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Original Article

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Effect of L-carnitine on the kinetics of carnitine, acylcarnitines and butyrobetaine in long-term haemodialysis

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Abstract

Background. The current study was performed to investigate the kinetics of carnitine, individual acylcarnitines and butyrobetaine in patients on haemodialysis.
Methods. Eight stable long-term haemodialysis patients were studied under basal conditions (no carnitine supplementation) and 3 weeks after intravenous supplementation with L-carnitine (10 or 20 mg/kg body weight) after each haemodialysis session. The kinetic studies included serial determinations of carnitine and metabolites just before, during or between haemodialysis sessions. Analysis was performed by liquid chromatography–tandem mass spectrometry.
Results. Before haemodialysis, the plasma concentrations were (μmol/l) 15.1 ± 0.6 (mean ± SEM) for carnitine, 5.9 ± 0.7 for acetylcarnitine, 0.66 ± 0.04 for propionylcarnitine and 0.98 ± 0.08 for butyrobetaine (basal conditions) or 142 ± 23 for carnitine, 69 ± 12 for acetylcarnitine, 6.0 ± 1.1 for propionylcarnitine and 2.6 ± 0.3 for butyrobetaine (carnitine 20 mg/kg). During haemodialysis, the plasma concentrations dropped by ~80% for all compounds determined, with extraction coefficients ranging from 0.65 to 0.86. In patients supplemented with 20 mg/kg carnitine, the amount of carnitine removed by haemodialysis equalled 42% of the dose administered, consisting of 2.08 mmol carnitine, 1.03 mmol acetylcarnitine and 0.051 mmol propionylcarnitine. Between the haemodialysis sessions, carnitine, acylcarnitines and butyrobetaine reached apparent steady-state concentrations within 1 day both under basal conditions and after supplementation.
Conclusions. Patients on haemodialysis have reduced carnitine, acylcarnitine and butyrobetaine plasma levels, which can be increased by supplementing carnitine. Propionylcarnitine, an important constituent of the acylcarnitine pool, can be removed by haemodialysis. Removal of potentially toxic acyl-groups may represent a mechanism for a beneficial effect of carnitine in these patients.

Keywords: acylcarnitines; butyrobetaine; carnitine; haemodialysis; liquid chromatography–tandem mass spectrometry

Introduction

Carnitine (L-3-hydroxy-4-*N*-trimethylaminobutyrate), an endogenous compound present in most mammalian tissues, is ingested by the diet and produced endogenously by biosynthesis. Carnitine is an essential factor for transport of long-chain fatty acids into the mitochondrial matrix, where they are metabolized via β-oxidation [1]. By reacting with activated fatty acids, carnitine can generate various acylcarnitines with different chain lengths. By accepting acyl-groups from acyl-CoAs, carnitine is also involved in the regulation of the cellular pool of free coenzyme A (CoASH), thus acting as a buffer [1]. This buffer effect of carnitine may be important for the detoxification and elimination of potentially toxic acyl-groups, originating from exposure to xenobiotics and/or from blockage of metabolic pathways. In contrast to acyl-CoAs, the corresponding acylcarnitines can be excreted in the urine [1,2].

In healthy subjects, carnitine and acetylcarnitine represent the major constituents of the body fluid and tissue carnitine pools [1,3]. Skeletal muscle contains >95% of the total carnitine body stores and the tissue concentrations are considerably higher than the concentration in plasma, necessitating active transport into tissues. Kidneys play a crucial role in carnitine homeostasis, since they reabsorb >90% of the filtered carnitine [1], so that the plasma levels of free carnitine are maintained at 30–40 μmol/l [4,5].

Since carnitine is a small molecule (molecular weight: 161.2) and not protein-bound, it is efficiently removed

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from blood through dialyser membranes [1]. Patients with end-stage renal disease undergoing long-term haemodialysis, therefore, have reduced carnitine plasma [6–8] and muscle levels [7,8] and may exhibit symptoms similar to those observed in patients with carnitine deficiency, e.g. skeletal muscle weakness and pain as well as impaired exercise performance [1]. In addition, such patients may suffer from intradialytic cramps and hypotensive episodes, which may also be related to low carnitine tissue stores [1]. In plasma of patients with haemodialysis, the carnitine concentration has been found to be decreased and the acylcarnitine concentration to be increased, so that the acylcarnitine to total carnitine ratio (free carnitine plus acylcarnitines) is higher than in healthy subjects [6,8].

Supplementation with carnitine, either orally or intravenously, at the end of haemodialysis has been shown to increase the carnitine plasma concentration [6,9] and carnitine skeletal muscle content [9]. Despite the fact that carnitine supplementation is clearly associated with an increase in carnitine plasma and tissue levels, carnitine administration has been shown to be associated with clinical improvement of muscular symptoms in some [7,10] but not all studies or patients [11]. In order to answer the question whether the administration of carnitine is beneficial to patients with haemodialysis, larger placebo-controlled studies with relevant clinical endpoints would be needed.

