Pharmacokinetics of L-carnitine in patients with end-stage renal disease undergoing long-term hemodialysis

Objective: **L-Carnitine is an endogenous molecule involved in fatty acid metabolism. Secondary carnitine deficiency may develop in patients with end-stage renal disease undergoing long-term hemodialysis because of dialytic loss. In these patients L-carnitine can be administered to restore plasma and tissue levels. The objective of this study was to evaluate the pharmacokinetics of intravenous L-carnitine in patients undergoing long-term hemodialysis.**

Methods: **Twelve patients undergoing three dialysis sessions/week received L-carnitine intravenously (20 mg · kg^{-1}) at the end of each dialysis session for 9 weeks. Plasma samples were analyzed for L-carnitine, acetyl-L-carnitine, and total carnitine by HPLC.**

Results: **Under baseline conditions, the mean ± SD predialysis plasma concentration of L-carnitine was 19.5 ± 5.6 μmol/L, decreasing to 5.6 ± 1.9 μmol/L at the end of the dialysis session. These concentrations were substantially lower than endogenous levels in healthy human beings. Under baseline conditions the extraction ratios of L-carnitine and acetyl-L-carnitine by the dialyser were 0.74 ± 0.07 and 0.71 ± 0.11, respectively. During repeated dosing, there was accumulation of L-carnitine in plasma, and after 9 weeks of dosing, the predialysis and postdialysis plasma levels were 191 ± 54.1 and 41.8 ± 13.0 μmol/L, respectively. The predialysis and postdialysis plasma levels of L-carnitine decreased once dosing was ceased but had not returned to pretreatment levels after 6 weeks.**

Conclusion: **The study demonstrated that removal of L-carnitine by hemodialysis is extremely efficient and that patients undergoing hemodialysis had plasma concentrations that were substantially lower than normal, particularly during dialysis. During repeated administration of L-carnitine, the predialysis and postdialysis concentrations of the compound increased steadily, reaching an apparent steady state after about 8 weeks. It is proposed that this accumulation arose from the distribution of L-carnitine into a deep tissue pool that includes skeletal muscle. (Clin Pharmacol Ther 2000;68:238-49.)**

Allan M. Evans, PhD, Randall Faull, PhD, Gianfranco Fornasini, PhD, Edward F. Lemanowicz, PhD, Antonio Longo, PhD, Silvia Pace, PhD, and Roger L. Nation, PhD *Adelaide, Australia*

Levocarnitine [(*R*)-3-carboxy-2-hydroxy-*N,N,N*-trimethyl-*l*-propanaminium, inner salt] is an endogenous compound with well established functions in intermediary metabolism.[1] The primary role of L-carnitine is to facilitate the transfer of long-chain fatty acids into the matrix of mitochondria, thereby delivering these substrates for β-oxidation and for subsequent energy production. Although L-carnitine is an important component of the human diet, it is also synthesized by the body, primarily in the liver and kidneys.[1] In addition to L-carnitine, the total body carnitine pool comprises various carnitine esters, the most prominent of which is the short-chain ester, acetyl-L-carnitine. The ability of L-carnitine to accept (and donate) acyl-groups from (and to) the corresponding acyl-coenzyme A thioester is integral to the physiological functions of the compound. More than 90% of the total carnitine pool resides within skeletal and myocardial muscle, with the remainder being present in the liver, kidneys, and other tissues.[2] This distribution pattern reflects the

From the Centre for Pharmaceutical Research, School of Pharmacy and Medical Sciences, University of South Australia and Department of Renal Medicine, Royal Adelaide Hospital.

Supported by Sigma-Tau Pharmaceuticals Inc, Gaithersburg, Md, and Sigma-Tau spa, Pomezia, Rome, Italy.

Received for publication Jan 18, 2000; accepted May 25, 2000.

Reprint requests: Allan M. Evans, PhD, Centre for Pharmaceutical Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide SA 5000.

E-mail: *allan.evans@unisa.edu.au*

0009-9236/2000/$12.00 + 0 **13/1/108850**

doi:10.1067/mcp.2000.108850

Table I. Demographic information and dosage and dialysis details for the 12 patients with end-stage renal disease that completed the study

Patient Number	*Age (y) at screening*	*Sex*	*Target weight (kg) at screening*	*Dose (μmol) of L-carnitine*	*Duration of dialysis session (min)*	*Dialysis blood flow rate (mL/min)*
1	30	Female	46.5	5831	210	300
2	51	Female	70.0	8685	210	350
3	53	Female	41.0	5087	180	400
4	66	Male	77.5	9677	240	250
5	53	Male	76.0	9429	240	350
6	28	Female	66.0	8189	210	300
7	61	Male	64.5	8065	270	300
8	59	Female	63.0	7816	210	350
9	26	Male	59.0	7320	270	300
10	26	Male	56.0	6948	240	250
11	54	Female	49.8	6203	240	250
12	55	Male	56.5	7072	240	300
Mean	47		60.5	7527	230	308

high requirement of muscle for fatty acid oxidation, for which L-carnitine is mandatory. However, because L-carnitine cannot be synthesized by muscle, the level of the compound in this tissue must be maintained by carrier-mediated uptake from blood.[1]

The healthy human kidney acts to conserve the s carnitine stores via carrier-mediated tubular orption of L-carnitine and its esters. At normal enous plasma concentrations (40 to 50 μmol/L), tent of this reabsorption is greater than 90%.[2] se it is a low-molecular-weight compound, L-car- is efficiently removed by hemodialysis,[3] and ore patients with end-stage renal disease under- long-term hemodialysis have low plasma concen- ns of L-carnitine. In some studies, low levels of itine in skeletal muscles have also been found, as ved by Brass[2] and Vacha et al. [4] Although this sec- y L-carnitine deficiency in patients undergoing erm hemodialysis is likely to result primarily from unrestricted loss from the body by the dialysis procedure, an impairment of biosynthesis or a reduced dietary intake of L-carnitine resulting from the modified diet of these patients may contribute to the deficiency. [5-8]

健康的人体肾小管的重吸收作用来保存体内的肉碱储存。在正常内源性血浆浓度(40-50 μ mol/L),肾小管重吸收的程度大于90%。由于左卡尼汀是一种小分子化合物，血液透析会大量丢失左卡尼汀。因此，长期血液透析的终末期肾病患者血浆中的左卡尼汀浓度较低。

L-Carnitine has been used both orally and intravenously for the treatment of primary and secondary carnitine-deficiency syndromes, and a number of clinical studies have been conducted to assess the benefits of L-carnitine supplementation in patients undergoing hemodialysis.[2,4,5-9] In the United States, the compound is currently approved for intravenous use in patients with secondary carnitine deficiency caused by hemodialysis. The recommended starting dose is 10 to 20 mg/kg after each dialysis session, decreasing to 5 mg/kg depending on predialysis levels. However, although a number of studies have been conducted to investigate the pharmacokinetics of L-carnitine in patients undergoing hemodialysis[9-16] and in healthy volunteers,[17-20] there has not been a systematic investigation of the pharmacokinetics of the compound under baseline conditions in patients undergoing hemodialysis and in the same patients after single and multiple doses of L-carnitine.

