansoura University Children's Hospital, Mansoura University, Mansoura, Egypt.