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Original Article

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Insulin action on glucose and protein metabolism during L-carnitine supplementation in maintenance haemodialysis patients

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Abstract

Background. Impaired protein anabolism and insulin resistance are characteristic features of maintenance haemodialysis patients. We have used a randomised, matched-paired, double-blind, placebo-controlled experimental design to determine the capability of intravenous L-carnitine supplementation to modify insulin resistance and protein catabolism in non-diabetic patients with end-stage renal disease (ESRD) undergoing chronic haemodialysis treatment.

Methods. L-carnitine (20 mg·kg^{-1}) ($n = 9$) or placebo ($n = 10$) were given intravenously at the end of seven consecutive dialysis sessions. Whole-body protein and glucose metabolism were assessed on interdialytic days by the L[1-^{13}C]leucine and the [2,2-$^{2}H_2$]glucose kinetic models in the postabsorptive state and during euglicemic hyperinsulinemic clamp studies at baseline and at the end of the treatment period.

Results. L-carnitine supplementation was associated with lower ($P < 0.05$) rates of leucine oxidation ($-11 \pm 12\%$) and appearance from proteolysis ($-6 \pm 2\%$) during the clamp studies than after placebo supplementation. The rates of glucose appearance in the postabsorptive state did not change significantly in the patients receiving L-carnitine treatment. Insulin-mediated glucose disappearance was improved by L-carnitine only in those patients ($n = 5$) ($+18 \pm 3\%$, $P < 0.05$ *vs* placebo group, $n = 5$) with greater baseline insulin resistance, selected according to the median value of insulin sensitivity before treatment.

Conclusions. L-carnitine supplementation was associated with protein-sparing effects in maintenance haemodialysis patients during hyperinsulinemia.

Keywords: carnitine; haemodialysis; insulin resistance; protein kinetics; stable isotopes; uremia

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Introduction

Impaired protein anabolism and insulin resistance are common metabolic alterations in patients with end-stage renal disease (ESRD) leading to serious comorbidities, such as cachexia, diabetes mellitus and coronary artery disease [1,2]. Chronic haemodialysis treatment does not restore to normal protein and glucose metabolism [3,4]. Haemodialytic sessions are net catabolic events because free amino acids are lost in the dialysate and proteolysis is accelerated [5].

A number of other low-molecular weight molecules are lost in the dialysate during each haemodialysis session. L-carnitine is a small compound (molecular weight, 162 D), derived from dietary intake and liver *de novo* synthesis, which is largely stored in skeletal muscle [6,7]. A large number of studies have reported low plasma and muscle-free L-carnitine levels in ESRD (end-stage renal disease) patients undergoing chronic haemodialysis [8,9]. This depletion occurs primarily within the first few months of haemodialysis and is mainly due to efficient removal of free L-carnitine via dialysate, possibly coupled with reduced dietary intake and endogenous synthesis [6,8].

L-carnitine is a key substrate in intermediary metabolism and participates in a series of reversible transesterification reactions in which organic acids are transferred from coenzyme A to the hydroxyl group of L-carnitine and vice versa. Acylcarnitines are imported in or exported out of mitochondria and contribute to free fatty acid transport and oxidation, ketogenesis and mitochondrial energy control [10,11]. In addition, L-carnitine, through regulation of availability of free coenzyme A, regulates the activity of key metabolic enzymes, such as pyruvate dehydrogenase and branched-chain alpha-keto acid dehydrogenase [12,13]. Consequences of free L-carnitine depletion may therefore involve not only lipid metabolism, but also insulin-mediated pathways of glucose utilisation, regulation of branched chain amino acid catabolism and, ultimately, whole-body protein balance [6].

Supplementation with L-carnitine in animals has been clearly associated with enhanced protein accretion in skeletal muscle [14,15]. In clinical settings, L-carnitine

supplementation improved biochemical markers of protein metabolism in some conditions [16,17] but not in others [18,19]. Concerning insulin sensitivity, L-carnitine administration to patients with type 2 diabetes reduced insulin resistance as assesses by the euglycemic hyperinsulinemic clamp [20]. However, it is still controversial whether restoring plasma L-carnitine will correct metabolic alterations observed in maintenance haemodialysis.