In several studies, the metabolism of carnitine in patients on long-term haemodialysis has been investigated. Most of them were restricted to the observation of free and acylcarnitines [12,13], but in some studies the individual acylcarnitines were differentiated [6,14]. In one recent study, the plasma concentration of carnitine, acetylcarnitine and total carnitine was investigated in patients on haemodialysis before and after intravenous supplementation of different doses of carnitine [6].

In the current study, we investigated the composition of the plasma carnitine, acylcarnitine and butyrobetaine pools in patients on long-term haemodialysis under baseline conditions and during intravenous supplementation with two different doses of carnitine. The kinetics of carnitine, individual acylcarnitines and butyrobetaine were evaluated during and between haemodialysis sessions. The analytical method utilized was high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [5], which allows the quantification of all analytes mentioned above. We were particularly interested to determine which acylcarnitines other than acetylcarnitine accumulate in the plasma of patients on long-term haemodialysis and how the plasma butyrobetaine pool is influenced by the administration of carnitine.

Subjects and methods

The study was approved by the Ethics Committee of the Cantons of Basel and patients provided written informed consent before the start of the study.

Study population

Patients aged between 20 and 80 years were recruited at the dialysis centre of the University Hospital of Basel. Enrolment requirements included treatment with haemodialysis three times a week for >6 months and the absence of acute illnesses. Patients already supplemented with carnitine, either orally or intravenously, were also excluded from the study.

Eight patients (four women and four men) with a mean age of 59 years (Table 1) were included. They underwent three dialysis sessions per week, each of them having a duration of 240 min. The dialysate fluid was based on bicarbonate (not acetate) and the dialysate flow was kept constant at 0.5 l/min. An HF 80 dialysis membrane (Fresenius, Bad Homburg, Germany), the most commonly used membrane in our haemodialysis centre, was utilized for all patients. The quality of haemodialysis was assessed by the urea reduction ratio, which was >0.6 for all patients on all haemodialysis sessions, with an average of 0.68 ± 0.02. In addition, Kt/V [15] and the protein equivalent of nitrogen appearance normalized per bodyweight (nPNA) [16] were calculated for each patient for one interdialytic period of all three trial phases. The mean values were 1.34 ± 0.09 for Kt/V and 1.20 ± 0.09 g/kg/day for nPNA.

Table 1. Characterization of the patients completing the study. Patients 1, 5, 7 and 8 were recruited for intra- and interdialysis kinetics (see Table 2). All patients were treated with parenteral epoetin and iron preparations and oral calcitriol, multivitamin preparations and calcium carbonate. Additional drugs for individual patients were acetylsalicylic acid, angiotensin-converting enzyme inhibitors and atorvastatin. None of these drugs is known to interfere with carnitine metabolism

Patient	Sex	Age (years)	Urine production per day (ml)	Target weight (kg)	Dialysis blood flow rate (l/min)	Carnitine dose (mmol) for 10 mg/kg
1	Female	46	0	59.0	0.30	3.66[a]
2	Male	75	200	83.5	NR[b]	5.17
3	Male	38	50	66.0	NR	4.08
4	Female	60	0	76.0	NR	4.69
5	Female	59	0	72.5[c]	0.30	4.39
6	Male	57	650	75.5	NR	4.69
7	Male	76	800	74.5	0.30	4.65
8	Female	58	0	62.0	0.30	3.83

[a]For 20 mg/kg the dose was doubled.
[b]Not relevant (NR), as patient did not participate in the intradialysis kinetic study.
[c]71.0 kg during the first 2 weeks of the study.

Study design

The study was performed over 7 weeks (21 haemodialysis sessions) and included three different observation periods. During the first week, patients were studied under baseline conditions (no carnitine supplementation; patients studied on days 1 and 6). During the next 3 weeks, all patients were supplemented after each dialysis session with 10 mg/kg body weight carnitine intravenously (low carnitine supplementation; patients studied on days 13, 20 and 27). During the last 3 weeks, carnitine supplementation was increased to 20 mg/kg body weight carnitine intravenously (high carnitine supplementation; patients studied on days 34, 41 and 48). Carnitine (Carnitene sigma-tau® Sigma-Tau Pharma AG, Zofingen, Switzerland) was administered by injection over 2 min using the venous line installed for haemodialysis, just before it was removed at the end of the haemodialysis session. After the administration of carnitine, the line was flushed with saline. Blood samples were collected in 2 ml heparinized tubes, which were centrifuged and the plasma was removed and stored in polystyrene tubes at −20°C until analysis.

Carnitine and acylcarnitine profiles between haemodialysis sessions

To establish the carnitine and acylcarnitine profiles over the entire study period, blood collections were performed in all patients twice under baseline conditions (before haemodialysis sessions on days 1 and 6), thrice under low carnitine supplementation (before haemodialysis sessions on days 13, 20 and 27) and thrice under high carnitine supplementation (before haemodialysis sessions on days 34, 41 and 48). Thus, the first blood sample collections under the respective supplementation conditions were performed after three and the last after nine administrations of the respective carnitine dosage. All samples were withdrawn immediately before the respective haemodialysis session was started.

