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Impact of hemodialysis on endogenous plasma and muscle carnitine levels in patients with end-stage renal disease

ALLAN M. EVANS, RANDALL J. FAULL, ROGER L. NATION, SHILPANJALI PRASAD, TONY ELIAS, STEPHANIE E. REUTER, and GIANFRANCO FORNASINI

Centre for Pharmaceutical Research, School of Pharmaceutical, Molecular and Biomedical Sciences, University of South Australia, Adelaide, South Australia, Australia; Department of Renal Medicine, Royal Adelaide Hospital, Adelaide, South Australia, Australia; and Regulatory Sciences, Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, Maryland

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Background. End-stage renal disease (ESRD) patients undergoing hemodialysis treatment have reduced plasma L-carnitine levels; however, the relationship between dialysis age and carnitine status is poorly understood. This study examined the relationship between duration of dialysis and plasma and skeletal muscle concentrations of L-carnitine and its esters in ESRD patients.

Methods. Blood samples were collected from 21 patients at baseline and throughout the first 12 months of hemodialysis. In 5 patients, muscle samples were obtained after 0, 6, and 12 months of hemodialysis. Blood and muscle samples were collected from an additional 20 patients with a mean dialysis age of 5.10 years. L-carnitine, acetyl-L-carnitine, and total L-carnitine were measured by high-performance liquid chromatography (HPLC).

Results. The mean ± SD plasma L-carnitine concentration in ESRD patients who had not yet started hemodialysis was 50.6 ± 20.0 μmol/L. Significantly lower concentrations were observed after 12 months (29.7 ± 10.5 μmol/L) and >12 months (22.0 ± 5.4 μmol/L) of hemodialysis treatment. Acetyl-L-carnitine also declined with dialysis age, while plasma nonacetylated acylcarnitines continued to increase with the progression of hemodialysis therapy. An inverse relationship between dialysis age and muscle L-carnitine concentrations was observed.

Conclusion. Long-term hemodialysis treatment is associated with a significant reduction in endogenous plasma and muscle L-carnitine levels and a significant increase in plasma acylcarnitines. The majority of the change in plasma L-carnitine concentrations occurs within the first few months of hemodialysis, while muscle levels continue to decline after 12 months of treatment.

L-carnitine is an endogenous substance required for the transfer of long-chain fatty acids across the inner matrix membrane of mitochondria, thereby delivering these substances for β-oxidation and energy production [1]. In healthy individuals, blood and tissue levels of L-carnitine are maintained within relatively narrow limits, reflecting the importance of the compound in intermediate metabolism. The healthy kidney plays a vital role in this homeostasis, first by conserving body L-carnitine stores through extensive reabsorption of the filtered load, and second by preferential excretion of short-chain carnitine esters [2, 3].

Patients with end-stage renal disease (ESRD) not undergoing hemodialysis tend to have higher plasma concentrations of L-carnitine than healthy individuals [4, 5, 6, 7]. In contrast, a large number of studies have reported low plasma and muscle L-carnitine levels in ESRD patients undergoing chronic hemodialysis [2, 3, 8]. This depletion in L-carnitine has been found to correlate with the length of time over which a patient has been undergoing hemodialysis—that is, their dialysis age, or dialysis vintage [4, 5, 6, 9, 10]. The decrease in endogenous L-carnitine concentrations that occurs during chronic hemodialysis is mainly due to the efficient removal of the compound via the dialysate, possibly coupled with a reduction in L-carnitine input via dietary intake and endogenous synthesis [2, 8, 11]. However, little is known about the time-course of L-carnitine in plasma and muscle during the first 12 months of hemodialysis and how these levels compare with concentrations in patients who have been receiving hemodialysis treatment for more than 12 months. With the approved clinical use of supplemental L-carnitine for the treatment of dialysis-related secondary carnitine deficiency and the clinical symptoms that have been associated with this disorder [8, 12], it is vital that the temporal relationship between dialysis age and carnitine status is examined.

In the present study, patients with ESRD were recruited prior to the time at which hemodialysis treatment was first initiated. In these patients, plasma and skeletal muscle levels of L-carnitine and its esters were

Key words: L-carnitine, acetyl-L-carnitine, end-stage renal disease, hemodialysis, skeletal muscle, plasma.

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examined over the first 12 months of hemodialysis treatment. L-carnitine levels were also determined in an additional group of ESRD patients who had been undergoing hemodialysis for more than 12 months. The primary objective of the study was to examine the relationship between plasma and skeletal muscle levels of L-carnitine, acetyl-L-carnitine, and total L-carnitine, and the duration of hemodialysis treatment. Additionally, changes in the contribution of nonacetylated forms of acylcarnitines to the total carnitine concentration were also evaluated.