In this randomised, matched-paired, double-blind, placebo-controlled study we have determined the capability of short-term L-carnitine supplementation to modify tissue insulin resistance and protein catabolism in non-diabetic patients with ESRD undergoing chronic haemodialysis treatment. L-carnitine was given intravenously at the end of seven consecutive dialysis sessions at the dose of 20 mg kg^{-1}. Whole-body protein and glucose metabolism were assessed on interdialytic days by the L[1-^{13}C]leucine and the [2,2-$^{2}H_2$]glucose kinetic models [21,22], in the postabsorptive state and during euglicemic hyperinsulinemic clamp at baseline and at the end of the treatment period.

Methods

Materials

L-[1-^{13}C]leucine, [^{13}C] sodium bicarbonate and D-[6,6-$^{2}H_2$]glucose were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Chemical and isotopic purity of the tracers was determined by gas chromatography–mass spectrometry (GC–MS). Before every infusion study, sterile solutions of the tracers were prepared using an aseptic technique. Accurately weighed amounts of the labelled compounds were dissolved in weighed volumes of sterile, pyrogen-free saline and filtered through a 0.22-μm Millipore filter before use. Solutions were prepared no more than 24 h before use and were kept at 4°C before administration.

Patients

The study protocol was approved by the Institutional Review Boards of the University Hospital of Trieste and of the Palmanova Hospital. All patients signed an informed, written consent before entering the study. This was a randomised, matched-paired, double-blind and placebo-controlled study. Patient matching was done according to body mass index, plasma-free L-carnitine and albumin concentrations. The two patients forming a matched-pair were assigned randomly to carnitine treatment or placebo in a parallel way. A total population of 19 ESRD patients on maintenance haemodialysis for at least 6 months was enrolled into the study. All patients were treated three times per week with conventional haemodialysis using modified cellulose (9 patients) of synthetic filter membranes (10 patients). In order to be eligible to enter the protocol and treatment period, patients (male or female) met the following criteria: age between 18 and 75 years; diagnosis of ESRD; being on regular haemodialysis three times a week for at least 6 months; equilibrated Kt/V above 1.2; absence of diabetes mellitus on the basis of fasting blood glucose or oral glucose tolerance test where appropriate [23]; stable clinical condition during the 4 weeks immediately prior to baseline as demonstrated by medical history, physical examination and laboratory testing; blood haemoglobin concentration above 9.5 g dl^{-1}. The following exclusion criteria were included: immunosuppressant, glucocorticoid, androgen or thyroid hormone therapy in the 6 months immediately prior entering the protocol; significant comorbidities, such as abnormal liver function test, alcohol abuse, infections, moderate-severe congestive heart failure (NYHA class III and IV), central nervous system diseases, cancer and immunologic diseases. The following medications were allowed if previously stabilised for at least 1 month and maintained stable during the study: antiplatelet and anticoagulation therapies, antihypertensive medications including angiotensin-converting enzyme inhibitors and beta-adenergic blockers, erythropoietin, calcium supplements, calcium or aluminium phosphate binders.

Experimental design

During a baseline period lasting no more than 1 week, whole-body protein and glucose metabolism were assessed on the interdialytic day in patients fulfilling all the enrolment criteria. The L-[1-^{13}C]leucine and the D-[6,6-$^{2}H_2$]glucose kinetic models in the postabsorptive state and during euglicemic hyperinsulinemic clamp studies were used to determine insulin action on whole-body protein kinetics and glucose metabolism [21,22]. After the overnight fast, polyethylene catheters were inserted into the arterial and venous ends of vascular accesses, the former for blood sampling (kept patent with a slow drip of 0.9% saline infusion) and the latter connected with a three-way stopcock, and used for isotope, insulin and glucose infusion. Blood and breath samples were taken before the start of isotope infusion to determine baseline natural enrichments of [1-^{13}C]α-ketoisocaproic acid and D-[6,6-$^{2}H_2$]glucose in plasma and of $^{13}CO_2$ in the expired air. Thereafter, priming solution consisting of 4.8 μmol kg^{-1} of L-[1-^{13}C]leucine, 0.11 mg kg^{-1} of [^{13}C] sodium bicarbonate and 2 mg kg^{-1} of D-[6,6-$^{2}H_2$]glucose were administered, followed by constant infusions of L-[1-^{13}C]leucine at a rate of 0.08 μmol kg^{-1} min^{-1} and of D-[6,6-$^{2}H_2$]glucose at a rate of 0.02 mg kg^{-1} min^{-1} and continued for 360 min. One hundred and fifty minutes were allowed to reach a steady-state condition for α-ketoisocaproic acid and glucose enrichments and expired $^{13}CO_2$. Thereafter, four breaths and plasma samples were taken over 30 min (150–180 min) at 10 min intervals each one from the following, to determine glucose and insulin concentrations as well as enrichment of labelled α-ketoisocaproic acid and glucose in plasma and $^{13}CO_2$ in expired air. Evaluation of total CO_2 production was performed by indirect calorimetry from min 140 to min 180. Thereafter, euglycemic insulin-clamp study was started and continued for 3 h. Regular insulin was continuously infused at a rate of 1 mU kg^{-1} min^{-1}. During this period plasma glucose concentration was held constant at baseline level by variable infusion of 20% dextrose solution. Endogenous glucose appearance was monitored by isotope dilution employing D-[6,6-$^{2}H_2$]glucose. Blood samples (10 μl) were taken every 5 or 10 min in order to measure glucose