Carnitine and acylcarnitine kinetics during dialysis sessions (intradialysis)

Intradialysis kinetics of carnitine was studied in four patients (patients 1, 5, 7 and 8) under baseline conditions (session on day 6), after 3 weeks of low carnitine supplementation (session on day 27) and after 3 weeks of high carnitine supplementation (session on day 48). Blood samples were collected before haemodialysis was started, during and at the end of haemodialysis just before the administration of carnitine. During haemodialysis, arterial blood (blood entering in the dialysis system), venous blood (blood leaving the dialysis system) and 10 ml dialysate were collected at 10, 30, 60, 120, 180 and 240 min after beginning haemodialysis. The arterial blood was obtained just before the venous one and the dialysate was collected just after the blood samples had been obtained.

Carnitine and acylcarnitine kinetics between two dialysis sessions (interdialysis)

Interdialysis kinetics of carnitine was studied in four patients (patients 1, 5, 7 and 8) under baseline conditions (between sessions on days 4 and 6) and at the end of the study (after 3 weeks of treatment with 20 mg/kg carnitine; between sessions on days 46 and 48). Blood samples were collected before the start of haemodialysis, immediately after haemodialysis and after the session on day 4, at 5, 10, 15, 20, 30 and 45 min and 1, 2, 4, 6, 20 and 44 h after termination of haemodialysis. After the session on day 46, a blood sample was obtained, 20 mg/kg carnitine was injected intravenously over 2 min and blood samples were obtained at the same time-points as indicated above, beginning after termination of the injection. At the end of haemodialysis, the venous line was kept open during 6 h for the collection of blood. The 20 h blood sample was obtained by venopuncture and the 44 h collection time-point corresponded to the beginning of the next haemodialysis session. Patients received a vegetarian meal between the blood collections at 1 and 2 h.

Analysis of carnitine and acylcarnitines

Carnitine, the different acylcarnitines and butyrobetaine present in plasma and dialysate samples were analysed by LC–MS/MS according to a method published previously for plasma [5]. Briefly, samples were subjected to a solid-phase extraction on a cation-exchange column and separation was performed using a reversed-phase column in the presence of a volatile ion-pairing reagent. Detection was carried out using an ion-trap mass spectrometer run in the MS/MS mode. The method linearity was 10–80 μmol/l for carnitine and 0.5–20 μmol/l for acetylcarnitine, propionylcarnitine, isovalerylcarnitine and butyrobetaine. Inter-day and intra-day plasma quality controls (determined at the extremes of the respective range of linearity) had precisions ranging from 2.2% to 13.7% and accuracies between 70.5% and 129.9%, for both carnitine and acylcarnitines.

Plasma samples having high carnitine and acetylcarnitine levels were diluted up to 40-fold with a 4% bovine serum albumin solution in water before analysis in order to reach the calibration range. Modified calibration ranges for carnitine (5–60 μmol/l) were used for the analysis of samples containing a low carnitine concentration.

The acylcarnitine concentration used to calculate total carnitine (sum of free and acylcarnitines) and the acylcarnitine to total carnitine ratios was obtained by summation of the concentration of each individual acylcarnitine.

Statistics and pharmacokinetic analysis

Data are presented as means ± SEM unless stated otherwise. Kinetics of carnitine, acylcarnitine and butyrobetaine in the interhaemodialysis period were analysed under baseline conditions (between haemodialysis sessions on days 4 and 6) and after 3 weeks of supplementation with 20 mg/kg body weight carnitine (between haemodialysis sessions on days 46 and 48). The area under the curve (AUC) from time 0 to 44 h (AUC_{0-44}) was determined with the linear trapezoidal rule using TopFit software [17]. The baseline-corrected AUC was calculated by subtracting the AUC obtained under baseline conditions from the corresponding AUC after carnitine substitution.

The intradialysis period was evaluated under baseline conditions (haemodialysis session on day 6), after 3 weeks of treatment with 10 mg/kg body weight carnitine (haemodialysis session on day 27) and after 3 weeks supplementation with 20 mg/kg body weight carnitine (haemodialysis session

Table 2. Kinetics of carnitine, acylcarnitines and butyrobetaine during and between haemodialysis sessions. Values are expressed as means ± SEM, $n = 4$ patients. AUC reflects the area under the curve between two dialysis sessions (interdialysis) or within one session (intradialysis). Cl_h represents the clearance by haemodialysis, E the extraction coefficient by haemodialysis and A_h and A_d the amount of carnitine or acylcarnitines removed by haemodialysis calculated using different methods. The calculation of these variables is described in the 'Subjects and methods'