METHODS

Study design

This study was conducted with the approval of the institutional human research ethics committees, and all patients provided written informed consent prior to participation. The patient population comprised 2 cohorts of adult men and women diagnosed with ESRD—21 patients were studied over the first 12 months of hemodialysis treatment (longitudinal component), and 20 patients who had been undergoing hemodialysis for more than 12 months were studied on one occasion only (single-session component).

Longitudinal component. Potential subjects were identified prior to the initiation of hemodialysis treatment. In order to be eligible for this component of the study, patients were required to be diagnosed with ESRD, and were expected to require hemodialysis for at least 12 months. Patients were not suitable if they had previously been receiving continuous ambulatory peritoneal dialysis (CAPD) or hemodialysis. Patients were excluded if they had used any products containing L-carnitine (and/or carnitine derivatives) during the 4 months prior to screening or throughout the course of the study. Any patient with a bleeding disorder or who was receiving anticoagulant therapy was excluded from the muscle biopsy-sampling component of the study.

Predialysis blood samples (2 mL) were collected from the inflow to the dialyzer prior to the first dialysis session, before the second and third dialysis sessions in the first week, and then prior to the second dialysis session of weeks 2, 3, 4, and months 2, 4, 6, 9, and 12. In a subset of consenting patients, a small sample (approximately 15 to 20 mg) of skeletal muscle was collected from the quadriceps prior to the first hemodialysis session, and after approximately 6 and 12 months of hemodialysis treatment. Muscle biopsies were performed by an appropriately trained physician using a Bard® Max Core® Disposable Biopsy Instrument (CR Bard, Inc., Covington, GA, USA). Vital signs were measured prior to and following muscle biopsy collection for safety monitoring.

Single-session component. An additional group of 20 ESRD patients who had been undergoing hemodialysis for more than 12 months were recruited to participate in the single-session component of the study. Patients who had used L-carnitine (and/or carnitine derivatives) in the previous 4 months, were receiving anticoagulant therapy, or were diagnosed with a bleeding disorder were excluded from the single-session component of the study.

From eligible patients, a predialysis blood sample was collected from the inflow of the dialyzer during a single (mid-week) hemodialysis session. In addition, a single muscle biopsy sample was collected using the technique described above within 3 days of the patient's corresponding blood sample collection.

Analytical methods

Plasma and muscle homogenate concentrations of (free) L-carnitine and acetyl-L-carnitine were determined by high-performance liquid chromatography (HPLC) based on a previously described analytical method [13, 14], and utilizing the muscle preparation steps described by Cederblad et al [15]. In addition, total L-carnitine was measured as L-carnitine after alkaline hydrolysis of esterified L-carnitine. The method involved purification by solid-phase extraction, derivatization, and fluorescence detection of HPLC eluant. The limits of quantification for plasma L-carnitine, acetyl-L-carnitine, and total L-carnitine were 2.5, 0.5, and 2.5 μmol/L, respectively. Muscle L-carnitine, acetyl-L-carnitine, and total L-carnitine limits of quantification were 0.25, 0.1, and 0.5 μmol/g of homogenized muscle. Quality control samples analyzed routinely with each analytical run returned mean measured concentrations relative to nominal values and precision values of within 10% and 8% for plasma and muscle, respectively. Acylcarnitines were calculated by subtraction of L-carnitine from total carnitine. Nonacetylated forms of acylcarnitines were derived by subtraction of acetyl-L-carnitine from the acylcarnitines level.

Plasma L-carnitine, acetyl-L-carnitine, and total L-carnitine concentrations were also obtained for a cohort of healthy volunteers (30 males and 30 females) with a mean age of 39 years. The normal range for each analyte was taken to be the 25th and 75th percentiles of the data obtained from these healthy volunteers.

Statistical methods

Statistical comparisons were performed using analysis of variance (ANOVA) with post-hoc analysis (contrasts) if significance was detected. Significance was set at $P < 0.05$. WinNonlin® Professional, Version 4.0 (Pharsight Corporation, Mountain View, CA, USA) was used for all statistical analyses. In order to examine the effect of hemodialysis on plasma and skeletal muscle L-carnitine, acetyl-L-carnitine, and total L-carnitine concentrations, baseline levels (prior to the first hemodialysis session) were compared to those measured at each

Table 1. Mean ± SD (range) subject demographic data

Parameter	Healthy adults	ESRD patients	
		Longitudinal	Single-session
No. of subjects	60	21	20
Age *years*	39 ± 13 (18-75)	51 ± 13 (23-78)	55 ± 14 (27-78)
Gender	30 m, 30 f	13 m, 8 f	12 m, 8 f
Weight *kg*	Not recorded	79 ± 21 (53-125)	71 ± 27 (36-146)
Height *cm*	Not recorded	169 ± 11 (151-190)	165 ± 12 (148-180)
Dialysis age *years*	0	0	5.10 ± 4.02 (1.25-16.92)

Data were recorded at the time of screening.