concentrations by a portable glucometer and to regulate, accordingly, infusion rate of the dextrose solution. Furthermore, blood and breath samples were taken every 10 min between min 330 and 360 to determine insulin concentrations as well as enrichment of labelled α-ketoisocaproic acid and glucose in plasma and $^{13}CO_2$ in expired air. Indirect calorimetry was performed from min 320 to min 360 to determine total CO_2 production.

After the first metabolic study, patients were randomised and underwent seven consecutive haemodialysis sessions under double-blind treatment period. During this period, they received experimental treatment at the end of each of the dialysis sessions. Medication consisted of ampules for intravenous administration containing L-carnitine or placebo (saline solution). Medications were prepared by Sigma-Tau i.f.r. S.p.A. Rome, Italy. The placebo ampules were identical in appearance to the ampules containing the active study medication. Levocarnitine or placebo was administered as a bolus injection via the venous return line. L-carnitine was administered at the dosage of 20 mg kg^{-1} dry body weight. Study medication was injected by study personnel without knowledge of the patient randomisation status. At the end of the double-blind treatment period, the day after the last drug treatment, patients underwent a second metabolic study as described.

Analytical methods

Whole blood glucose concentrations were measured immediately after blood drawing using glucose oxidase paper strips (One Touch, Lifescan, Milpitas, CA, USA) and portable glucometers (One Touch II, Lifescan, Milpitas, CA, USA). During the study, precision of blood glucose determinations was evaluated every 30 min using a calibrated paper strip. Mean and standard deviation of 20 determinations of glucose concentrations from the same eparinised blood sample were 78.6 mg% and 1.5, respectively. Plasma insulin was measured by radioimmunoassay. Plasma levels of free and total L-carnitine were determined by high-pressure liquid-chromatography. Free carnitine was not determined during carnitine supplementation because the amount of plasma was not sufficient for the analysis. Plasma D-[6,6-$^2H_2$]glucose enrichments were measured as butylboronate glucose derivatives [21] by GC–MS (Agilent—HP 5973 Mass Spectrometer, Albertville, MN, USA) using electron impact ionisation and selected ion monitoring [21]. Plasma [1-^{13}C]α-ketoisocaproic acid and breath $^{13}CO_2$ isotopic enrichments were determined by GC–MS and by isotope ratio mass spectrometry (Delta S, Finnigan, MAT Bremen, Germany) as previously described [22]. During the euglycemic hyperinsulinemic clamp studies, background enrichment in breath $^{13}CO_2$ increased due to the presence of natural ^{13}C abundance in the dextrose solution infused [21]. We used the mean changes in breath $^{13}CO_2$ enrichment obtained in five healthy control subjects (male/female: 4/1; age: 54 ± 5 years; body mass index: 25.0 ± 1.4 kg m^{-2}) during 3-h euglycemic hyperinsulinemic clamp studies with no tracer infusions to correct background breath $^{13}CO_2$ enrichment during the clamp studies in the patients. In the haemodialysed patients receiving L-carnitine, basal natural $^{13}CO_2$ enrichment was not significantly different before and after treatment.