	Carnitine	Acetylcarnitine	Propionylcarnitine	Butyrobetaine
Interdialysis				
$AUC_{0-44\,h}$ (μmol/l × h) – no substitution	540 ± 18	200 ± 13	ND[a]	45.0 ± 4.3
$AUC_{0-44\,h}$ (μmol/l × h) – 20 mg/kg carnitine	9250 ± 550	2690 ± 200	242 ± 23	108 ± 10
Baseline-corrected $AUC_{0-44\,h}$ (μmol/l × h)	8710 ± 550	2490 ± 190	242 ± 23	62.3 ± 5.8
Intradialysis				
No substitution				
$AUC_{art;\,0-240\,min}$ (μmol/l × h)	24.7 ± 1.9	7.59 ± 0.75	ND	2.18 ± 0.05[b]
Cl_h (l/h)	8.2 ± 0.3	9.4 ± 0.3	ND	8.3 ± 0.6[b]
E	0.65 ± 0.02	0.75 ± 0.02	ND	0.66 ± 0.05[b]
A_h (μmol)	203 ± 15	71.4 ± 7.1	ND	18.2 ± 1.8[b]
A_d (μmol)	229 ± 19	ND	ND	ND
10 mg/kg carnitine				
$AUC_{art;\,0-240\,min}$ (μmol/l × h)	64.4 ± 2.4	25.1 ± 0.5	3.03 ± 0.12[b]	3.53 ± 0.10
Cl_h (l/h)	10.0 ± 0.58	10.4 ± 0.3	9.89 ± 0.22[b]	9.67 ± 0.25
E	0.76 ± 0.03	0.80 ± 0.03	0.79 ± 0.02[b]	0.77 ± 0.02
A_h (μmol)	640 ± 15	262 ± 11	30.0 ± 1.9[b]	34.1 ± 1.3
A_d (μmol)	634 ± 50	226 ± 47	ND	32.0 ± 4.0[b]
A_h (% of carnitine administered)[c]	23.8 ± 1.1			
20 mg/kg carnitine				
$AUC_{art;\,0-240\,min}$ (μmol/l × h)	197 ± 25	88.9 ± 17.0	4.96 ± 0.16	3.45 ± 0.31
Cl_h (l/h)	10.7 ± 0.42	11.3 ± 0.5	10.4 ± 0.4	10.5 ± 0.4
E	0.82 ± 0.01	0.86 ± 0.01	0.79 ± 0.01	0.80 ± 0.01
A_h (μmol)	2080 ± 220	1030 ± 250	51.4 ± 1.5	35.9 ± 2.4
A_d (μmol)	1950 ± 230	915 ± 162	ND	34.2 ± 5.7[b]
A_h (% of carnitine administered)[c]	42.4 ± 5.1			

[a]Not determined (ND) (below the limit of quantification).
[b]$n = 2$.
[c]Includes carnitine, acetylcarnitine and propionylcarnitine.

on day 48). The extraction coefficient (E) was calculated as follows:

$$E = (C_{pre} - C_{post})/C_{pre} \quad (1)$$

where C_{pre} is the concentration of the analyte in arterial blood plasma (entering the dialysis system) at the start of haemodialysis and C_{post} is the concentration in arterial blood plasma at the end of haemodialysis. Alternatively, E was calculated as:

$$E' = (C_{art} - C_{ven})/C_{art} \quad (2)$$

where C_{art} is the arterial and C_{ven} is the venous plasma concentration at a given time-point. Since the values for E and the average of E' (average of several determinations during haemodialysis) were not different, E is given in Table 2 and was used for the following calculations.

The clearance by haemodialysis was calculated as:

$$Cl_h = Q \times E \times (1 - \text{haematocrit}) \quad (3)$$

where Q is the haemodialysis blood flow (Table 1) and E is the extraction coefficient (equation 1). The term (1 – haematocrit) was introduced due to the publication of a study by Leschke *et al.* [12], showing that the carnitine content of erythrocytes does not change significantly during haemodialysis. The haematocrit values obtained before haemodialysis were used for the calculation (they were all in the range of 0.30–0.35).

The amount of carnitine and acylcarnitines eliminated by haemodialysis (A_h) was calculated as follows:

$$A_h = AUC_{art;\,0-240} \times Cl_h \quad (4)$$

with $AUC_{art;\,0-240}$ being the AUC in arterial blood from time zero until completion of haemodialysis after 240 min.

Alternatively, the amount eliminated by haemodialysis was calculated from the carnitine and acylcarnitine concentrations in the dialysate:

$$A_d = AUC_{d;\,0-240} \times Q_d \quad (5)$$

with $AUC_{d;\,0-240}$ being the AUC in the dialysate calculated using the trapezoidal rule and Q_d the dialysate flow (0.5 l/min).

Results

Using a sensitive LC–MS/MS method, we investigated the intra- and interdialysis kinetics of carnitine in patients with chronic renal failure treated by haemodialysis under baseline conditions and during treatment with different amounts of intravenous carnitine. Under baseline conditions, the plasma free carnitine concentrations ranged from 12.1 to 16.6 μmol/l. These levels are in the same range as the 19.5 ± 5.6 μmol/l reported

by Evans *et al.* [6] in similar patients, but lower as compared with the 34.3 ± 1.2 μmol/l obtained in healthy persons [5]. The acylcarnitine to total carnitine ratio was 0.30 ± 0.02 (range: 0.22–0.39), a value which is higher than the ratio of 0.16 ± 0.02 observed in healthy persons [5]. Acetylcarnitine was the most prominent short-chain acylcarnitine, but propionylcarnitine was also detectable in five and isovalerylcarnitine in two patients. Other acylcarnitines were not detectable. The carnitine precursor butyrobetaine could be found in the plasma of all patients at a concentration of 0.98 ± 0.08 μmol/l (range: 0.61–1.38 μmol/l).