Table 2. Mean ± SD (range) dialysis prescription details at each nominal dialysis session

Dialysis session	No. of subjects	Duration of dialysis session *hours*	Blood flow rate *mL/min*	Dialysate flow rate *mL/min*
Longitudinal patients				
Week 1 DS1	21	2.08 ± 0.26 (1.92-3.17)	164 ± 21 (100-200)	500 ± 0 (500-500)
Week 1 DS2	21	2.55 ± 0.23 (2.00-3.13)	173 ± 25 (100-200)	500 ± 0 (500-500)
Week 1 DS3	21	3.12 ± 0.48 (2.50-5.00)	192 ± 30 (150-290)	500 ± 0 (500-500)
Week 2 DS2	18	3.37 ± 0.36 (2.58-4.00)	194 ± 25 (133-250)	500 ± 0 (500-500)
Week 3 DS2	18	3.86 ± 0.29 (3.50-4.27)	211 ± 37 (160-325)	500 ± 0 (500-500)
Week 4 DS2	20	3.85 ± 0.38 (2.50-4.17)	226 ± 48 (150-350)	500 ± 0 (500-500)
Month 2 DS2	20	3.96 ± 0.30 (3.47-4.58)	244 ± 49 (160-350)	500 ± 0 (500-500)
Month 4 DS2	19	4.18 ± 0.65 (2.25-5.08)	272 ± 50 (167-400)	516 ± 69 (500-800)
Month 6 DS2	18	4.35 ± 0.59 (3.32-5.33)	298 ± 43 (200-400)	533 ± 97 (500-800)
Month 9 DS2	18	4.52 ± 0.62 (3.50-5.77)	308 ± 53 (240-450)	535 ± 100 (500-800)
Month 12 DS2	18	4.28 ± 0.58 (3.08-5.22)	282 ± 75 (100-450)	535 ± 100 (500-800)
Single-session patients				
>12 months	20	3.97 ± 0.73 (2.50-5.22)	320 ± 39 (280-400)	560 ± 123 (500-800)

subsequent sample collection time for those patients participating in the longitudinal component of the study. In addition, for each analyte, plasma and muscle levels at baseline and after 6, 12, and >12 months of dialysis were compared, and in the case of plasma levels, comparison with data from 60 healthy adults was also performed.

RESULTS

A total of 41 ESRD patients (25 males, 16 females) were enrolled into the study. Of these, 21 patients were enrolled into the longitudinal evaluation and 20 patients into the single-session evaluation. Eight patients enrolled in the longitudinal component consented for muscle biopsy collection, while muscle samples were collected from all patients participating in the single-session evaluation.

Thirty-eight patients completed the study, with 2 patients withdrawing due to kidney transplants, and another patient dying from a cerebrovascular event. The 3 patients who did not complete the study were enrolled in the longitudinal component. An additional 2 patients were withdrawn from the muscle biopsy component of the longitudinal evaluation before the 6-month sample could be collected because they no longer met the inclusion/exclusion criteria for muscle biopsy collection. Demographic data for all subjects, including the healthy volunteers, are presented in Table 1. During the first 4 months of treatment, the duration of the dialysis session, together with the blood flow rate, tended to increase, thereafter becoming constant (Table 2).

For those subjects who participated in the longitudinal component of the study, the mean plasma levels of L-carnitine, acetyl-L-carnitine, and total L-carnitine prior to the commencement of hemodialysis were 50.6 ± 20.0, 19.8 ± 8.9, and 75.3 ± 21.9 μmol/L, respectively. In comparison, the mean levels found in healthy adults were 43.3 ± 8.6, 6.65 ± 2.11, and 49.2 ± 9.3 μmol/L, respectively, with the difference between ESRD patients and healthy adults being significant ($P < 0.05$) in all cases (Table 3). Most notably, the plasma concentration of acetyl-L-carnitine was almost 3 times higher in the ESRD patients. It is also notable that while free L-carnitine comprised about 88% of the total L-carnitine in the plasma of healthy adults (12% esterified), in the ESRD patients only 67% of the total L-carnitine was in the unesterified form (33% esterified) (Table 3).