Calculations and statistics

Estimates of whole-body leucine kinetics were made at isotopic steady-state, effectively attained at min 150–180 in the postabsorptive state and at min 340–360 at the end of the clamp study. Mean values of [1-^{13}C]α-ketoisocaproic acid and $^{13}CO_2$ enrichments and of total CO_2 production in each study period were used for data calculation. In the postabsorptive state, leucine appearance rate is the result of amino acid release from proteolysis. Intracellular leucine rate of appearance from proteolysis was calculated according to the reciprocal pool model [21,22], i.e. by dividing L-[1-^{13}C]leucine infusion rate by plasma [1-^{13}C]α-ketoisocaproic acid tracer–tracee ratio. α-ketoisocaproic acid is the leucine deamination product and, during labelled leucine infusion, provides a direct estimate of the intracellular leucine enrichment [21]. To calculate leucine oxidation rate, the rate of $^{13}CO_2$ production in the expired air was determined by multiplying $^{13}CO_2$ enrichment in breath by total CO_2 production (as determined by indirect calorimetry). This figure was divided by correction factors of 0.74 in the postabsorptive state and of 0.84 during the euglycemic hyperinsulinemic clamp studies, accounting for the incomplete recovery of CO_2 in breath [24]. We assumed that L-carnitine administration did not affect CO_2 recovery in the expired air [19]. Leucine oxidation was then calculated by dividing $^{13}CO_2$ production by plasma α-ketoisocaproic acid tracer–tracee ratio.

Estimates of whole-body glucose kinetics were made at isotopic steady-state, effectively attained at min 150–180 in the postabsorptive state and at min 350–380 at the end of the clamp study. Mean values of plasma D-[6,6-$^2H_2$]glucose tracer–tracee ratio and of exogenous unlabelled glucose infusion rate were used for data calculation. Total glucose appearance rate is equal to rate of disappearance under steady-state conditions; these figures were calculated by dividing D-[6,6-$^2H_2$]glucose infusion rate by plasma D-[6,6-$^2H_2$]glucose tracer–tracee ratio both in the postabsorptive state and during the clamp studies. In the postabsorptive state, total glucose appearance reflects endogenous glucose appearance from liver production in ESRD patients, while glucose disappearance reflects whole-body utilisation. During the clamp studies, endogenous glucose appearance was calculated by subtracting exogenous unlabelled glucose infusion rate from total glucose appearance. Total glucose disappearance reflects whole-body utilisation. The combination of the D-[6,6-$^2H_2$]glucose kinetic model with the hyperinsulinemic euglycemic clamp allowed to determine insulin ability to suppress hepatic glucose production and promote whole-body glucose utilisation. The homeostasis model assessment (HOMA), an index of insulin resistance in epidemiological studies, was calculated by the formula: fasting plasma insulin (microinternational units per milliliter) × fasting plasma glucose (millimoles per liter)/22.5 [25].

All data were expressed as mean ± standard error of the mean. Results of the L-carnitine or placebo groups studied before and after treatments at baseline and during

Table 1. Clinical and demographic characteristics of treatment groups

	L-carnitine	Placebo
No.	9	10
Gender (male/female)	7/2	9/1
Age (years)	63 ± 3	57 ± 4
Body mass index (kg m^{-2})	23.2 ± 0.9	24.9 ± 1.5
Duration of dialysis (months)	75 ± 33	104 ± 32
Plasma free L-carnitine (μmol l^{-1})		
Before treatment	22 ± 3	25 ± 3
Plasma total L-carnitine (μmol l^{-1})		
Before treatment	56 ± 3	50 ± 3
After treatment	451 ± 46	50 ± 4
Blood haemoglobin (g l^{-1})		
Before treatment	129 ± 3	12.8 ± 4
After treatment	129 ± 4	125 ± 3
Plasma C-reactive protein (mg l^{-1})		
Before treatment	1.6 ± 0.6	1.2 ± 0.3
After treatment	2.0 ± 0.9	1.5 ± 0.7
Plasma albumin (g l^{-1})		
Before treatment	43 ± 1	42 ± 1
After treatment	43 ± 1	42 ± 1

euglycemic insulin clamp were analyzed with repeated measures ANOVA with interactions. Post hoc analysis was performed, when appropriate, by *t*-test with Bonferroni's adjustment. Changes from baseline of leucine and glucose kinetics mediated by L-carnitine or placebo were compared using the two-sample Wilcoxon test or the Wilcoxon matched-pair signed-rank test where appropriate. Correlation studies were assessed by standard method. $P \leq 0.05$ was considered statistically significant.