The aim of this study was to evaluate the pharmacokinetics of L-carnitine in patients undergoing long-term hemodialysis for end-stage renal disease. The patients were studied under baseline conditions (before the first dose) and after single and multiple (9 weeks) intravenous administration of L-carnitine (20 mg · kg^{-1}). In addition to intensive monitoring of the plasma and dialysate concentrations of L-carnitine, acetyl-L-carnitine, and total carnitine under baseline conditions and after the first and last dose of L-carnitine, blood samples were collected on a weekly basis before and after dialysis throughout the study to follow the approach to steady state. In a subgroup of patients, blood was also collected for 6 weeks after cessation of dosing to assess the washout pharmacokinetics of L-carnitine.

METHODS

This longitudinal study incorporated baseline observation, single- and multiple-dose administration of L-carnitine, and a washout phase. Approval for the study was obtained from the Human Research Ethics Committees of the University of South Australia and the

Royal Adelaide Hospital, and the study was conducted in accordance with the Declaration of Helsinki, as revised. Subjects were required to provide written informed consent before they were included in the study.

Study population

The patients (6 men, 6 women) had been on maintenance hemodialysis for a minimum of 6 months and were in a stable clinical condition, with uremia well controlled by dialysis therapy. Demographic details are presented in Table I. Before the study was begun, none of the patients had been receiving L-carnitine supplementation, either orally or intravenously. All patients underwent three hemodialysis treatments per week throughout the study.

Study design

Baseline. Patients were admitted to the study center on the morning of the second dialysis session of week 1, having been instructed to fast overnight. Patients were allowed to consume fluids within the limits of their usual fluid restriction, and a standardized light breakfast was provided before dialysis. Within 5 minutes after the end of hemodialysis, 0.1 mL · kg^{-1} normal saline solution was administered intravenously. The saline solution was administered over 2 minutes into the venous return line after disconnection of the line from the dialyzer. For the purpose of defining postdose pharmacokinetics, time zero was taken to be the midpoint of this 2-minute infusion period. Immediately after administration of saline solution, the venous return line was flushed with 5 mL sterile saline solution. Blood samples (2 mL) were collected from the arterial line (of the fistula) 5 minutes before the onset of hemodialysis, immediately after the hemodialysis had stopped (predose sample), and 5, 10, 15, 30, and 45 minutes, and 1, 2, 4, 6, 8, 20, and 44 hours after administration of saline solution. The 44-hour blood sample coincided with the beginning of the third hemodialysis session of week 1. During this third dialysis session, blood was collected simultaneously (within 10 seconds) from the arterial and venous lines of the dialyser at 0.25, 0.5, 1, 2, and 3 hours. At the same times, 10 mL dialysate was collected from the outflow of the dialyzer.

First dose of L-carnitine. A solution of L-carnitine (Carnitor 1 g in 5 mL; Sigma Tau Pharmaceuticals, Inc. Gaithersburg, Md), in a dose volume of 0.1 mL · kg^{-1} (corresponding to a dose of 20 mg · kg^{-1}), was administered intravenously at the end of the second dialysis session of week 2 of the study. Patient restrictions and the protocol for administration of the L-carnitine and the collection of blood and dialysate samples was exactly the same as that for the administration of normal saline solution in the baseline period.

Multiple dose treatment. Starting from the third dialysis session of week 2 until the first dialysis session of week 10, within 5 minutes after the end of every hemodialysis session (ie, three times a week), L-carnitine was administered intravenously at a dose of 20 mg · kg^{-1}, as described above. At the second dialysis session of weeks 3 to 9, blood samples (2 mL) were collected before and after dialysis, with the postdialysis blood sample collected before L-carnitine administration.

Final dose of L-carnitine. The final dose of L-carnitine (20 mg · kg^{-1}) was administered at the second dialysis session of week 10, as described above. Patient restrictions and the collection of blood and dialysate samples followed the same schedule that was used for the baseline period and the first dose of L-carnitine.

Washout evaluation. After the completion of L-carnitine administration, 6 volunteers (patients 7 through 12 in Table I) participated in a washout evaluation. Arterial blood samples (2 mL) were collected before and after dialysis at the first and second dialysis session of week 11 and the second dialysis sessions of weeks 12, 14, and 16.

Analytical methods

Plasma and dialysate samples were analyzed for L-carnitine, acetyl-L-carnitine, and total carnitine with use of an HPLC procedure with fluorescence detection, as described by Longo et al.[16] The method involved solid-phase extraction, derivatization of L-carnitine and acetyl-L-carnitine with 1-aminoanthracene, separation of the derivatives on a reverse-phase column, and fluorometric detection. Total carnitine levels were determined as L-carnitine after hydrolysis of the acylcarnitines contained in plasma and dialysate samples.[16] The limits of quantification were 0.5 and 0.25 μmol/L in plasma and dialysis fluid for L-carnitine and acetyl-L-carnitine, respectively. During the analysis of study samples, the accuracy for both analytes ranged from –8% to 11%, and the reproducibility was within 9%, as assessed by the repeated analysis of quality control samples spanning the relevant concentration ranges.

Pharmacokinetic analysis

For the purposes of defining pharmacokinetic parameters, two specific intervals are defined; the interdialysis interval and the intradialysis interval. The interdialysis interval is defined as the time between the end of the second dialysis session and the start of the third dialysis session of week 1 (baseline), week 2 (first dose), and week 10 (last dose). The parameters defined for the

interdialysis period were the maximum observed plasma concentration (C_{max}) and the time of its occurrence (t_{max}) and the area under the plasma concentration versus time curve from time zero (time of dosing) until 44 hours after dosing (immediately before the next dialysis session). This area (AUC_0^{44}), was calculated with the linear trapezoidal method. The differences between the AUC_0^{44} values obtained after the first and last dose of L-carnitine and the corresponding value obtained under baseline conditions were taken to represent the baseline-corrected areas.

