Toward personalized hemodialysis by low molecular weight amino-containing compounds: future perspective of patient metabolic fingerprint

Vittorio Sirolli[1], Claudia Rossi[2,3], Augusto Di Castelnuovo[4], Paolo Felaco[1], Luigi Amoroso[1], Mirco Zucchelli[2,3], Domenico Ciavardelli[2,5], Carmine Di Ilio[2,3], Paolo Sacchetta[2,3], Sergio Bernardini[7,8], Arduino Arduini[6], Mario Bonomini[1], Andrea Urbani[7,8]

[1]Nephrology Clinical Institute, Department of Medicine, "G. d'Annunzio" University, Chieti-Pescara; [2]Centre of Study on Aging (Ce.S.I.), University Foundation, Chieti; [3]Department of Biomedical Science, "G. d'Annunzio" University, Chieti-Pescara; [4]Laboratory of Genetic and Environmental Epidemiology, "Giovanni Paolo II" Research Foundation, Campobasso; [5]Faculty of Engineering, Architecture, and Motor Science, Kore University, Enna, Italy; [6]Department of Research and Development, CoreQuest Sagl, Tecnopolo, Bioggio, Switzerland; [7]Department of Internal Medicine, University "Tor Vergata", Rome; [8]IRCCS-Santa Lucia Foundation, Rome, Italy

Background. L-carnitine deficiency is commonly observed in chronic hemodialysis patients, and this depletion may cause clinical symptoms like muscle weakness, anaemia, and hypotension.

Materials and methods. We pursued a targeted metabonomics investigation in 28 hemodialysis patients (13 non diabetics and 15 diabetics) and in 10 age-matched healthy controls, on plasma levels of all carnitine esters and of several amino acids. Samples were taken before and after the first hemodialysis treatment of the week. Multiplexed data were collected in LC-MRM (Multiple Reaction Monitoring) and analysed by unsupervised multivariate analysis.

Results. In diabetic uremic patients, we observed lower values of propionylcarnitine than in other groups, while acylcarnitine concentration was higher in uremics compared to controls. The hemodialysis session induced a decline in free, short-chain, medium-chain and dicarboxylic acylcarnitines, whereas the long chain acylcarnitines remained unaffected. Plasma levels of amino acid proline, ornithine, citrulline and serine were significantly elevated in uremic patients before dialysis compared to controls. For most tested plasma amino acids, a significant reduction after hemodialysis session was found.

Discussion. Our study is the first that investigated on possible modifications of the system of carnitine in diabetic patients in hemodialysis not only in relation to the condition of deficiency but also compared to lipid and glucose homeostasis alteration typical of diabetics. We proposed the application of targeted metabolic fingerprint in the management of the hemodialysis patients.

Keywords: amino acid, carnitine, hemodialysis, metabolic fingerprint.

Introduction

Patients with end-stage renal disease (ESRD) on chronic hemodialysis generally develop carnitine deficiency, as defined by subnormal plasma and tissue free carnitine and/or elevated acylcarnitine concentrations[1]. These deficiencies may stem from a reduction in renal synthesis, insufficient dietary intake, or elevated intradialytic losses[2], and may contribute to several abnormalities encountered in dialysis patients including muscle weakness, cardiomyopathy, cramps, and abnormal lipid profile[3].

L-carnitine (LC) is a compound widely distributed in nature and obtained primarily from the diet. It is involved in intermediary metabolism and is important to mammalian bioenergetic processes. It has been shown that LC plays an essential role in multiple primary functions including transport of long-chain fatty acids into the mitochondrial matrix, export products of β-oxidation from peroxisomes, and release of mitochondrial coenzyme A (CoA) from acyl-CoA when free CoA supply is limited[4].