Results

The clinical and demographic characteristics of the treatment groups were not significantly different at randomisation (Table 1). In particular, there were no significant differences with respect to plasma concentrations of free and total L-carnitine. L-carnitine supplementation increased total plasma L-carnitine concentrations by 7 ± 1 times. No complications were reported during either L-carnitine or placebo treatment. Neither L-carnitine supplementation nor placebo changed significantly blood haemoglobin or plasma concentrations of albumin and C-reactive protein. Before treatment, in the two groups of patients receiving L-carnitine supplementation or placebo, baseline rates of leucine appearance from proteolysis and oxidation were not significantly different both in the postabsorptive state and during the euglycemic hyperinsulinemic clamp (Table 2). As expected, leucine rate of appearance was significantly decreased by insulin infusion in all conditions. There was significant treatment effect and treatment × group interaction for leucine rate of appearance. L-carnitine-mediated changes of leucine appearance were significantly different from those of placebo both in the postabsorptive state and during the clamp. There was significant treatment effect and treatment × group interaction for leucine oxidation. L-carnitine-mediated changes of leucine oxidation were significantly different from those of placebo during the hyperinsulinemic clamp.

Table 3 shows the effects of L-carnitine supplementation on glucose metabolism in the basal postabsorptive state and during the euglycemic hyperinsulinemic clamp in the ESRD patients and in group of age- and BMI-matched healthy volunteers. Before treatment, the two groups of patients receiving L-carnitine supplementation or placebo exhibited similar values of plasma glucose and insulin concentrations as well as of glucose rate of appearance and disappearance in the basal postabsorptive state. During the clamp study, plasma glucose concentrations were maintained at basal level by glucose infusion at a variable rate. At the end of the clamp studies, at steady-state, glucose infusion rate and plasma insulin concentrations were not significantly different in all conditions. The rates of glucose appearance were completely suppressed during the euglycemic hyperinsulinemic clamp studies in all conditions. Before treatment, the two groups of patients receiving L-carnitine supplementation or placebo exhibited similar rates of glucose disappearance during the clamp studies. L-carnitine supplementation or placebo did not change significantly the rates of glucose disposal during the hyperinsulinemic clamp studies. In healthy volunteers, plasma glucose and insulin concentrations during clamp studies were not different from the patients. The rate of glucose infusion during clamp studies, an index of insulin sensitivity, tended to be greater in healthy controls than in patients. Nonetheless, differences did not achieve statistical significance.

Figure 1 shows individual values of insulin sensitivity, as determined by glucose disposal rates during hyperinsulinemic clamp studies, before and after L-carnitine supplementation or placebo. In the group of patients supplemented with L-carnitine, baseline values of insulin