In the ESRD patients starting hemodialysis, the predialysis plasma levels of L-carnitine, acetyl-L-carnitine, and total L-carnitine had decreased significantly ($P < 0.05$) within the first week of hemodialysis treatment, with a continual decline over the first 12 months of treatment (Fig. 1). After 12 months of dialysis, plasma L-carnitine, acetyl-L-carnitine, and total L-carnitine had reached values of 29.7 ± 10.5, 10.0 ± 2.7, and 44.7 ± 11.1 μmol/L, respectively (Table 3). Upon examination

Table 3. Mean ± SD plasma concentrations (μmol/L) of L-carnitine, acetyl-L-carnitine, and total L-carnitine in healthy adults, and ESRD patients at baseline and after 6, 12, and >12 months of hemodialysis treatment

Analyte	Healthy adults	ESRD patients			
		Baseline	6 months	12 months	>12 months
L-carnitine	$43.3 \pm 8.6^{b,c,d,e}$	$50.6 \pm 20.0^{a,c,d,e}$	$34.6 \pm 12.9^{a,b,d,e}$	$29.7 \pm 10.5^{a,b,c}$	$22.0 \pm 5.4^{a,b,c}$
Acetyl-L-carnitine	$6.65 \pm 2.11^{b,c,d,e}$	$19.8 \pm 8.9^{a,c,d,e}$	$12.4 \pm 4.7^{a,b,d}$	$10.0 \pm 2.7^{a,b,c}$	$9.23 \pm 3.47^{a,b}$
Total L-carnitine	49.2 ± 9.3^{b}	$75.3 \pm 21.9^{a,c,d,e}$	48.9 ± 18.5^{b}	44.7 ± 11.1^{b}	42.6 ± 11.9^{b}

[a] Compared to healthy adults, $P < 0.05$.
[b] Compared to ESRD patients at baseline, $P < 0.05$.
[c] Compared to ESRD patients after 6 months of dialysis, $P < 0.05$.
[d] Compared to ESRD patients after 12 months of dialysis, $P < 0.05$.
[e] Compared to ESRD patients after >12 months of dialysis, $P < 0.05$.

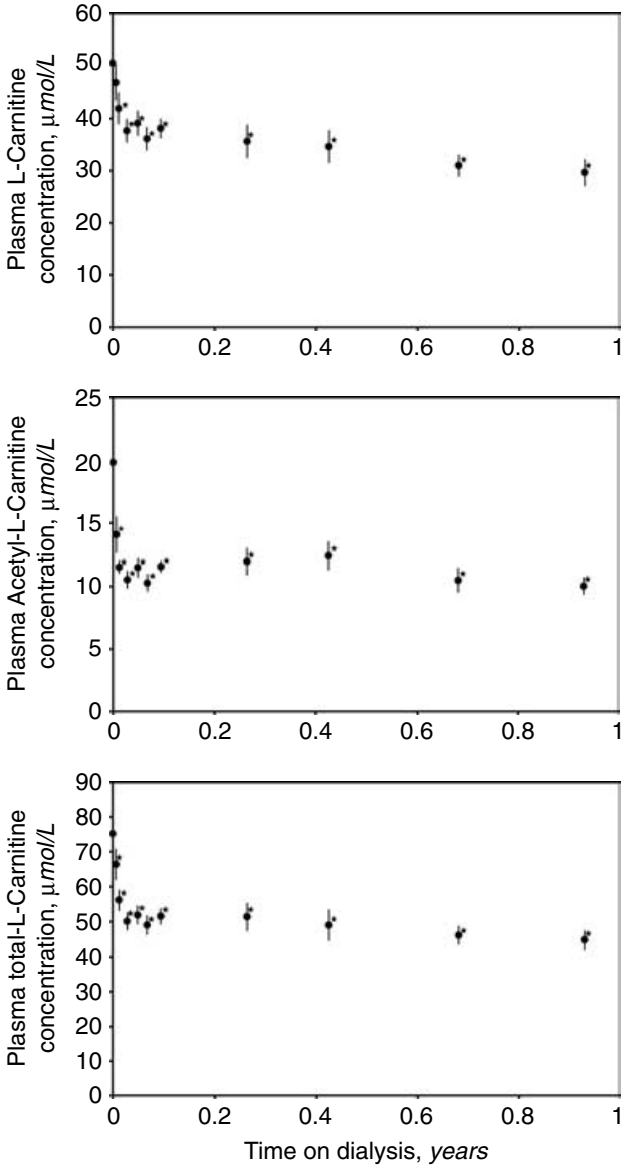

Fig. 1. Mean ± SEM plasma concentrations of L-carnitine (upper panel), acetyl-L-carnitine (middle panel), and total L-carnitine (lower panel) in patients with end-stage renal disease (ESRD) during the first 12 months of hemodialysis treatment. *Compared with baseline, $P < 0.05$.

of data from individual patients from each cohort (longitudinal and single-session), a clear relationship between plasma L-carnitine, acetyl-L-carnitine, and total L-carnitine concentrations and dialysis age is evident, whereupon the majority of the decrease from baseline is seen to occur over the first 12 months (Fig. 2). Indeed, when the patients who had been dialyzed for 12 months were compared to those who had been undergoing an average of 5.10 years of treatment, no statistically significant differences were detected (Table 3), even though the mean concentrations were lower for all 3 analytes in the >12 month group.