Carnitine and acylcarnitines profiles

The evolution of the pre-dialytic carnitine and acylcarnitine pools over the entire study is depicted in Figure 1. After 1 week of supplementation with 10 mg/kg carnitine (low dosage) at the end of each haemodialysis session, the carnitine pre-dialysis concentration increased in all patients between 130% and 440% as compared with baseline (mean plasma concentration: 40.2 ± 5.3 μmol/l; range: 21.1–70.8 μmol/l). The acetylcarnitine concentration increased from 5.9 ± 0.4 to 17.8 ± 3.0 μmol/l, propionylcarnitine from 0.7 ± 0.1 to 1.20 ± 0.2 μmol/l and butyrobetaine from 0.94 ± 0.09 to 1.83 ± 0.17 μmol/l. After 3 weeks of low dosage carnitine supplementation, the carnitine, acetylcarnitine and propionylcarnitine plasma levels had increased further in most patients, while the isovalerylcarnitine and butyrobetaine levels remained constant. After 1 week of carnitine supplementation at low dosage, the acylcarnitine to total carnitine ratio had reached a value of 0.31 ± 0.02, which dropped to 0.29 ± 0.01 at the end of the low carnitine supplementation period.

After 1 week of supplementation with 20 mg/kg carnitine (high dosage) at the end of each haemodialysis session, the carnitine plasma concentration showed a further increase to 114 ± 17 μmol/l (range: 46.4–186 μmol/l). Similar to carnitine, acetylcarnitine increased to 50.7 ± 7.9 μmol/l (range: 13.4–77.6 μmol/l), propionylcarnitine to 4.57 ± 0.82 μmol/l (range: 1.23–8.58 μmol/l) and butyrobetaine to 2.69 ± 0.36 μmol/l (range: 1.56–4.21 μmol/l). Isovalerylcarnitine appeared in the plasma of five patients, reaching a concentration of 0.75 ± 0.04 μmol/l (range: 0.66–0.95 μmol/l). At the end of the study, after 3 weeks of high-dose carnitine supplementation, seven patients showed further increases in their carnitine levels, reaching concentrations of 78–229 μmol/l. In contrast, patient 4 showed a constant carnitine plasma concentration of 47 μmol/l over the entire 3 weeks of high-dose carnitine supplementation. Further increases were also observed for acetylcarnitine, propionylcarnitine and isovalerylcarnitine, reaching plasma concentrations of 69.0 ± 11.9, 6.0 ± 1.1 and 0.88 ± 0.08 μmol/l, respectively. The mean plasma concentration of butyrobetaine reached 2.58 ± 0.28 μmol/l at the end of the high-supplementation period. After 1 week of carnitine supplementation at high dosage, the acylcarnitine to total carnitine ratio had reached a value of 0.32 ± 0.02, which increased to 0.34 ± 0.01 at the end of the high carnitine supplementation period. The fact that the plasma carnitine concentration tended to increase during the individual supplementation periods (Figure 1) suggests that 3 weeks of carnitine supplementation were not sufficient for reaching steady-state conditions. This may be explained by a slow turnover of carnitine in skeletal muscle, which has been estimated to be in the range of 7–10 days [18].