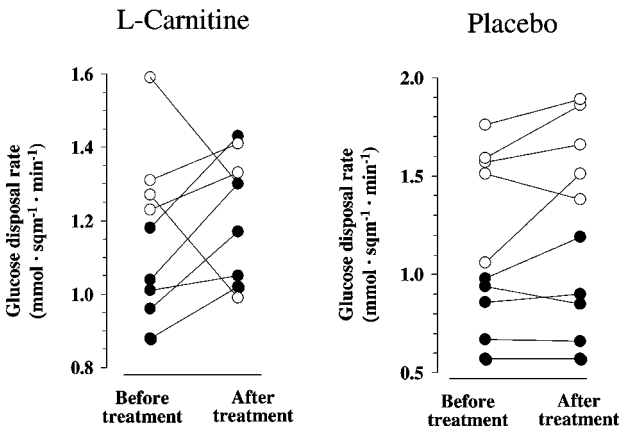

Fig. 1. Effects of L-carnitine supplementation and placebo on insulin-mediated glucose disposal in individual haemodialysis patients with higher (○) or lower (●) insulin sensitivity stratified according to median values before treatment. Patients with lower insulin sensitivity (●) were selected for statistical analysis with two-factor ANOVA with interaction (treatment × group). $P = 0.02$ for treatment effect; $P = 0.03$ for group effect; $P = 0.06$ for interaction. Post hoc analysis with Bonferroni's adjustment indicated significant ($P = 0.01$) effect of L-carnitine supplementation (mean ± SEM; from 1.02 ± 0.05 to 1.19 ± 0.08 mmol·sqm^{-1}·min^{-1}), while no significant changes were observed after placebo (from 0.80 ± 0.08 to 0.83 ± 0.11 mmol·sqm^{-1}·min^{-1}). L-carnitine-mediated changes of glucose disposal (0.18 ± 0.05 mmol·sqm^{-1}·min^{-1}) were greater than those in the placebo group (0.03 ± 0.05 mmol·sqm^{-1}·min^{-1}) ($P < 0.05$; two-sample Wilcoxon test).

Table 2. Whole-body leucine kinetics in haemodialysis patients in the basal postabsorptive state and during euglycemic hyperinsulinemic clamp before and after L-carnitine supplementation or placebo

	L-carnitine		Placebo	
	Basal	Clamp	Basal	Clamp
Rate of appearance (μmol·sqm^{-1}·min^{-1})				
Before treatment	68 ± 3	56 ± 2*	68 ± 3	55 ± 3*
After treatment	62 ± 3**	53 ± 3*	68 ± 4	56 ± 3*
Changes	−5.1 ± 1.5***	−3.4 ± 1.5***	0.4 ± 1.2	1.0 ± 1.0
Oxidation (μmol·sqm^{-1}·min^{-1})				
Before treatment	5.3 ± 0.3	4.7 ± 0.3	5.1 ± 0.6	3.9 ± 0.2
After treatment	4.6 ± 0.4	3.9 ± 0.2	5.0 ± 0.5	3.8 ± 0.3
Changes	−0.7 ± 0.4	−0.8 ± 0.4***	−0.2 ± 0.4	−0.1 ± 0.3

Leucine rate of appearance is a marker of proteolysis; leucine oxidation is a marker of net protein catabolism. Kinetic data before and after treatment were analysed with repeated measures ANOVA with interaction. There were significant insulin ($P < 0.001$) and treatment ($P = 0.05$) effects and treatment × group interaction ($P = 0.01$) for leucine appearance. There were significant insulin ($P = 0.01$) and treatment ($P = 0.04$) effects and treatment × group interaction ($P = 0.05$) for leucine oxidation.
*$P < 0.01$, insulin clamp *vs* basal (Bonferroni's post hoc analysis).
**$P < 0.01$, after treatment *vs* before treatment (Bonferroni's post hoc analysis). Changes from before treatment mediated by L-carnitine or placebo were analysed with two-sample Wilcoxon test.
***$P < 0.05$, L-carnitine *vs* placebo.

Table 3. Whole-body glucose metabolism in basal postabsorptive state and during euglycemic hyperinsulinemic clamp in healthy controls and in haemodialysis patients before and after L-carnitine supplementation or placebo

	Haemodialysis patients					
	L-carnitine		Placebo		Healthy controls	
	Basal	Clamp	Basal	Clamp	Basal	Clamp
Plasma glucose (mmol·l^{-1})						
Before	5.3 ± 0.1	5.2 ± 0.2	5.3 ± 0.1	5.2 ± 0.3	4.8 ± 0.1	4.6 ± 0.1
After	5.0 ± 0.4	5.1 ± 0.2	5.2 ± 0.2	5.2 ± 0.1		
Plasma insulin (μU·ml^{-1})						
Before	11 ± 1	137 ± 15	12 ± 2	141 ± 11	10 ± 1	142 ± 4
After	16 ± 2	137 ± 8	15 ± 8	133 ± 9		
HOMA						
Before	2.6 ± 0.3		3.8 ± 0.7		2.1 ± 0.2	
After	3.0 ± 0.4		3.2 ± 0.6			
Glucose infusion during the clamp (mmol·sqm^{-1}·min^{-1})						
Before		1.17 ± 0.07		1.17 ± 0.16		1.39 ± 0.08
After		1.21 ± 0.06		1.26 ± 0.1		
Glucose appearance (mmol·sqm^{-1}·min^{-1})						
Before	0.39 ± 0.01	−0.01 ± 0.03	0.45 ± 0.04	−0.01 ± 0.04		
After	0.40 ± 0.02	0.01 ± 0.02	0.43 ± 0.03	−0.02 ± 0.05		
Glucose disposal (mmol·sqm^{-1}·min^{-1})						
Before	0.39 ± 0.01	1.16 ± 0.07	0.45 ± 0.04	1.15 ± 0.13		
After	0.40 ± 0.02	1.22 ± 0.06	0.43 ± 0.03	1.25 ± 0.15		