In the majority of ESRD patients who had been receiving hemodialysis for more than 12 months, plasma L-carnitine levels were substantially lower than the normal range observed in healthy subjects (37.7 to 48.5 μmol/L), as shown in Figure 2. Conversely, plasma acetyl-L-carnitine concentrations in these patients tended in most cases to be within or higher than the normal range (5.27 to 8.30 μmol/L), while there was no consistent trend for total L-carnitine (normal range of 43.1 to 55.3 μmol/L) once patients had been dialyzed for 12 months. Statistical comparisons of the various groups (Table 3) confirm these findings—notably, once patients had been undergoing hemodialysis for 12 months, mean plasma L-carnitine concentrations were about 30% to 50% below the average value for healthy subjects, while acetyl-L-carnitine was about 50% higher than normal, and total L-carnitine was not significantly different.

By comparing plasma L-carnitine and acetyl-L-carnitine levels to those of total L-carnitine (Fig. 3), it is evident that long-term hemodialysis in ESRD patients is associated with a progressive increase in the relative contribution of other L-carnitine esters (nonacetyl acylcarnitines, which mainly represent medium- and long-chain acylcarnitines). Indeed, as a percent of the total plasma carnitine pool, the proportion of these nonacetyl esters increases from negligible levels in healthy patients to about 26% in longer-term ESRD patients (Fig. 3). The accumulation of these esters probably explains why total L-carnitine levels are relatively normal in long-term dialysis patients despite a significant reduction in free L-carnitine (Fig. 2).

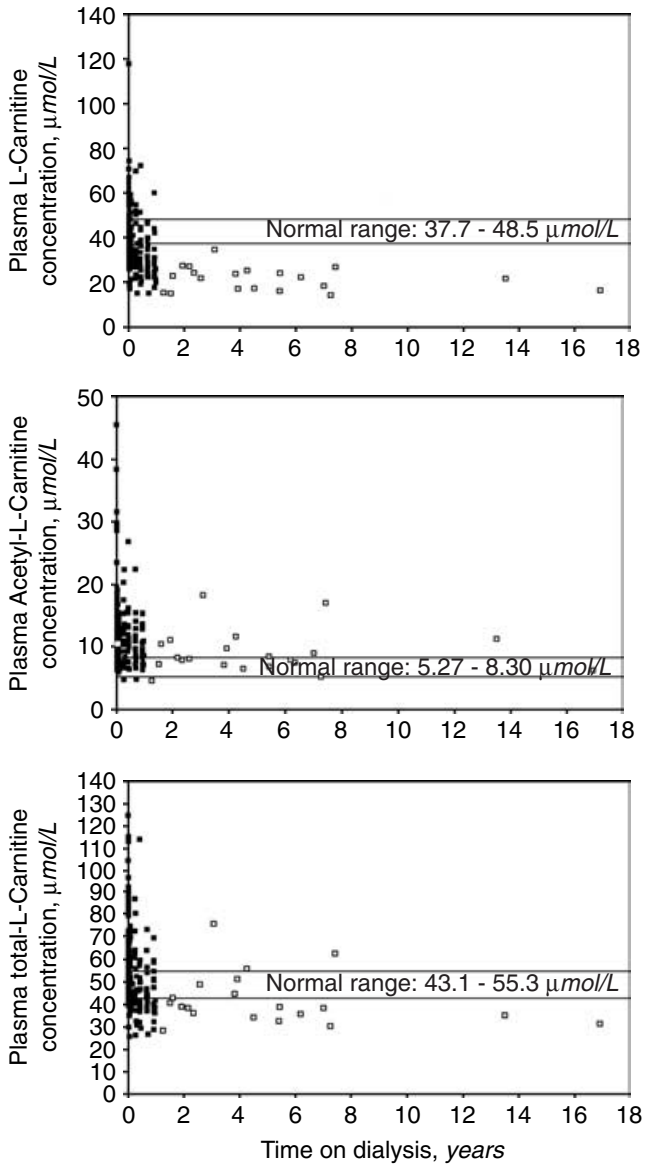

Fig. 2. Plasma concentrations of L-carnitine (upper panel), acetyl-L-carnitine (middle panel), and total L-carnitine (lower panel) from longitudinal (■) and single-session (□) patients as a function of dialysis age.