The intradialysis interval covered the time from the start to the completion of the third dialysis session of week 1 (baseline), week 2 (first dose), and week 10 (final dose). For L-carnitine and acetyl-L-carnitine, the area under the arterial plasma concentration versus time curve from the start until the end of dialysis ($AUC_{ART}^{0\text{-end}}$) was calculated with the linear trapezoidal method; this required the estimation of the predicted concentration in arterial plasma at the end of dialysis (C_{PRED}) to estimate the area under the curve from 3 hours until the end of dialysis. The following equation was used to calculate C_{PRED}:

$$C_{PRED} = I \cdot e^{-k_{DIAL} \cdot T_{DIAL}} \quad (1)$$

in which the zero-time intercept (I) and the intradialysis rate constant (k_{DIAL}) were estimated by use of ln-linear regression of the intradialysis arterial plasma concentration versus time data, and T_{DIAL} is the dialysis time. The half-life of the substrate during the dialysis session ($t_{½}DIAL$) was calculated as $0.693/k_{DIAL}$.

The instantaneous plasma clearance of each species by the dialyzer at each collection time (CL_{DIAL}^{i}) was calculated with the following equation:

$$CL_{DIAL}^{i} = \frac{Q_{DIAL} \cdot C_{DIAL}^{i}}{C_{ART}^{i}} \quad (2)$$

in which Q_{DIAL} is the flow rate of dialysate, and C_{DIAL}^{i} and C_{ART}^{i} are the concentrations of substrate in dialysate and arterial plasma, respectively. The numerator of equation 2 is the instantaneous rate of excretion of substrate into dialysate. The average plasma clearance value for each subject during a dialysis session (CL_{DIAL}^{ave}) was calculated as the arithmetic mean of instantaneous clearance values.

The apparent volume of distribution of L-carnitine and acetyl-L-carnitine during dialysis (V_{app}), referenced to arterial plasma, was estimated from the following equation:

$$V_{app} = \frac{CL_{DIAL}^{ave}}{k_{DIAL}} \quad (3)$$

The amount of substrate eliminated from the body by the dialyzer during an entire dialysis session (Ae_{DIAL}) was calculated by equation 4:

$$Ae_{DIAL} = AUC_{ART}^{0\text{-end}} \cdot CL_{DIAL}^{ave} \quad (4)$$

The fraction of the dose of L-carnitine removed from the body in the form of the selected substrate during a single hemodialysis session was taken to be Ae_{DIAL} divided by the dose of L-carnitine administered. All values were in molar equivalents.

Although the dialysis clearance of each compound could be measured from plasma and dialysis concentration measurements (equation 2), the hemodialysis clearance of each substrate with respect to blood ($CL_{A\text{-}V}^{i}$) was also calculated at each sample collection time:

$$CL_{A\text{-}V}^{i} = \frac{Q_{ART} \cdot (C_{ART}^{i} - C_{VEN}^{i})}{C_{ART}^{i}} \quad (5)$$

in which Q_{ART} is the arterial blood flow and C_{ART}^{i} and C_{VEN}^{i} are the arterial and venous plasma concentrations at time i. Equation 5 assumes that the arterial and venous blood flows are the same and that the blood-to-plasma concentration ratio of the substrate does not differ between arterial and venous blood. The time-averaged blood clearance of each substrate by hemodialysis $CL_{A\text{-}V}^{ave}$ was calculated from the following equation:

$$CL_{A\text{-}V}^{ave} = \frac{Q_{ART} \cdot (AUC_{ART}^{0.25\text{-}3} - AUC_{VEN}^{0.25\text{-}3})}{AUC_{ART}^{0.25\text{-}3}} \quad (6)$$

in which $AUC_{ART}^{0.25\text{-}3}$ and $AUC_{VEN}^{0.25\text{-}3}$ are the areas under the concentration-time curves between 0.25 and 3 hours, for plasma derived from arterial and venous blood, respectively. These area terms were estimated by use of the trapezoidal method.

The extraction ratio of the substrate by the dialyzer (E_{dial}) was taken to be the time-averaged blood clearance by hemodialysis relative to the blood flow rate through the dialyzer:

$$E_{dial} = \frac{CL_{A\text{-}V}^{ave}}{Q_{ART}} \quad (7)$$

Statistics

The Student *t* tests for paired data and ANOVA were used, as appropriate, to determine whether there were any differences between the plasma concentrations and the pharmacokinetic parameters for each species during the interdialysis and intradialysis intervals, with $P < .05$ taken to represent statistical significance. All data are presented as mean and standard deviation.