HOMA, homeostasis model assessment, an index of insulin resistance in the postabsorptive state. Glucose disposal is determined by D-[6,6-$^2H_2$]glucose dilution technique and is an index of insulin sensitivity. Glucose appearance equals glucose disposal in the postabsorptive state, whereas it is calculated from the difference between glucose disposal and glucose infusion during the clamp. Glucose appearance indicates hepatic glucose production. Glucose infusion during euglycemic hyperinsulinemia is an index of insulin sensitivity when glucose appearance is suppressed. Kinetic data before and after treatment were analysed with repeated measures ANOVA with interaction. There were not significant treatment effects and treatment × group interaction.

sensitivity inversely correlated ($n = 9$; $r = -0.68$; $P < 0.05$) with L-carnitine-mediated changes in insulin sensitivity. No relationships were found between baseline values of insulin sensitivity and changes in insulin sensitivity in the placebo group ($r = 0.08$). In order to determine the effects of L-carnitine supplementation in patients with lower baseline insulin sensitivity, we have selected for statistical analysis only those patients exhibiting values of insulin-mediated glucose disposal before treatment lower than median values in L-carnitine and placebo groups (Figure 1). In these patients, glucose infusion rates during baseline clamp studies (L-carnitine group: 1.03 ± 0.05 mmol·sqm^{-1}·min^{-1}; placebo group: 0.74 ± 0.10 mmol·sqm^{-1}·min^{-1}) were significantly lower ($P < 0.001$) than those in healthy controls (Table 3). In addition, patients with lower baseline insulin sensitivity exhibited greater ($P < 0.05$) HOMA index for insulin resistance (3.8 ± 1.0) than healthy controls (2.1 ± 0.3). In

these sub-groups of insulin-resistant ESRD patients, mean insulin-mediated glucose disposal significantly improved ($P = 0.01$) by 18 ± 4% after L-carnitine supplementation (from 1.02 ± 0.05 to 1.19 ± 0.08 mmol·sqm^{-1}·min^{-1}) while it did not change significantly after placebo (from 0.80 ± 0.08 to 0.83 ± 0.11 mmol·sqm^{-1}·min^{-1}) (Figure 1). L-carnitine-mediated changes of glucose disposal during clamp studies (0.18 ± 0.05 mmol·sqm^{-1}·min^{-1}) were significantly ($P < 0.05$) greater than those in the placebo group (0.03 ± 0.05 mmol·sqm^{-1}·min^{-1}).

Discussion

ESRD patients undergoing maintenance haemodialysis often present with decreased plasma and muscle L-carnitine [8,9]. Potential consequences include abnormalities of protein kinetics and insulin action leading to wasting of lean body mass and development of the metabolic syndrome [1,5]. We have used a double-blind, placebo-controlled design to investigate the effects of L-carnitine supplementation on glucose and protein metabolism in the postabsorptive state and during euglycemic, physiologic hyperinsulinemia in non-diabetic ESRD patients undergoing chronic haemodialysis treatment. We found that, during hyperinsulinemia, L-carnitine supplementation was associated with significant protein-sparing effects caused by down-regulation of whole-body proteolysis. In regard to sensitivity of glucose metabolism to insulin, we have observed beneficial effects of L-carnitine in those ESRD patients exhibiting greater insulin resistance.

Mechanisms leading to changes of lean body mass and body protein content involve regulation of catabolic response to fasting and/or of anabolic response to feeding. Insulin is the main postprandial anabolic hormone; its whole-body anabolic action is mainly due to suppression of proteolysis [26]. Rates of whole-body proteolysis have been determined as intracellular rate of leucine appearance by standard steady-state tracer kinetics. Net protein catabolism was determined as rate of irreversible leucine oxidation [21]. L-carnitine supplementation significantly decreased rates of whole-body leucine appearance from proteolysis both in the postabsorptive state and during hyperinsulinemia. L-carnitine supplementation was associated with decreased leucine oxidation during hyperinsulinemia. These results indicate that L-carnitine supplementation has a protein-sparing effect in ESRD patients in the postprandial state.