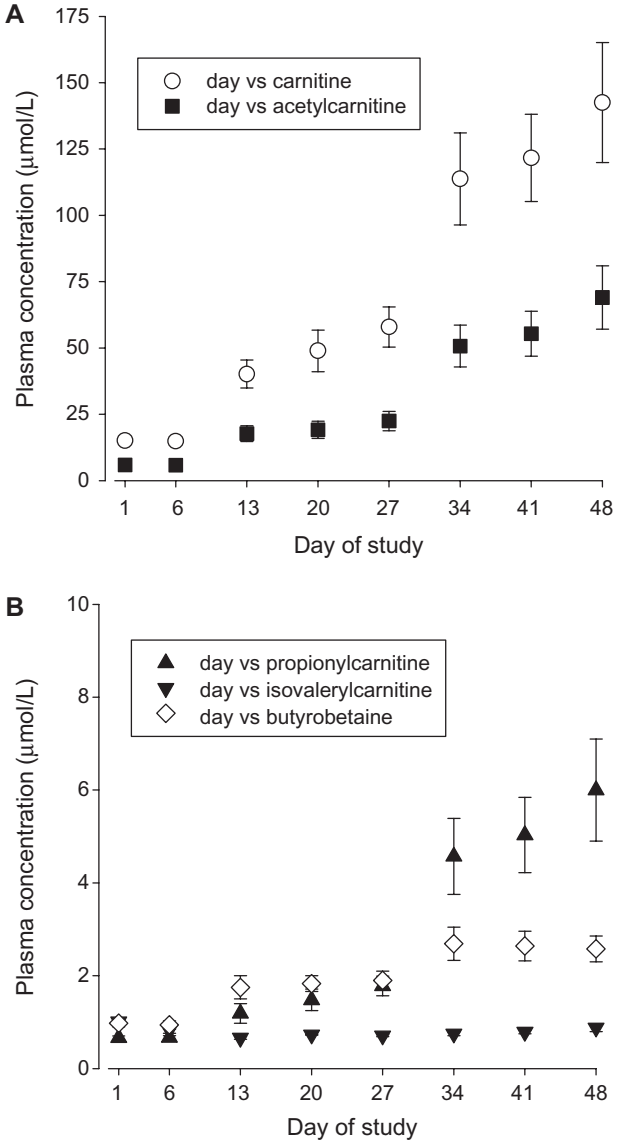

Fig. 1. Pre-dialysis plasma concentrations of carnitine, acylcarnitines and butyrobetaine. Values are expressed as means ± SEM, $n = 8$ patients. Analysis was performed by LC–MS/MS as described in the 'Subjects and methods'. Carnitine supplementation (intravenous after each haemodialysis session) was as follows. Days 1 and 6: no supplementation (baseline); days 13, 20 and 27: 10 mg/kg (low carnitine supplementation); days 34, 41 and 48: 20 mg/kg (high carnitine supplementation).

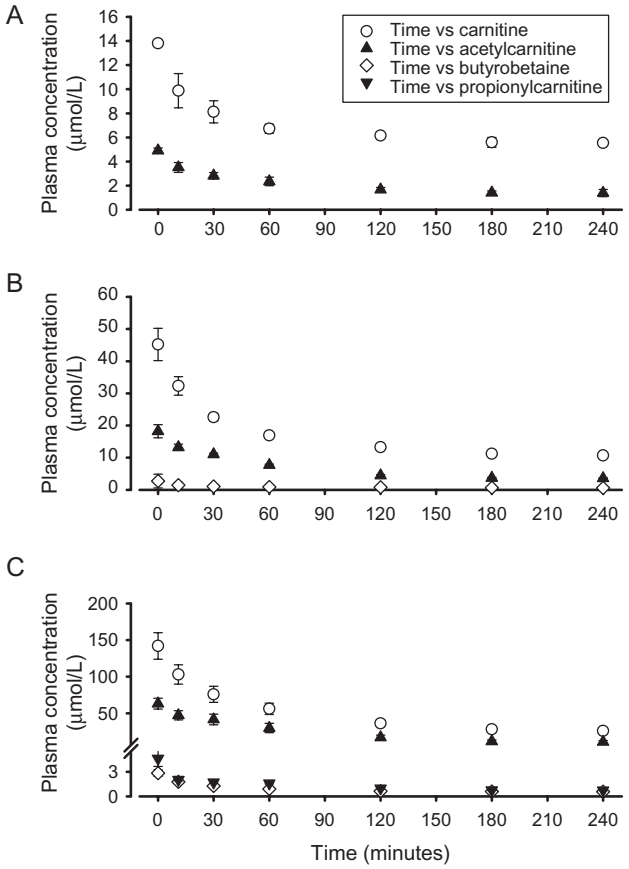

Fig. 2. Kinetics of carnitine, acylcarnitines and butyrobetaine during haemodialysis (intradialysis kinetics). Values are expressed as means ± SEM, $n = 4$ patients. Analysis was performed by LC–MS/MS as described in the 'Subjects and methods'. (**A**) Baseline conditions (day 6). (**B**) During supplementation with 10 mg/kg body weight carnitine (day 27). (**C**) During supplementation with 20 mg/kg body weight carnitine (day 48). All analytes are removed efficiently by haemodialysis, reaching a new apparent steady state after ~2 h.

Intradialysis kinetics

The plasma concentration–time curves for carnitine, acylcarnitines and butyrobetaine are given in Figure 2. As known from previous studies [6,12], carnitine and acetylcarnitine can be removed efficiently by haemodialysis. As could be expected, this is also the case for propionylcarnitine and butyrobetaine (Figure 2B and 2C). For carnitine, acylcarnitines and butyrobetaine, apparent steady-state plasma concentrations are reached after 2–3 h of haemodialysis. This apparent steady-state concentration is ~80% lower for all analytes than the respective pre-dialysis plasma concentrations, demonstrating efficient removal by haemodialysis.

The kinetic analysis of the plasma concentration–time curves during haemodialysis allowed the calculation of the extraction (E) by haemodialysis, haemodialytic clearance (Cl_h) and the amount of analytes removed by haemodialysis (A_h and A_d) (Table 2). These calculations show that supplementation with increasing doses of carnitine is associated with a dose-dependent removal of acyl groups by haemodialysis. The good agreement between A_h and A_d suggests that the equilibration between the carnitine pool within the erythrocytes and plasma is minimal during haemodialysis.

Interdialysis kinetics

At the end of haemodialysis, the plasma levels of all analytes were reduced by ~80% as compared with pre-dialysis values (Figure 2). When carnitine was not supplemented, carnitine, acetylcarnitine and butyrobetaine plasma concentrations started to increase already 5 min after the end of haemodialysis (Figure 3A). This increase was more marked during the first 6 h after haemodialysis and apparently stable concentrations were reached after 8–24 h.