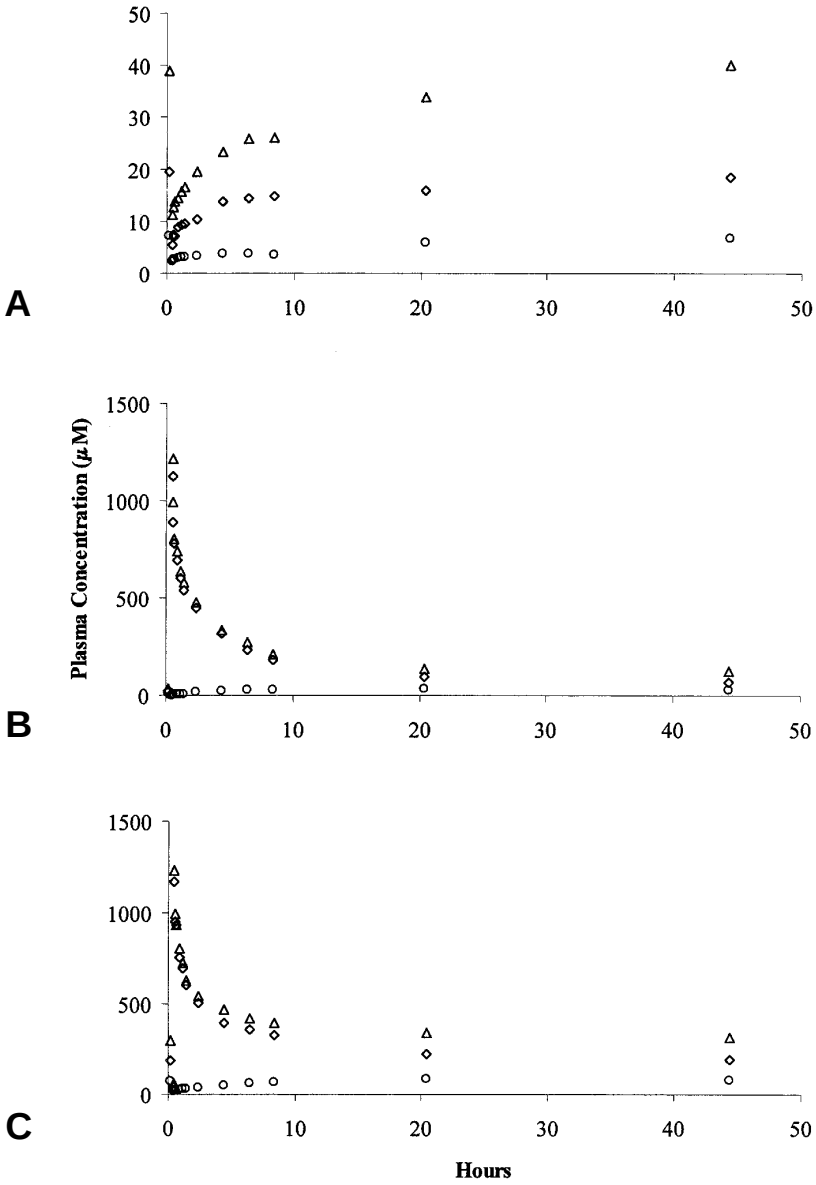

Fig 1. Mean (n = 12) plasma concentration versus time profiles for L-carnitine *(diamonds)*, acetyl-L-carnitine *(circles)*, and total carnitine *(triangles)* during the interdialysis interval under baseline conditions **(A)** and after the first **(B)** and final **(C)** dose of L-carnitine (20 mg · kg^{-1}).

RESULTS

Demographic information and details of dialysis for the 12 patients who entered and completed this study are provided in Table I. Patients 7 through 12 participated in the washout component of the study. An additional patient was enrolled but was withdrawn because of an adverse event that was judged subsequently to be unrelated to the study medication.

During the baseline period, a single intravenous dose of normal saline solution was administered immediately after the second dialysis session of the week. Before this dialysis session, the plasma concentration of L-carnitine was 19.5 ± 5.6 μmol/L, and that of acetyl-L-carnitine was 7.2 ± 1.9 μmol/L. At the end of the dialysis session, immediately before the dose of normal saline solution, the corresponding plasma concentra-

Table II. Mean ± SD interdialysis pharmacokinetic parameters for L-carnitine, acetyl-L-carnitine and total carnitine*

	Baseline	*First dose of L-carnitine*	*Final dose of L-carnitine*
L-carnitine			
AUC_0^{44} (μmo/L · h)	703 ± 190	6592 ± 1178	12093 ± 2502†
Baseline-corrected AUC_0^{44} (μmol/L · h)	—	5889 ± 1142	11390 ± 2452†
C_{max} (μmol/L)	19 ± 6	1139 ± 240	1190 ± 270
t_{max} (h:min)	40:24 ± 9:29	0:06 ± 0:03	0:06 ± 0:03
Acetyl-L-carnitine			
AUC_0^{44} (μmol/L · h)	241 ± 63	1265 ± 312	3234 ± 820†
Baseline-corrected AUC_0^{44} (μmol/L · h)	—	1024 ± 313	2993 ± 810†
C_{max} (μmol/L)	7 ± 2	36 ± 11	90 ± 22†
t_{max} (h:min)	40:24 ± 9:29	19:00 ± 12:59	29:06 ± 13:46
Total carnitine			
AUC_0^{44} (μmol/L · h)	1433 ± 421	8406 ± 1397	16444 ± 3697†
Baseline-corrected AUC_0^{44} (μmol/L · h)	—	6973 ± 1339	15011 ± 3627†
C_{max} (μmol/L)	40 ± 12	1235 ± 233	1233 ± 223
t_{max} (h:min)	40:24 ± 9:29	0:06 ± 0:03	0:05 ± 0:00

The interdialysis parameters shown relate to the pharmacokinetics of each species in the interval commencing immediately before dosing (at the end of dialysis) until 44 hours after dosing (before the next dialysis session).

*Under baseline conditions and after the first and final dose of nine weeks of administration of L-carnitine at a dose of 20 mg · kg^{-1}, administered at the end of each dialysis session.

†Indicates a statistically significant difference ($P < .05$) in the parameter between the first and final dose of L-carnitine.

Table III. Mean ± SD intradialysis pharmacokinetic parameters for L-carnitine and acetyl-L-carnitine*

	Baseline	*First dose of L-carnitine*	*Final dose of L-carnitine*
L-Carnitine			
CL_{DIAL}^{ave} (L/h)	7.8 ± 1.7	7.5 ± 2.0	8.1 ± 1.8
$CL_{A\text{-}V}^{ave}$ (L/h)	13.4 ± 1.0	13.4 ± 1.7	13.5 ± 1.3
E_{DIAL}	0.74 ± 0.07	0.73 ± 0.08	0.73 ± 0.08
$t_{½dial}$ (h)	2.34 ± 0.69	1.76 ± 0.47	1.98 ± 0.79
V_{app} (L)	25.7 ± 7.7	18.7 ± 5.4	22.8 ± 9.0
Ae_{DIAL} (μmol)	250 ± 76	758 ± 268	2390 ± 856†
Fraction of dose recovered by dialysis	—	0.10 ± 0.03	0.32 ± 0.10†
Acetyl-L-carnitine			
CL_{DIAL}^{ave} (L/hr)	8.2 ± 2.7	8.9 ± 3.0	9.2 ± 1.8
$CL_{A\text{-}V}^{ave}$ (L/h)	13.0 ± 2.2	12.7 ± 1.5	12.5 ± 1.3
E_{DIAL}	0.71 ± 0.11	0.69 ± 0.10	0.68 ± 0.10
$t_{½dial}$ (h)	2.70 ± 1.78	2.07 ± 0.56	2.04 ± 0.62
V_{app} (L)	29.4 ± 17.9	26.5 ± 10.5	26.7 ± 8.9
Ae_{DIAL} (μmol)	95 ± 21	424 ± 195	1210 ± 473†
Fraction of dose recovered by dialysis	—	0.06 ± 0.03	0.16 ± 0.05†

*Under baseline conditions and after the first and final dose of 9 weeks of administration of L-carnitine at a dose of 20 mg · kg^{-1}, administered at the end of each dialysis session. The parameters relate to the pharmacokinetics of each species during the hemodialysis session that was conducted 44 hours after dosing with normal saline solution (baseline) or L-carnitine (first dose and final dose).