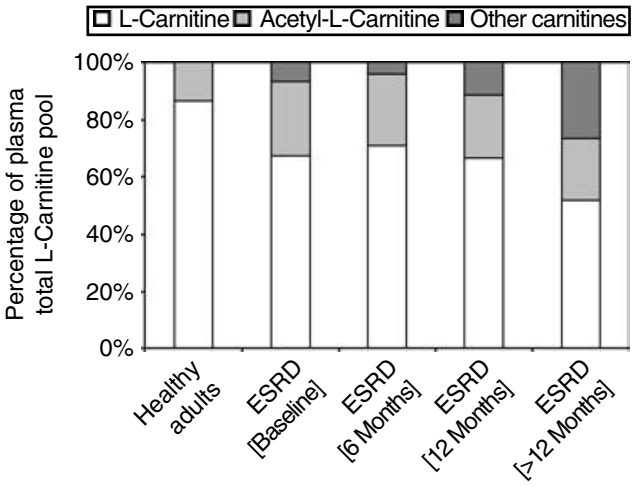

Fig. 3. The impact of hemodialysis on the relative composition of the plasma total L-carnitine pool.

A clear decline in skeletal muscle L-carnitine, acetyl-L-carnitine, and total L-carnitine levels was associated with increasing dialysis age (Fig. 4). In patients who had been undergoing hemodialysis for more than 12 months, the average muscle L-carnitine concentration (1.87 ± 0.67 µmol/g) was 38% lower than that in patients who had not yet started hemodialysis (3.01 ± 0.47 µmol/g) (Table 4). The duration of dialysis treatment required to cause a statistically significant decline in skeletal muscle L-carnitine levels was greater than 12 months, whereas for acetyl-L-carnitine a significant reduction was detected after 12 months (Table 4).

DISCUSSION

It has been widely reported that plasma L-carnitine levels in ESRD patients receiving hemodialysis are lower than in the general population [6, 7, 16–21]. However, there is very little information on the time frame over which levels decrease once dialysis therapy commences. The primary aim of the present study was to closely monitor L-carnitine levels in ESRD patients over their first 12 months of hemodialysis to provide definitive data on the temporal relationship between dialysis age and the quantitative and qualitative composition of the plasma and skeletal muscle pools. The results from these first-time hemodialysis patients were complemented by data from ESRD patients receiving long-term (>12 months) hemodialysis and healthy control patients.

Blood samples collected from the ESRD patients prior to the first dialysis session gave plasma levels of free L-carnitine (50.6 µmol/L) that were significantly higher than healthy adults (43.3 µmol/L). This finding is supported by other studies in which the impact of renal disease on L-carnitine levels has been examined, as reviewed recently by Ahmad [8] and Evans [2]. Once the ESRD patients commenced hemodialysis (twice or three times a week) there was a progressive decline in plasma L-carnitine levels (Fig. 1). Plasma L-carnitine concentrations declined by approximately 30% during the first 4 weeks of dialysis and by 40% over the first 12 months. A previous study demonstrated a decrease of about 28% over the first 6 months of hemodialysis treatment [6]. In the current study, mean plasma L-carnitine levels in those patients with a mean dialysis age of 5.10 years (22 µmol/L) was not significantly different to those patients who had been dialyzed for 12 months (29.7 µmol/L), suggesting that the majority of the change occurs during the initial 12 months of hemodialysis. In a study involving a larger