In patients supplemented with 20 mg/kg carnitine, the plasma carnitine concentration reached 1350 ± 160 μmol/l 5 min after termination of the intravenous administration of carnitine (Figure 3B). After having reached the peak concentration, the plasma carnitine concentration decreased with a half-life of ~2 h, attaining apparently stable concentrations 8–24 h after injection. After intravenous administration of carnitine, the plasma concentrations of acetylcarnitine, propionylcarnitine and butyrobetaine increased rapidly, reaching apparently stable concentrations after 8 h (Figure 3B). In patients 1 and 8, isovalerylcarnitine was also detected and showed a similar kinetic behaviour as the other acylcarnitines.

The AUCs for the interdialysis period obtained without carnitine supplementation and after 3 weeks of supplementation with 10 or 20 mg/kg intravenous carnitine are given in Table 2. In comparison to baseline, the AUCs increased by a factor of 17.1 ± 1.0 for carnitine, 13.5 ± 1.0 for acetylcarnitine and 2.4 ± 0.2 for butyrobetaine after supplementation with 20 mg/kg carnitine.

Discussion

Our study demonstrates that patients on long-term haemodialysis have reduced plasma carnitine concentrations before and after haemodialysis, that carnitine, acylcarnitines and butyrobetaine are removed efficiently by haemodialysis and that the plasma carnitine and butyrobetaine levels can be increased to physiological or even supraphysiological levels by supplementation of carnitine following each haemodialysis session.

Under baseline conditions, all patients investigated were carnitine-deficient with a mean plasma free carnitine concentration of 15.1 ± 0.6 μmol/l, which is well below the concentration of 34.3 ± 1.2 μmol/l found in healthy persons [5]. The acylcarnitine to total carnitine ratio was 0.30 ± 0.02, indicating a redistribution of the plasma carnitine pool towards acylcarnitines [5]. Acylcarnitines were represented mainly by acetylcarnitine, as reported in other studies [6,7,14].

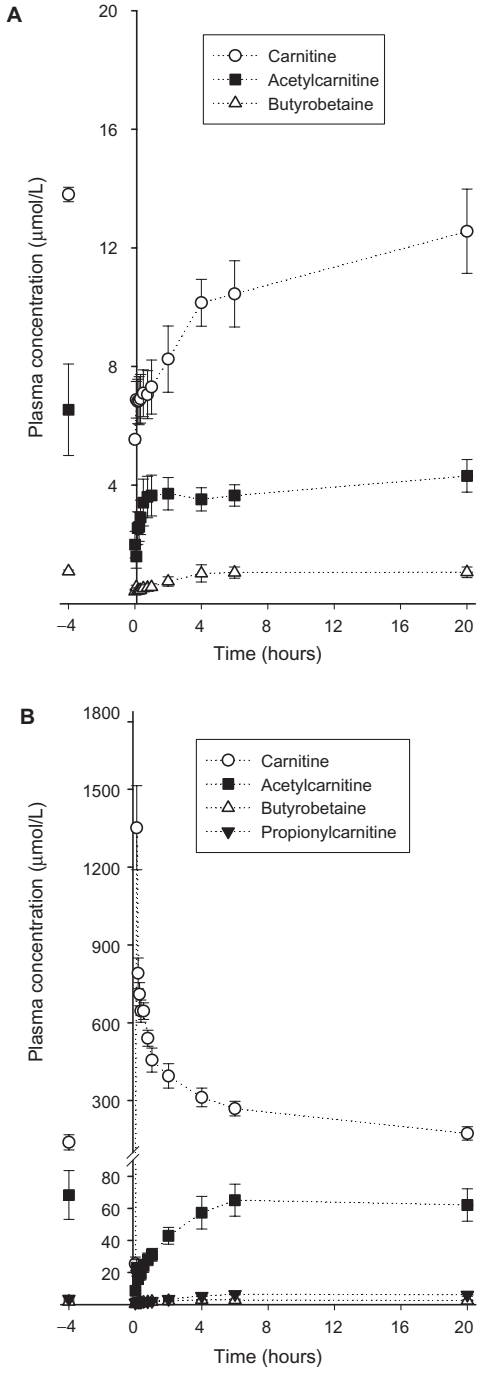

Fig. 3. Kinetics of carnitine and acylcarnitines between two haemodialysis sessions (interdialysis kinetics). Values are expressed as means ± SEM, $n = 4$ patients. Analysis was performed by LC–MS/MS as described in the 'Subjects and methods'. (**A**) Baseline conditions between haemodialysis sessions on days 4 and 6 (no carnitine supplementation). (**B**) Supplementation with carnitine (20 mg/kg body weight) between haemodialysis sessions on days 46 and 48. The period between –4 and 0 h reflects the haemodialysis session on day 4 (A) or on day 46 (B), respectively. The values at 44 h (pre-dialysis concentrations before haemodialysis sessions on days 6 or 48, respectively) are not given in this figure, but can be obtained from Figure 1. They do not differ significantly from the respective values obtained at –4 h. After a drop during haemodialysis (see Figure 2 for details), the plasma carnitine, acylcarnitine and butyrobetaine concentrations reach apparently stable values 8–20 h after termination of haemodialysis.