†Indicates a statistically significant difference ($P < .05$) in the parameter between the first and final dose of L-carnitine. For all other parameters, there was no significant difference among the three study periods.

tions of L-carnitine and acetyl-L-carnitine were 5.6 ± 1.9 and 2.3 ± 0.9 μmol/L, respectively. Fig 1 shows the mean plasma concentration versus time profiles for each species during the 44-hour period after the dose of normal saline solution. The mean pharmacokinetic parameters for this "interdialysis" phase of the baseline period are given in Table II.

Pharmacokinetic parameters for the hemodialysis session studied under baseline conditions (commencing 44 hours after the dose of normal saline solution)

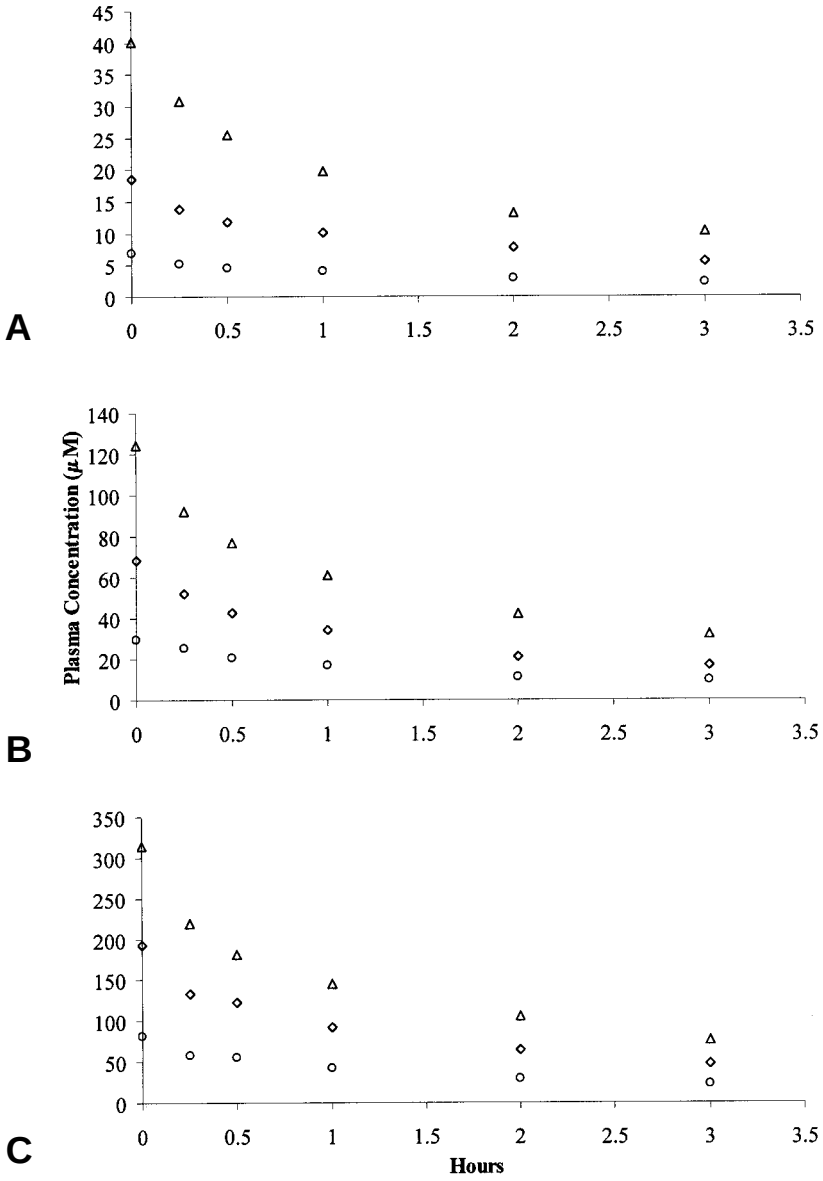

Fig 2. Mean (n = 12) plasma concentration versus time profiles for L-carnitine *(diamonds)*, acetyl-L-carnitine *(circles)*, and total carnitine *(triangles)* during the hemodialysis session that was conducted 44 hours after dosing with normal saline solution, corresponding to baseline period **(A)**, and 44 hours after the first **(B)** and final dose of L-carnitine **(C)**.

are summarized in Table III, and mean concentrations in plasma obtained from blood entering (arterial) and leaving (venous) the dialyser, and outflow dialysate, are presented in Fig 2. The arterial and venous plasma levels of all species were found to decline in a log-linear manner during hemodialysis. The dialyser extraction ratios for L-carnitine and acetyl-L-carnitine, were 0.74 ± 0.07 and 0.71 ± 0.11, respectively. The apparent volume of distribution values for L-carnitine and acetyl-L-carnitine were 25.7 ± 7.7 and 29.4 ± 17.9 L, respectively (Table III), and during the dialysis procedure, the mean amount of L-carnitine and acetyl-L-carnitine removed via the dialysate was estimated to be 250 and 95 μmol, respectively.

After the first intravenous dose, the plasma concentrations of L-carnitine increased substantially (Fig 1), and the C_{max} value was 1139 ± 240 μmol/L (Table II). The plasma levels then decreased rapidly, halving