In several studies, the metabolism of carnitine in patients on long-term hemodialysis has been investigated. Most of them were however restricted to the measurement of free and acylcarnitines, although in some studies the individual acylcarnitines were differentiated[5]. In particular, it was noted that plasma levels of propionyl-L-carnitine were significantly reduced in patients affected by type 2 diabetes (whereas acetyl-L carnitine levels proved to be elevated), and this decrease could compromise the ability of organs such as skeletal muscle and liver to adapt to metabolic changes[1]. Moreover low serum carnitine concentrations may be associated with poor clinical outcomes in certain patients on HD. Furthermore, there is increasing evidence linking poor nutritional status to increased mortality and morbidity in hemodialysis population[6]. Several factors including anorexia, decreased nutrient intake, and losses of amino acids, may contribute to this problem.

The aim of this study was to apply targeted metabolic fingerprint in order to evaluate the metabolic status of hemodialysis patients. Plasma levels of carnitine and its esters (short-medium- and long-chain) have been quantified in a LC-MS/MS multiplex experimental setup in uremic patients, diabetics and non diabetics, on chronic hemodialytic treatment, and the possible influence on these levels of the hemodialysis session have been addressed by multivariate analysis. In the same cohort of subjects plasma amino acid levels were also quantified.

This work is the first study to provide evidences on the possible modifications of the system of carnitine in diabetic patients on HD, not only in relation to the condition of deficiency, but also compared to lipid and glucose homeostasis alteration typical of diabetes.

Materials and methods

Study design and population

This observational, prospective study, included two periods of time work for a total of twenty-one days: a period of time screening of two weeks in which eligibility of patients was assessed, followed by a week of observation in which two blood samples were withdrawn, before and after the first dialysis treatment of the week.

The procedures were in accordance with the ethical standards of the Institutional Committee on Human Experimentation and with the Declaration of Helsinki Principles. After approval of the protocol by the institutional review board, signed informed consent was obtained from each participating subject.

Only clinically stable patients older than 18 years and on chronic hemodialysis since at least 6 months were included in the study. Other exclusion criteria were represented by current or previous treatment (within the last 3 months) with L-carnitine and/or its derivates, severe liver disease, acute infection, haemoglobin concentration <9 g/dL, and pregnancy.

A total of 28 ESRD patients (15 diabetics and 13 non diabetics) was enrolled in the study. Main characteristics of study population are reported in Table I. Glycated haemoglobin levels in diabetics were 7.1%±1.1%. All patients underwent three HD sessions per week each having a duration of 4 hours. The dialyzers used were represented by polysulfone (PS, 18 pts) and polymethylmethacrylate (PMMA, 10 pts). Plasma levels of carnitine species and aminoacids were also determined in ten age- and gender-matched healthy controls (Table I).

Methods

Whole blood samples obtained after a 12-h fasting period were collected in K_3EDTA vials (3 mL) and

Table I - Main characteristics of study population.

	Controls	Non diabetics	Diabetics
Number	10	13	15
Age (years)	70 (65-73)	74 (68-76)	73 (67-79)
Gender (male/female)	6/4	7/6	12/3
Duration of dialysis (months)	=	42 (9-81)	66 (31-75)
Glycemia (mg/dl)	78 (70-86)	75 (62-106)	147 (137-184)*
Haemoglobin (g/dl)	13.2 (12.9-14)*	10.5 (9.3-10.9)	10.6 (9.8-11.5)

Values are expressed as medians (25%-75% percentiles). *Significantly different from the other study groups.

centrifuged at 4,000 rpm for 20 minutes. Plasma samples were stored at –80 °C until analysis. Plasma samples (3.2 μL) were transferred into 1.5 mL tubes (Eppendorf, Hamburg, Germany) and then extracted with a solution of Methanol/Water (75:25, v:v) and 0.01% oxalic acid (100 mL) containing the stable isotope labeled internal standards. The stable isotope labeled internal standards as well as the extraction solution were obtained from the NeoBase Non-derivatized MSMS Kit (Perkin Elmer Life and Analytical Sciences, Turku, Finland)[7]. The tubes were then capped and vortex mixed. The samples were centrifuged (15,600 rpm at 4 °C for 15 minutes), and the supernatant was analyzed by direct infusion mass spectrometry (DIMS).