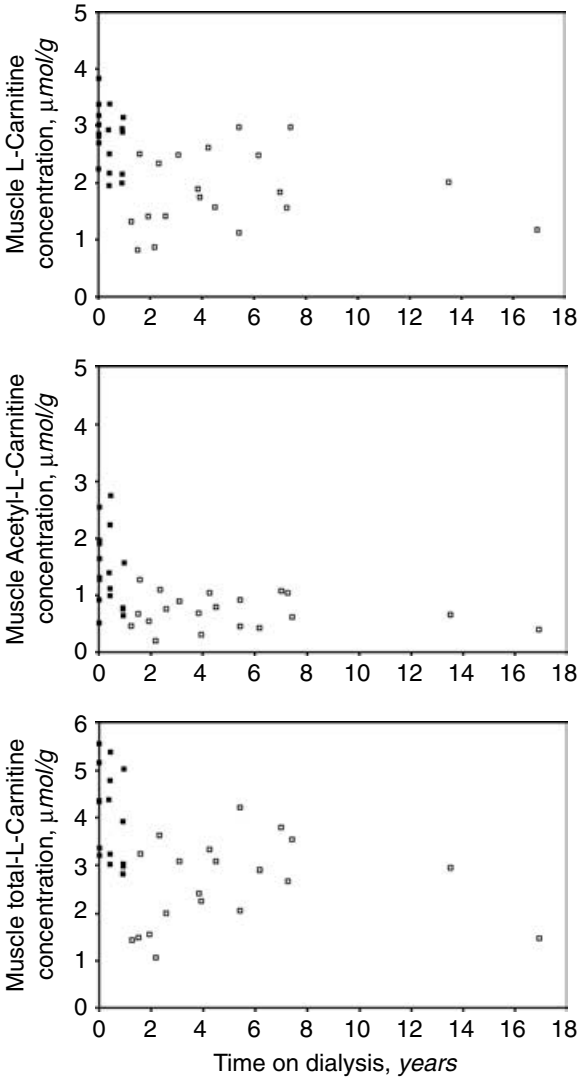

Fig. 4. Skeletal muscle concentrations of L-carnitine (upper panel), acetyl-L-carnitine (middle panel), and total L-carnitine (lower panel) from longitudinal (■) and single-session (□) patients as a function of dialysis age.

cohort ($N = 107$) of patients who had been undergoing hemodialysis for 1 to 25 years, Sakurachi et al [10] reported a significant negative correlation between plasma free L-carnitine levels and months of dialysis. However, the slope of the regression line in that study was very low, suggesting a net reduction of only 7 μmol/L over about 20 years of hemodialysis. Taken together with published data [6, 10], the results of the current study provides compelling evidence that the reduction in plasma L-carnitine levels in patients receiving chronic hemodialysis occurs primarily in the first 12 months of dialysis treatment. After 12 months, variability between patients tends to obscure any further changes, which may explain the failure of a previous study [9] to find a statistically significant correlation between plasma L-carnitine levels and dialysis age—most patients included in this latter study were long-term (>12 months) dialysis patients.

In a previous study, we demonstrated that there was no difference in the efficiency with which L-carnitine and acetyl-L-carnitine are removed from the body by hemodialysis, with plasma concentrations of both compounds falling by about 70% during a single 3.5-hour session [13], and this would explain the seemingly parallel decline (of about 40% to 50%) in L-carnitine and acetyl-L-carnitine over the first 12 months of hemodialysis. However, while plasma L-carnitine falls below normal within the first 6 months of hemodialysis, the levels of acetyl-L-carnitine actually remain above the normal range, even in long-term dialysis patients (Table 3, Fig. 2). Similarly, the acylated forms (excluding acetyl-L-carnitine) were substantially higher in the long-term dialysis patients (Fig. 3). The net effect of lower L-carnitine concentrations and elevated acylcarnitine levels is that the ESRD patients receiving hemodialysis tend to have relatively normal total L-carnitine levels in plasma (Table 3, Fig. 2). While patients commencing dialysis treatment had minimal residual kidney function, the effects of worsening kidney disease on the changes in the carnitine profile during the ensuing hemodialysis period are unknown.

A large number of studies have measured L-carnitine and related compounds in human skeletal muscle [9, 15, 17, 18, 22–27]. Direct comparisons between the results of these studies are complicated by the various reporting methods (per g wet weight, per g dry weight, per g of non-collagen protein), as well as differences between the sites and methods of muscle biopsy sampling [28]. A significant reduction in muscle L-carnitine, acetyl-L-carnitine, and total L-carnitine concentrations were observed as a function of dialysis age (Table 4). The mean muscle level of L-carnitine in long-term hemodialysis patients (>12 months) was 38% lower than the baseline level, while the acetyl-L-carnitine and total L-carnitine levels were 52% and 43% lower than the respective values prior to the commencement of hemodialysis. In keeping with our findings, in a previous study [18], the muscle concentrations of L-carnitine were 36% lower in long-term dialysis patients compared with healthy control patients.