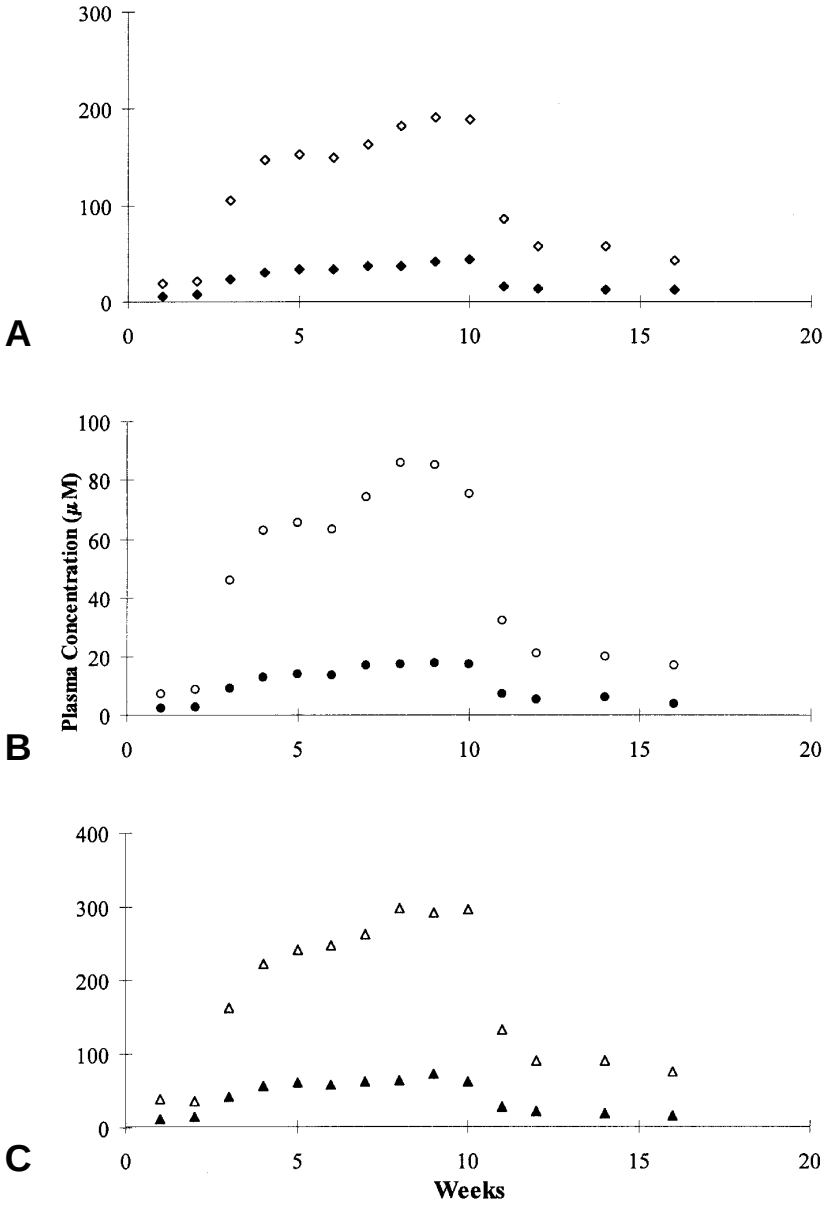

Fig 3. Mean (n = 12) predialysis *(open symbols)* and postdialysis *(filled symbols)* plasma concentrations of L-carnitine **(A)**, acetyl-L-carnitine **(B)**, and total carnitine **(C)** under baseline conditions (week 1 and 2), during L-carnitine administration at dose of 20 mg · kg^{-1} after each dialysis session (weeks 3 through 10) and for 6 weeks after cessation of L-carnitine administration (in 6 of 12 patients only).

within 1 hour and reaching a mean of 318 μmol/L after 4 hours and 68.4 μmol/L at 44 hours (ie, immediately before the next dialysis session). During this same period, the plasma concentrations of acetyl-L-carnitine increased steadily, reaching a mean value of 29.2 μmol/L after 44 hours. The area-under-the-curve for L-carnitine during the interdialysis period was about 9 times higher than the corresponding value measured under baseline conditions. The first dose of L-carnitine also produced a fivefold increase in the AUC_0^{44} value for acetyl-L-carnitine (Table II). The pattern for total carnitine was, in essence, a reflection of what was found for L-carnitine (Fig 1).

The mean C_{max} value for L-carnitine, observed after the final dose (1190 μmol/L) was not significantly different to that observed after the first dose (1139

μmol/L). However, from 15 minutes until 44 hours after dosing, the plasma concentrations of L-carnitine were significantly higher ($P < .05$) than those encountered after the first dose (Fig 1). The uncorrected and baseline-corrected area-under-the-curve values for L-carnitine after the final dose were about twice those observed after the first dose (Table II). For acetyl-L-carnitine, there was a clear increase in the interdialysis plasma concentrations during long-term administration of L-carnitine (Fig 1).

The pharmacokinetic parameters for the hemodialysis sessions that were conducted 44 hours after the first and final doses of L-carnitine are given in Table III. The various dialysis clearance parameters were not significantly different to the corresponding values reported under baseline conditions. However, the amount of L-carnitine recovered in dialysate after the last dose of L-carnitine represented about 32% of the dose, compared with about 10% after the first dose ($P < .05$). For acetyl-L-carnitine, the amount recovered in dialysate after the last dose of L-carnitine represented 16% of the dose, compared with about 6% after the first dose ($P < .05$).

The predialysis and postdialysis arterial plasma concentrations of L-carnitine, acetyl-L-carnitine, and total carnitine, as determined on a weekly basis throughout the study, are presented in Fig 3. For the 6 patients who participated in the washout phase of the study, the mean predialysis L-carnitine concentration observed 6 weeks after L-carnitine administration was discontinued (43.4 μmol/L) was significantly higher ($P < .05$) than the corresponding concentration under baseline conditions (19.5 μmol/L). Similarly, for acetyl-L-carnitine, the plasma concentration 6 weeks after cessation of L-carnitine (16.9 μmol/L) was significantly higher ($P < .05$) than the baseline value (7.23 μmol/L).

DISCUSSION

This study was conducted in 12 patients who had received at least 6 months of hemodialysis for the management of end-stage renal disease. In all cases, the predialysis plasma concentration of L-carnitine, under baseline conditions, was less than 30 μmol/L. During dialysis there was a marked reduction in the plasma levels of L-carnitine, and the mean postdialysis concentration was 5.61 μmol/L. In the subsequent 44-hour interdialysis period, the plasma levels of L-carnitine returned to the predialysis level (Fig 1). The average plasma concentration of L-carnitine during the interdialysis period, calculated by dividing the mean AUC_0^{44} value (703 μmol/L · h) by the time interval (44 hours), was 16 μmol/L. In contrast, the normal plasma concentration of L-carnitine in healthy individuals is reported to be in the range of 40 to 50 μmol/L.[21] For example, in six healthy subjects with a mean age of 47 years, the plasma concentration of L-carnitine was 47 ± 6.3 μmol/L, whereas the mean plasma concentration in four subjects with symptomatic primary systemic carnitine deficiency was 13 ± 6.8 μmol/L.[22] This study confirms the results of earlier studies reporting low plasma levels of L-carnitine in patients undergoing hemodialysis who were not receiving L-carnitine supplementation.[2,4,9,13-15]

The low plasma L-carnitine concentrations in the patients undergoing long-term hemodialysis is likely to be partly due to the efficient removal of the compound via hemodialysis. Thus the extraction ratio of the compound, which represents the fractional removal of L-carnitine from blood during a single passage through the dialyser, was about 70%. This efficient removal has been reported previously[4,23,24] and is not unexpected given that L-carnitine is a low-molecular-weight compound that does not bind to plasma proteins.[25]

The apparent volume of distribution of L-carnitine during hemodialysis was estimated from the decline in the arterial plasma concentrations of L-carnitine during the dialysis procedure. The derived value during the baseline period (25.7 L) represents the apparent volume of body fluid that is in rapid equilibrium with the plasma component of circulating blood. However, because the total body store of L-carnitine is located primarily in a deep tissue compartment (muscle tissue) that equilibrates very slowly with L-carnitine in plasma,[2,26] the true equilibrium volume of distribution with respect to plasma is likely to be much larger. For instance, in a healthy 70-kg man, the total amount of carnitine in the body is about 128 mmol[2]; therefore, assuming an average plasma concentration of 40 μmol/L, one would estimate a true steady-state volume of distribution with respect to plasma of about 3200 L. However, because the movement of L-carnitine into and out of the deep compartment, representing muscle, is very slow, the volume of this deep compartment would not influence intradialysis plasma concentrations of L-carnitine. This explains why the apparent volume of distribution measured in this study is substantially lower than the expected value.