A quality control (QC) plasma pool was prepared from the plasma patient samples, then extracted and analyzed as described for the patient plasma samples. A total of 10 QC plasma pool were analyzed during the run. Method accuracy was assessed for each analyte, precision being evaluated as repeatability in terms of coefficient of variation for the QC samples. The calculated mean coefficient of variation for the amino acids and acylcarnitines was 6.90% and 8.15%, respectively.

The DIMS analysis for the evaluation of metabolite profile, specifically carnitine species and amino acids, in plasma samples was performed using a Liquid Chromatography-tandem Mass Spectrometry (LC/MS/MS) system consisting of an Alliance HT 2795 HPLC Separation Module coupled to a Quattro Ultima Pt ESI tandem quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). The instrument operated in positive electrospray ionisation mode using MassLynx V4.0 Software (Waters) with auto data processing by NeoLynx (Waters Corporation, Milford, MA, USA).

Autosampler injections of 30 μL were made into the ion source directly by a narrow peek tube, and the mobile phase was methanol/water 75:25 (v/v) plus 0.01% oxalic acid (Perkin Elmer). The total run time was 1.8 min, injection-to-injection. The mass spectrometer ionisation source settings were optimized for maximum ion yields for each analyte. Capillary voltage was 3.25 kV, source temperature was 120 °C, desolvation temperature was 350 °C, and the collision cell gas pressure was 3-3.50 e^{-3} mbar Argon.

Statistical analyses

Baseline characteristics were computed for different groups at baseline and after treatment and presented as mean values and standard deviation as well as median values and 25%-75% percentiles. Between-individuals comparison at baseline among the three groups (controls, non diabetics, and diabetics) have been conducted using non parametric analysis of variance (Kruskall-Wallis test). Pairwise contrasts were corrected for multiple comparison following Bonferroni approach.

Baseline *versus* post treatment within-individual comparisons in non diabetic and diabetic patients were performed using both absolute and relative differences. We first constructed the difference Δ = (measure after intervention) *minus* (measure at baseline), for each individual and variable, and the relative percentage difference %Δ = 100*Δ/(measure at baseline). Then, the two measures Δ and %Δ were tested for the null hypothesis they were equal zero, using the Wilcoxon test. This approach is equivalent to the non parametric Friedman test. At the end, differences of effects between diabetics and non diabetics groups (i.e. if Δ or %Δ distributions were different in the two groups of patients) were tested contrasting both Δ and %Δ in the two groups with Kruskall-Wallis test. A two tailed p-value <0.05 was considered as statistical significant.

The data analysis was generated using SAS/STAT software, Version 9.1.3 of the SAS System for Windows©2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA. Principal component analysis (PCA) was performed using Metabonalayst 2.0 statistical analysis module[8]. Autoscaling of data was performed before analysis by mean-centering and dividing by the standard deviation of each variable.

Results

Basal values of plasma carnitine species are reported in Table II. Levels were significantly increased in both diabetic and non diabetic patients as compared to controls for the following species: acetylcarnitine (C2), butyrylcarnitine (C4), tiglylcarnitine (C5:1), isovalerylcarnitine (C5), malonylcarnitine/3-hydroxy-butyrylcarnitine (C4OH/C3DC), hexanoylcarnitine (C6), methylmalonylcarnitine/3-hydroxy-isovalerylcarnitine (C5OH/C4DC),

Table II - Plasma basal levels of carnitine species. Data are expressed in mmol L^{-1}.