Interestingly, propionylcarnitine could be detected in five patients with plasma concentrations ranging between 0.52 and 0.81 µmol/l. Propionylcarnitine has also been detected in plasma of healthy persons, but at lower concentrations as compared with the patients in our study [19,20]. In addition, still under baseline conditions, isovalerylcarnitine was present in two patients at levels of 0.98 and 1.04 µmol/l, also higher concentrations than the 0.11 ± 0.06 µmol/l reported in healthy sportive subjects [19]. Since acylcarnitines are formed from the respective acyl-CoAs [1], it can be assumed that the tissue concentrations of propionyl-CoA and isovaleryl-CoA are increased at least in some patients on long-term haemodialysis. Depending on the metabolic situation, the plasma carnitine profile can reflect both the carnitine pool of the liver [1,2] or of skeletal muscle [3]. In the case of propionate, the plasma carnitine profile primarily reflects the liver carnitine and coenzyme A pools, because propionate is metabolized mainly by liver mitochondria [21]. It is well known that propionate and other short- and medium-chain fatty acids can impair mitochondrial function, in particular mitochondrial β-oxidation [1,22]. This inhibition may at least partially explain increased plasma concentrations of free fatty acids in patients on long-term haemodialysis [1,23] and suggests that removal of short- and medium-chain acyl-groups may be potentially beneficial for this group of patients. Since the administration of exogenous carnitine is associated not only with an increase in free carnitine, but also in the respective acylcarnitine fractions, and since acylcarnitines are removed efficiently by haemodialysis, carnitine supplementation is indeed associated with an increased removal of acyl groups by haemodialysis (Table 2). In support of the hypothesis that carnitine may improve mitochondrial metabolism in patients on long-term haemodialysis, carnitine supplementation has been associated with a drop in the plasma free fatty acid concentration and with an increase in cardiac fatty acid metabolism in this group of patients [1,23]. In addition, beneficial results on hepatic mitochondrial function have also been reported in patients suffering from methylmalonic aciduria, another condition with hepatic accumulation of toxic acyl-CoAs, who were treated with high doses of carnitine [2]. The administration of carnitine to patients on long-term haemodialysis may, therefore, act in at least two ways, namely by increasing the low carnitine plasma and tissue levels and by eliminating potentially toxic acyl-groups, mainly from liver and possibly from other organs.

Butyrobetaine, the direct carnitine biosynthesis precursor [1,24], was present in all patients at concentrations between 0.61 and 1.38 µmol/l under baseline conditions. These values are below reported mean butyrobetaine plasma concentrations in healthy persons of 1.80 µmol/l determined by tandem mass spectrometry [19] or 4.66 µmol/l determined by an enzymatic assay [25]. Similar to carnitine, butyrobetaine is stored mainly in skeletal muscle [24]. In order to be converted to carnitine, butyrobetaine has to be transported out from skeletal muscle into the blood and

then into liver, kidney or testis, where butyrobetaine hydroxylase is expressed and carnitine can be synthesized [24]. Since butyrobetaine can be removed by haemodialysis (Table 2 and Figure 2), patients on long-term haemodialysis have lower butyrobetaine plasma concentrations than healthy subjects. Considering that butyrobetaine is taken up actively by tissues and that the K_m value of this transport is in the range of 5 μmol/l [26], it can be assumed that transport into tissues shows an almost linear relationship with the plasma concentrations, suggesting that patients on haemodialysis have reduced tissue butyrobetaine concentrations. Impaired carnitine biosynthesis may, therefore, contribute to the low carnitine plasma and tissue concentrations [6–8] in patients on long-term haemodialysis. In support of this hypothesis, patients on long-term haemodialysis have lower tissue concentrations of carnitine as compared with healthy subjects, despite the fact that haemodialysis removes less carnitine than renal excretion in healthy subjects [12]. Our study therefore supports the hypothesis that carnitine biosynthesis is reduced in patients on long-term haemodialysis and offers a possible mechanism for this assumption.

Interestingly, supplementation of carnitine in patients on long-term haemodialysis is not only associated with an increase in plasma carnitine and acylcarnitines, but also in butyrobetaine (Figure 1). As explained above, in order to be converted to carnitine, butyrobetaine has to be transported out of skeletal muscle and to be taken up by the kidney, liver or testis, where it is hydroxylated to carnitine [24]. Since butyrobetaine inhibits the transport of carnitine by OCTN2 [27], an interaction between the transport of carnitine and butyrobetaine into tissues could explain our findings. An interaction on the level of the kidney can be excluded in patients with end-stage renal disease, but carnitine may inhibit the transport of butyrobetaine into other tissues, e.g. testis and/or liver, where OCTN2 is expressed [27].

In conclusion, our study shows that carnitine, short-chain acylcarnitines and butyrobetaine are removed efficiently by haemodialysis and that supplementation with exogenous carnitine increases the plasma concentrations of carnitine itself, but also of acylcarnitines and butyrobetaine in patients on long-term haemodialysis. Removal of potentially toxic acyl-groups as acylcarnitines by haemodialysis may explain some of the beneficial effects associated with the administration of carnitine in this group of patients. The increase in the plasma butyrobetaine concentration during carnitine supplementation may be explained by an inhibition by carnitine of the transport of butyrobetaine into tissues such as testis and liver.

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Conflict of interest statement. None declared.

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