During the baseline dialysis session, the total amount of carnitine lost from the body as L-carnitine and acetyl-L-carnitine was about 345 μmol. Although this amount represents a significant fraction of the carnitine in the rapidly equilibrating pool of the body, it is only a small fraction of the total body content (128 mmol[2] in a healthy adult, although it may be significantly lower in patients undergoing long-term hemodialysis). There-

fore, even though hemodialysis does cause a dramatic reduction in the L-carnitine and acetyl-L-carnitine levels in plasma, a single dialysis session would not result in a significant loss of carnitine from the body. However, the summed effect of repeated hemodialysis in patients with end-stage renal disease could lead to a depletion in skeletal muscle stores of L-carnitine and its esters that may lead to a secondary carnitine deficiency.[2] This is in keeping with the observation that skeletal muscle concentrations of L-carnitine decreased significantly after 4 months of long-term hemodialysis in patients not receiving L-carnitine supplementation.[4]

The interdialysis plasma concentration versus time profile for L-carnitine, under baseline conditions, shows that the depleted L-carnitine plasma levels are slowly restored during the interdialysis interval (Fig 1). This restoration is likely to be due to L-carnitine moving into plasma from organs of synthesis and storage, although dietary intake is also likely to be important.

The administration of the first dose of L-carnitine (20 $mg \cdot kg^{-1}$) resulted in a substantial increase in the plasma concentrations of the compound. The plasma levels observed are comparable to those reported previously after the intravenous administration of similar doses of L-carnitine to healthy subjects.[16] Although a formal pharmacokinetic evaluation of the interdialysis disappearance profile of L-carnitine was not conducted, the logarithmically transformed plasma concentration versus time profiles suggested two or three distinct components. This multiexponential behavior of L-carnitine has been observed previously in healthy individuals who were given bolus doses of the compound[17,18,26,27] and is believed to be due to the fact that L-carnitine distributes into rapidly and slowly equilibrating tissue pools. Acetyl-L-carnitine is formed intracellularly via the ubiquitous enzyme acylcarnitine transferase.[1,28] The fact that there was a progressive increase in the plasma concentrations of acetyl-L-carnitine during the interdialysis interval is therefore in keeping with the concept that a component of the dose of L-carnitine must have distributed into cells and become incorporated into the total body carnitine pool.

During repeated administration of L-carnitine, there was a progressive increase in the predialysis and postdialysis plasma concentrations of L-carnitine and acetyl-L-carnitine (Fig 3). This slow accumulation is consistent with the fact that the administration of L-carnitine results in a net movement of the compound into a compartment that is in slow equilibration with plasma. It is important to recognize that the turnover time for L-carnitine in skeletal muscle is in the order of 191 hours,[26] and therefore slow equilibration is to be expected on pharmacokinetic grounds. Toward the end of the treatment period, the plasma concentrations of both L-carnitine and acetyl-L-carnitine had stabilized, suggesting that an apparent steady-state condition had arisen at this time (Fig 3). The approximate steady-state predialysis and postdialysis plasma levels of L-carnitine were 190 and 40 μmol/L, respectively, whereas the corresponding plasma levels of acetylL-carnitine were about 80 and 17 μmol/L.

The pharmacokinetics of L-carnitine after the final dose followed a pattern similar to that observed after the first dose (Fig 2), but the baseline-corrected area-under-the-curve values increased twofold and threefold for L-carnitine and acetyl-L-carnitine, respectively. This result signifies that accumulation of both species occurs during repeated administration, and, on the basis of previously published kinetic models,[26] most of this accumulation would be due to the prolonged retention of the compound in skeletal muscle. However, it is interesting to note that the C_{max} value for L-carnitine after the final dose was not significantly different to that determined after the first dose. This is because the postdialysis plasma concentrations of L-carnitine, during long-term administration, are low relative to the concentrations that arise immediately after dosing of the compound via an intravenous bolus.

The sum of L-carnitine and acetyl-L-carnitine eliminated via dialysate after the last dose of L-carnitine represented about 48% of the dose, compared with about 16% of the dose after the first dialysis session. This increase was due to the higher plasma concentrations of L-carnitine and acetyl-L-carnitine concentrations entering the dialyser after multiple dosing, because the clearance of both compounds by the dialyser remained unaltered (Table III).

During the washout phase of this study, the predialysis and postdialysis concentrations of L-carnitine and acetyl-L-carnitine decreased with time. However, even 6 weeks after the last dose of L-carnitine, the plasma levels of both compounds were significantly higher than the respective baseline concentrations. This finding is significant because it suggests that a significant portion of the administered L-carnitine had distributed into a compartment that can replenish plasma levels once administration is discontinued. Our current understanding of the distribution of carnitine within the body indicates that skeletal muscle represents the major slow-equilibrating pool of endogenous L-carnitine,[1,2,26,28] and it is possible that the slow progressive increase (during dosing) and decrease (on cessation of dosing) in the concentrations of L-carnitine in plasma reflects the slow movement of the compound into and out of skeletal muscle. One

would anticipate that had the washout component of the study continued indefinitely, L-carnitine concentrations would have returned to their pretreatment plasma levels.