Carnitine species	Controls (n=10)	Non diabetics (n=13)	Diabetics (n=15)	p VALUE*
Short-chain acylcarnitines				
Free carnitine	42.81 35.94-47.43	40.91 33.88-48.23	36.24 28.08-60.97	n.s.
C2 carnitine	6.66 3.28-8.35	12.68 8.64-16.30	12.05 7.11-26.04	0.012°
C3 carnitine	0.47 0.37-0.55	0.53 0.38-0.73	0.42 0.28-0.94	n.s.
C4 carnitine	0.28 0.26-0.32	0.86 0.60-1.00	0.66 0.50-1.36	0.0007°
C5:1 carnitine	0.03 0.03-0.04	0.08 0.06-0.09	0.06 0.04-0.09	0.0005°
C5 carnitine	0.13 0.12-0.14	0.32 0.23-0.38	0.33 0.19-0.55	0.0011°
Medium-chain acylcarnitines				
C6 carnitine	0.04 0.02-0.06	0.10 0.09-0.14	0.10 0.05 0.17	0.0027°
C8:1 carnitine	0.04 0.03-0.05	0.11 0.10-0.20	0.10 0.08 0.14	<0.0001°
C8 carnitine	0.14 0.07-0.24	0.35 0.28-0.41	0.34 0.19 0.66	0.0054°
C10:2 carnitine	0.01 0.01-0.02	0.05 0.03-0.06	0.04 0.02-0.06	0.0041°
C10:1 carnitine	0.09 0.03-0.17	0.22 0.16-0.28	0.15 0.14-0.27	0.014°
C10 carnitine	0.19 0.05-0.36	0.33 0.27-0.40	0.33 0.17 0.57	n.s.
C12:1 carnitine	0.07 0.02-0.12	0.09 0.08-0.16	0.08 0.06-0.14	n.s.
C12 carnitine	0.06 0.02-0.13	0.08 0.07-0.14	0.08 0.05-0.14	n.s.
Long-chain acylcarnitines				
C14:1 carnitine	0.07 0.02-0.13	0.09 0.07-0.15	0.10 0.05-0.13	n.s.
C16 carnitine	0.11 0.07-0.12	0.10 0.09-0.13	0.09 0.08-0.11	n.s.
C18:1 carnitine	0.16 0.09-0.19	0.16 0.14-0.20	0.15 0.13-0.17	n.s.
Dicarboxylic acylcarnitines				
C3DC/C4OH	0.02 0.01-0.03	0.07 0.06-0.08	0.07 0.04-0.10	0.0001°
C4DC/C5OH	0.04 0.04-0.06	0.19 0.16-0.20	0.14 0.09-0.19	<0.0001°
C5DC/C6OH	0.13 0.10-0.15	2.35 1.40-3.00	1.85 1.05-2.03	<0.0001°
C6DC	0.13 0.13-0.14	2.39 1.51-3.47	1.94 1.37-2.27	<0.0001°

*Comparison between the three study groups. °Controls versus both patient groups. n.s.: not significant.

glutarylcarnitine/3-hydroxy-hexanoylcarnitine (C5DC/C6OH), octenoylcarnitine (C8:1), octanoylcarnitine (C8), adipylcarnitine (C6DC), decadienoylcarnitine (C10:2), decenoylcarnitine (C10:1). Otherwise, no significant changes were observed for other esters (Table II, Figure 1A).