The degree of L-carnitine depletion negatively correlates with markers of muscle function and protein catabolism in ESRD patients undergoing maintenance haemodialysis [16]. In these patients, 6-month L-carnitine supplementation decreased by 15–20% predialysis concentrations of serum urea nitrogen and increased by 10% mid-arm muscle area [25]. Moreover, consistent data exist on beneficial effects of L-carnitine supplementation on muscle strength [14,15], exercise capacity [27] and quality of life [28,29] of haemodialysis patients. All these effects are potentially related to improved protein kinetics leading to increased lean body mass. Evidence indicates that long-term administration of carnitine in haemodialysis patients increased the skeletal muscle carnitine content significantly [14]. Besides in ESRD patients, L-carnitine supplementation exhibited anticatabolic effects in patients with advanced cancer [17] and surgical trauma [30]. Nonetheless, the usefulness for protein metabolism of L-carnitine supplementation was not apparent in other clinical conditions [18,31]. In regard to potential mechanisms of L-carnitine anticatabolic effects, animal studies have shown that L-carnitine supplementation directly suppresses branched-chain alpha-keto acid dehydrogenase activity [13] possibly through increases of regulatory ratios of acetyl CoA/free enzyme A and ATP/ADP. As a consequence of this decreased dehydrogenase complex flux, intracellular levels of branched-chain amino acids increased. These effects were associated with enhanced protein accretion in skeletal muscle [13]. Branched-chain amino acids, leucine, valine and isoleucine, play a key role in body protein turnover regulation. Leucine directly initiates stimulation of protein synthesis and inhibits proteolysis in skeletal muscle [32,33].

Insulin-mediated glucose disposal was quantified in ESRD patients by the hyperinsulinemic euglycemic clamp combined with glucose tracer infusion which enables, in addition, to determine endogenous glucose production rate [21]. This latter figure reflects hepatic glucose production, because kidney contribution is negligible in ESRD patients. L-carnitine supplementation in ESRD patients had no effect on hepatic glucose production in the postabsorptive state. In agreement with previous evidence [34], hepatic glucose production was completely suppressed in patients by the high physiological insulin levels achieved during the clamp studies. We can certainly predict the same effect in healthy controls even if the glucose tracer was not infused in these subjects [21,34]. Thus, assuming that the rate of whole-body glucose disposal was virtually equal to the rate of glucose infusion required to maintain euglycemia in all subjects, our data indicated that ESRD patients before treatment with L-carnitine or placebo were not significantly more resistant to insulin glucoregulatory effects than healthy controls. This is in agreement with the fact that chronic haemodialysis treatment improves insulin sensitivity in non-diabetic, ESRD patients [35,36]. When the effects of L-carnitine supplementation on insulin sensitivity were determined in the whole group of patients, we did not observe significant improvement of insulin-mediated glucose disposal as compared to the placebo group. Nonetheless, L-carnitine-mediated changes in insulin sensitivity correlated with baseline degree of insulin resistance. We tested, therefore, the hypothesis that L-carnitine effects on insulin action in ESRD patients could depend on the degree of insulin resistance. We found that L-carnitine supplementation improved insulin-mediated glucose disposal by 18 ± 4% in a subgroup of patients exhibiting baseline insulin resistance selected according to the median value of insulin sensitivity before treatment. Our results suggest that L-carnitine supplementation improves insulin action on glucose metabolism only in insulin-resistant patients. This conclusion needs to be confirmed in a larger study in haemodialysis patients with severe insulin resistance. Nonetheless, this observation is in agreement with a previous study conducted in patients with type 2 diabetes who are characterised by severe insulin resistance. In these

patients, L-carnitine administration improved insulin sensitivity by about 40% [20].

The results of this short-term explorative study justify new randomised controlled trials to evaluate the utility of adjuvant L-carnitine treatment in the management of lean body mass wasting and insulin resistance in haemodialysis patients. Haemodialysis patients could take advantage of L-carnitine supplementation to reduce catabolism of body protein, whereas patients with insulin resistance and type 2 diabetes could, possibly, improve insulin sensitivity as a consequence of L-carnitine supplementation.

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