In patients who had been undergoing hemodialysis for up to 16 years, a statistically significant negative correlation between muscle total L-carnitine and duration of dialysis was observed [18], with similar muscle concentrations to the present study (1 to 4 μmol/g tissue). Bellinghieri et al [22] also reported that the levels of free- and acetyl-L-carnitine in muscle from hemodialysis patients were about half those observed in control patients, with similar findings being reported by Savica et al [26]. In the most recent study involving muscle analysis, Dębska-Slizień et al [9] reported muscle free and total L-carnitine levels that were, on average, 30% lower

Table 4. Mean ± SD skeletal muscle concentrations (μmol/g) of L-carnitine, acetyl-L-carnitine, and total L-carnitine in ESRD patients at baseline and after 6, 12, and >12 months of hemodialysis treatment

		ESRD patients		
Analyte	Baseline	6 months	12 months	>12 months
L-carnitine	3.01 ± 0.47^{d}	2.60 ± 0.57^{d}	2.64 ± 0.52^{d}	$1.87 \pm 0.67^{a,b,c}$
Acetyl-L-carnitine	$1.52 \pm 0.64^{c,d}$	$1.71 \pm 0.76^{c,d}$	$0.914 \pm 0.377^{a,b}$	$0.727 \pm 0.297^{a,b}$
Total L-carnitine	4.54 ± 0.88^{d}	4.17 ± 1.01^{d}	3.56 ± 0.93^{d}	$2.61 \pm 0.91^{a,b,c}$

[a]Compared to ESRD patients at baseline, $P < 0.05$.
[b]Compared to ESRD patients after 6 months of dialysis, $P < 0.05$.
[c]Compared to ESRD patients after 12 months of dialysis, $P < 0.05$.
[d]Compared to ESRD patients after > 12 months of dialysis, $P < 0.05$.

than normal in a group of long-term hemodialysis patients. In contrast, Fagher et al [29] found that the levels of L-carnitine in skeletal muscle from patients who had been undergoing hemodialysis for an average of 3 years were within the range of values encountered for healthy adults. However, the authors of this paper warn that the analytical method used for the 2 populations differed and so no statistical comparisons were performed. Therefore, the results of this latter study should not be taken as evidence for a lack of effect of hemodialysis on skeletal muscle L-carnitine content. Mingardi et al [30] also reported that total and free muscle L-carnitine in hemodialysis patients was similar to control values but this was based on data from just 4 patients, which do not allow a meaningful comparison, particularly with the variability normally encountered with muscle L-carnitine analysis.

For L-carnitine, the magnitude of the reduction (between baseline and the long-term dialysis patients) was similar (30% to 40%) in both plasma and muscle. However, while the reduction in plasma levels occurred over the first 12 months of hemodialysis, the most significant change in muscle L-carnitine concentrations were detected after 12 months. This is consistent with the concept that L-carnitine in skeletal muscle equilibrates very slowly with that in plasma [31]. This slow equilibration arises because more than 98% of the body's L-carnitine is stored inside skeletal muscle, and the rate constant for the efflux of the compound from muscle tissue is extremely small [2, 31]. The other notable finding in the present study is that in muscle, most of the total L-carnitine could be accounted for as the sum of directly measured L-carnitine and acetyl-L-carnitine, irrespective of the sampling time (Table 4), whereas in plasma there was a progressive accumulation of nonacetyl acylcarnitines (Fig. 3). The implications of the accumulation of longer-chain acylcarnitines in the plasma of these hemodialysis patients are unknown.

The delayed temporal relationship between plasma and muscle L-carnitine levels, described above, may explain why previous attempts to correlate plasma and skeletal muscle levels of L-carnitine and related species have met with limited success [9, 27, 32]. The disparate time-courses can be explained by the compartmentalization of L-carnitine in skeletal muscle and the slow kinetics of L-carnitine distribution between plasma and tissue [2, 8]. While the plasma compartment becomes dramatically depleted of L-carnitine during a single dialysis session [3, 8], the movement of L-carnitine from the rich muscle stores during the intervening interdialysis period serves to partially (but not totally) replenish the plasma levels. With continued dialysis, there is a net removal of L-carnitine from muscle tissue, leading to a gradual depletion in this compartment. While acetyl-L-carnitine levels also decline during hemodialysis treatment, the plasma levels of this ester remain well above the normal range. Other carnitine esters accumulate in plasma during long-term hemodialysis treatment, meaning that there is a significant change with time in the composition of a dialysis patient's plasma carnitine pool.

CONCLUSION

The present study provides definitive data showing a dramatic reduction in plasma L-carnitine and acetyl-L-carnitine levels upon the commencement of hemodialysis treatment of ESRD patients. While these changes occur primarily within the first few months of hemodialysis, it may take 12 months or more for significant accumulation of plasma (nonacetyl) esters of L-carnitine, and for a significant decline in the skeletal muscle L-carnitine pool. The lack of previous studies to find significant correlations between plasma and muscle carnitine levels is likely to be due, in large, to the different kinetic profiles for the 2 carnitine pools.

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Reprint requests to Professor Allan M. Evans, Centre for Pharmaceutical Research, University of South Australia, Frome Road, Adelaide, SA, 5000, Australia.
E-mail: allan.evans@unisa.edu.au

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