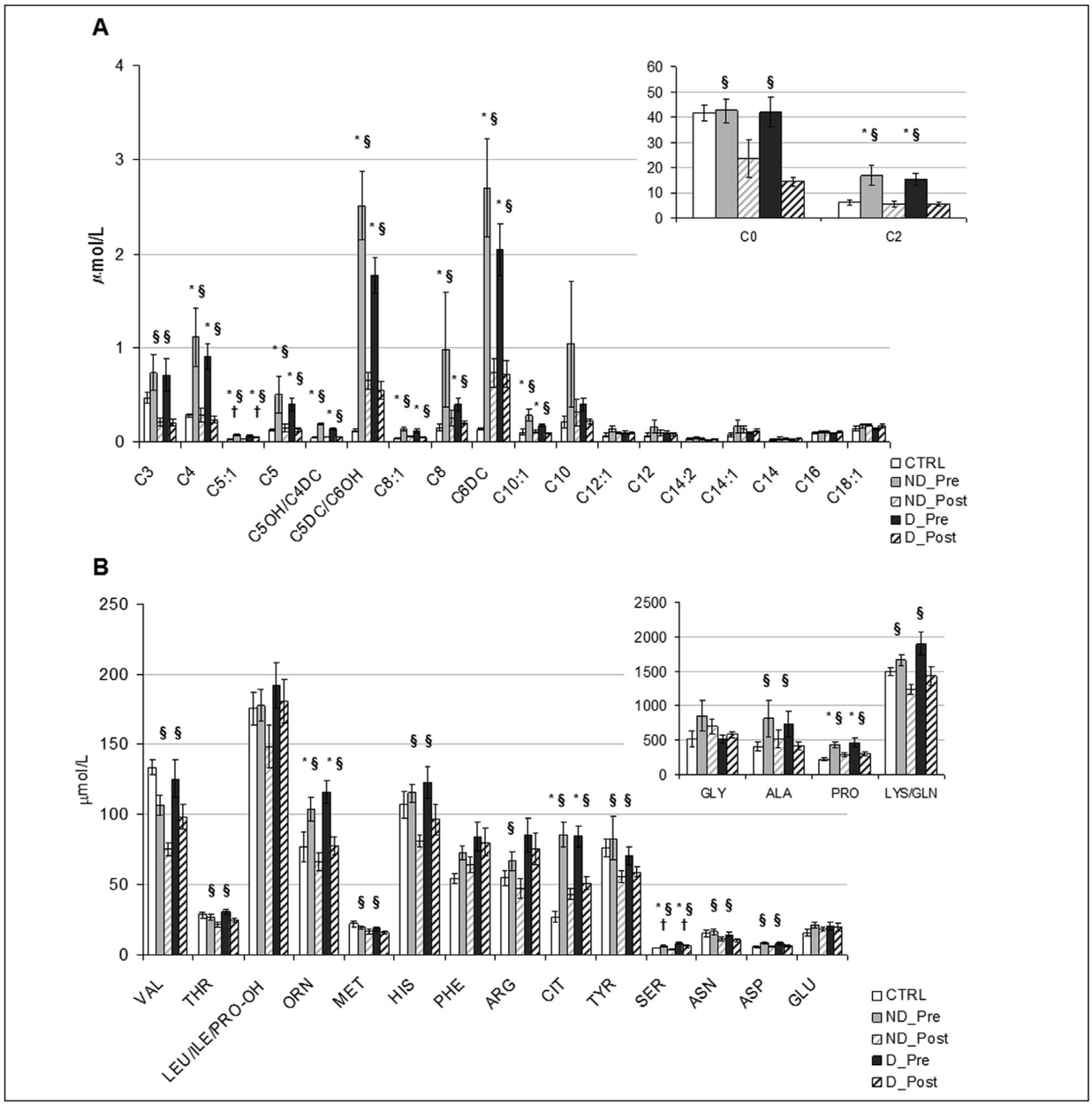

Figure 1 - Plasma concentrations of short, medium, and long-chain acylcarnitines (A) and amino acids (B) in control (CTRL) and uremic subjects (ND_Pre: non diabetics pre-dialysis; ND_Post: non diabetics post-dialysis; D_Pre: diabetics pre-dialysis; D_Post: diabetics post dialysis). Data are mean values (μmol L-1) and bars represent the standard error of the mean. *Significantly different from control. §Significantly different from post-dialysis concentration. †Significant different effect of the treatment. (Abbreviations: C0: free carnitine; C3: propionylcarnitine; C10: decanoylcarnitine; C12:1: dodecenoylcarnitine; C12: dodecanoylcarnitne; C14:2: tetradecadienoylcarnitine; C14:1: tetradecenoylcarnitine; C14: tetradecanoylcarnitine; C16: hexadecanoylcarnitine; C18:1: octadecenoylcarnitine; Ala: alanine; Lys/Gln: lysine/glutamine; Val: valine; Thr: threonine; Met: methionine; His: histidine; Tyr: tyrosine; Asn: asparagine; Asp: aspartic acid).