Collectively, the study has provided a number of important findings. First, the study confirms that the removal of L-carnitine and acetyl-L-carnitine by hemodialysis is extremely efficient and that the hemodialysis procedure causes a substantial reduction in the plasma concentrations of L-carnitine and acetyl-L-carnitine. After dialysis the plasma levels of L-carnitine and acetyl-L-carnitine are restored by what is likely to include movement of the compound out of slowly-equilibrating tissue stores. Second, the repeated administration of L-carnitine, given at a dose of 20 $mg \cdot kg^{-1}$ after each hemodialysis session, results in accumulation of L-carnitine and acetyl-L-carnitine in plasma, reaching an apparent steady state after about 8 weeks. Finally, on cessation of L-carnitine administration, the predialysis and postdialysis plasma levels of L-carnitine and acetyl-L-carnitine decrease progressively but do not return to pretreatment levels after 6 weeks. It is suggested that during this period there is movement of L-carnitine out of a slowly equilibrating pool (probably muscle) into a pool that is in rapid equilibrium with plasma.

References

1. Rebouche CJ, Paulson DJ. Carnitine metabolism and function in humans. Ann Rev Nutr 1986;6:41-66.
2. Brass EP. Pharmacokinetic considerations for the therapeutic use of carnitine in hemodialysis patients. Clin Ther 1995;17:176-85.
3. Bohmer T, Bergrem H, Eiklid K. Carnitine deficiency induced during intermittent hemodialysis for renal failure. Lancet 1978;1(8056):126-8.
4. Vacha GM, Corsi M, Giorcelli G, D'Iddio S, Maccari F. Serum and muscle L-carnitine levels in hemodialyzed patients, during and after long-term L-carnitine treatment. Curr Ther Res 1985;37:505-13.
5. Ahmad S. Carnitine, kidney and renal dialysis. In: Ferrari R, DiMauro S, Sherwood G, editors. L-Carnitine and its role in medicine; from function to therapy. London, Academic Press; 1992. p. 381-400.
6. Borum PR, Taggart EM. Carnitine nutriture of dialysis patients. J Am Diet Assoc 1986;86:644-7.
7. Guarnieri G, Taigo G, Crapesi L, Situlin R, Del Bianco MA, Corsi M et al. Carnitine metabolism in chronic renal failure. Kidney Int 1987;32(Supp 22):S116-S127.
8. Wanner C, Hörl WH. Carnitine abnormalities in patients with renal insufficiency: pathophysiological and therapeutic aspects. Nephron 1988;50:89-102.
9. Bertoli M, Battistella PA, Vergani L, Naso A, Gasparotto ML, Romagnoli GF et al. Carnitine deficiency induced during hemodialysis and hyperlipidemia: effect of replacement therapy. Am J Clin Nutr 1981;34:1496-500.
10. Glöggler A, Bulla M, Fürst P. Kinetics of intravenously administered carnitine in haemodialysed children. J Pharm Biomed Anal 1990;8:411-4.
11. Golper TA, Wolfson M, Ahmad S, Hirschberg R, Kurtin P, Katz LA, et al. Multicentre trial of L-carnitine in maintenance hemodialysis patients: I—carnitine concentrations and lipid effects. Kidney Int 1990;38:904-11.
12. König B, McKaigney E, Conteh S, Ross B. Effect of a lipid load on blood and urinary carnitine in man. Clin Chim Acta 1978;88:121-5.
13. Moorthy AV, Rosenblum M, Rajaram R, Shug AL. A comparison of plasma and muscle carnitine levels in patients on peritoneal or hemodialysis for chronic renal failure. Am J Nephrol 1983;3:205-8.
14. Panzetta G, Bonadonna G, Giovene P, De Grandis D. Carnitine kinetics during dialysis: evidence of unilateral transport from tissues to plasma. Nephron 1985;41:230-4.
15. Wanner C, Förstner-Wanner S, Rössle C, Fürst P, Schollmeyer P, Hörl WH. Carnitine metabolism in patients with chronic renal failure: effects of L-carnitine supplementation. Kidney Int 1987;32 Suppl 22:S-132-5.
16. Longo A, Bruno G, Curti S, Mancinelli A, Miotto G. Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by high-performance liquid chromatography after pre-column derivatization with 1-aminoanthracene. J Chromatogr 1996;686:129-39.
17. Harper P, Elwin C-E, Cederblad G. Pharmacokinetics of bolus intravenous and oral doses of L-carnitine in healthy subjects. Eur J Clin Pharmacol 1988;35:69-75.
18. Rizza V, Lorefice R, Rizza N, Calabrese V. Pharmacokinetics of L-carnitine in human subjects. In: Ferrari R, DiMauro S, Sherwood G, editors. L-Carnitine and its role in medicine; from function to therapy. London: Academic Press; 1992. p. 63-77.
19. Sahajwalla CG, Helton ED, Purich ED, Hoppel CL, Cabana BE. Comparison of L-carnitine pharmacokinetics with and without baseline correction following administration of single 20-mg/kg intravenous dose. J Pharm Sci 1995;84:634-9.
20. Uematsu T, Itaya T, Mishimoto Y, Mizuno A, Nakashima M, Yoshinobu K. Pharmacokinetics and safety of L-carnitine infused i.v. in healthy subjects. Eur J Clin Pharmacol 1988;34:213-6.
21. Rebouche CJ. Metabolic fate of dietary carnitine in humans. In: Lee Carter A, editor. Current concepts in carnitine research. London, CRC Press; 1992. p. 37-48.
22. Engel AG, Rebouche CJ, Wilson DM, Glasgow AM, Romshe CA, Cruse RP. Primary systemic carnitine deficiency: II—renal handling of carnitine. Neurology 1981;31:819-25.
23. Bartel LL, Hussey JL, Shrago E. Perturbation of serum carnitine levels in human adults by chronic renal disease and dialysis therapy. Am J Clin Nutr 1981;34:1314-1320.
24. Broquet PE, Von Moos C, Frei J, Gobelet C, Wauters JP. Carnitine depletion during chronic hemodialysis: effect

of substitution on free carnitine plasma levels. Adv Exp Med Biol 1985;223:287-90.
25. Marzo A, Arrigoni Martelli E, Mancinelli A, Cardace G, Corbelletta C, Bassani E, et al. Protein binding of L-carnitine family components. Eur J Drug Metab Pharmacokinet 1991;Spec No 3:364-8.
26. Rebouche CJ, Engel AG. Kinetic compartmental analysis of carnitine metabolism in the human carnitine deficiency syndromes: evidence for alterations in tissue carnitine transport. J Clin Invest 1984;73:857-67.
27. Segre G, Bianchi E, Corsi M, D'Iddio S, Ghirardi O, Maccari F. Plasma and urine pharmacokinetics of free and short-chain carnitine after administration of carnitine in man. Arzneimittelforschung 1988;38:1830-4.
28. Bieber LL. Carnitine. Ann Rev Biochem 1988;57: 261-83.