Basal plasma levels of amino acid proline (Pro), ornithine (Orn), citrulline (Cit) and serine (Ser) were significantly elevated in uraemic patients compared to controls (Table III, Figure 1B). No differences were observed for the other amino acids tested (Table III).

Regarding the role of hemodepurative treatment on plasma carnitine species, after hemodialysis it was found a significant reduction as compared to predialysis for short-chain acylcarnitines, medium-chain acylcarnitines and dicarboxylic acylcarnitines (Table IV, Figure 1A). Approximately 70% of the short-chain and dicarboxylic acylcarnitines and 50% of the medium-chain of the circulating pool were removed in both diabetic and non diabetic patients. Levels of long-chain acylcarnitines at variance were significantly modified after dialysis in diabetic patients only (Table IV, Figure 1A).

Table III - Plasma basal levels of amino acids. Data are expressed in mmol L^{-1}.

Amino acids	Controls (n=10)	Non diabetics (n=13)	Diabetics (n=15)	p value*
Glycine	423.57 318.09-784.53	621.54 507.95-1006.83	500.79 363.08-634.45	n.s.
Alanine	338.41 302.87-601.04	520.58 353.19-762.94	428.11 282.47-891.69	n.s.
Proline	200.31 180.42-222.17	377.81 333.91-464.54	413.34 307.21-629.73	0.0053°
Valine	130.28 122.78-145.96	97.83 84.44-127.68	107.90 88.59-145.87	n.s.
Threonine	27.89 24.43-33.05	29.08 20.93-32.09	30.03 27.78-36.38	n.s.
Leucine/Isoleucine/HydroxyProline	169.68 146.98-207.89	170.84 162.88-186.18	186.28 134.52-241.43	n.s.
Ornithine	67.10 60.76-92.22	96.35 87.43-114.87	118.14 99.90-138.22	0.017°
Methionine	22.92 16.29-26.95	19.40 16.41-23.10	18.17 14.83-19.20	n.s.
Histidine	104.27 93.78-121.50	118.54 97.67-124.71	132.31 83.32-159.82	n.s.
Phenylalanine	55.33 45.62-60.77	70.44 60.50-79.68	62.90 51.14-125.79	n.s.
Arginine	52.51 42.06-67.90	66.04 49.42-77.98	62.11 52.94-133.63	n.s.
Citrulline	24.29 18.19-37.80	83.89 65.96-89.64	91.41 57.25-111.81	0.0002°
Tyrosine	71.77 62.10-83.22	62.41 55.54-92.26	63.94 55.38-79.26	n.s.
Serine	4.69 3.97-5.19	6.42 4.75-7.53	8.39 5.11-10.04	0.012°
Asparagine	14.77 9.58-18.53	15.99 11.82-19.69	11.51 11.33-17.72	n.s.
Aspartic acid	5.76 4.73-6.60	8.55 5.95-10.16	6.95 5.78-10.06	n.s.
Lysine-Glutamine	1,575.29 1,334.41-1635.44	1,629.40 1,517.93-1,780.31	1,873.13 1,384.94-2,566.95	n.s.
Glutamic acid	16.94 8.90-21.56	20.86 14.25-25.14	18.63 12.30-24.05	n.s.

*Comparison between the three study groups. °Controls versus both patient groups. n.s.: not significant.

Table IV - Percent change of plasma carnitine species after hemodialysis as compared to predialysis*.

Carnitines	Non diabetics (n=13)	p value	Diabetics (n=15)	p value	p value for differences of effects°
Short-chain acylcarnitines					
Free carnitine	–64.71 –68.49; –61.52	0.027	–66.60 –74.08; –62.26	<0.0001	n.s.
C2 carnitine	–68.83 –74.31; –56.46	0.0002	–61.54 –70.79; –48.52	<0.0001	n.s.
C3 carnitine	–70.00 –75.71; –63.57	0.0002	–71.43 –75.95; –57.14	<0.0001	n.s.
C4 carnitine	–75.00 –77.78; –72.63	0.0002	–70.69 –80.00; –63.64	<0.0001	n.s.
C5:1 carnitine	–57.14 –66.67; –50.00	0.0002	–25.00 –40.00; –16.67	0.0160	0.004
C5 carnitine	–65.79 –71.88; –62.86	0.0002	–65.63 –76.92; –52.94	<0.0001	n.s.
Medium-chain acylcarnitines					
C6 carnitine	–63.16 –66.67; –50.00	0.0002	–58.82 –71.43; –42.86	0.0005	n.s.
C8:1 carnitine	–52.17 –63.64; –50.00	0.0002	–54.55 –62.50; –45.45	<0.0001	n.s.
C8 carnitine	–51.72 –60.71; –40.00	0.0002	–45.83 –63.64; –17.79	0.0004	n.s.
C10:2 carnitine	–66.67 –71.43; –66.67	0.0005	–50.00 –66.67; –25.00	0.0190	n.s.
C10:1 carnitine	–61.54 –70.00; –40.91	0.0005	–51.52 –59.09; –28.57	0.0002	n.s.
C10 carnitine	–57.14 –60.61; –33.33	0.0005	–41.67 –52.63; –7.14	0.0010	n.s.
C12:1 carnitine	–33.33 –52.00; –12.50	n.s.	0 –25.00; 33.33	n.s.	n.s.
C12 carnitine	–37.5 –50.00; –12.50	0.01	–12.5 –28.57; 0.00	n.s.	n.s.
Long-chain acylcarnitines					
C14:1 carnitine	–14.29 –44.44; 36.36	0.95	40.00 0.00; 100.00	0.0240	n.s.
C16 carnitine	0.00 –7.69; 8.33	0.82	16.67 0.00; 30.00	0.0220	n.s.
C18:1 carnitine	6.25 –7.69; 27.78	0.27	20.00 0.00; 46.67	0.0130	n.s.
Dicarboxylic acylcarnitines					
C3DC/C4OH	–71.43 –71.43; –66.67	0.0002	–66.67 –71.43; –50.00	0.0002	0.044
C4DC/C5OH	–68.75 –75.00; –64.29	0.0002	–64.71 –72.73; –55.56	0.0001	n.s.
C5DC/C6OH	–75.21 –78.02; –68.09	0.0002	–74.51 –79.35; –62.00	<0.0001	n.s.
C6DC	–73.51 –76.07; –70.42	0.0002	–67.5 –75.33; –58.73	0.0001	n.s.

*Ratio post- to pre-dialysis (%) was calculated as follows: 100 x (post-pre)/pre. °Comparison between the two patient groups. n.s., not significant.

The effect of the hemodialysis session on plasma amino acid profile is reported in Table V and Figure 1B. In both diabetic and non diabetic subjects, a significant reduction was observed for most tested amino acids with the exception of glycine (Gly), leucine/isoleucine/hydroxyproline (Leu/Ile/Pro-OH), phenylalanine (Phe), arginine (Arg) and glutamic acid (Glu).

Then, to examine whether the plasma carnitine species and amino acids had been modified in relation to hemodialysis treatment in a different way in non diabetic as compared to diabetics subjects, an analysis for differences of effects was done. A significant difference was only found for short-chain C5:1 carnitine and for dicarboxylic C3DC/C4OH carnitine (Table IV), and for Ser (Table V). Finally, we performed a PCA in order to highlight the separation of the study groups. As can be seen in Figure 2, plasma acetylcarnitne and amino acid profiles discriminate both diabetics and non-diabetics uremic patients from control subjects. As expected, the differences between control and post-dialysis uremic subjects decrease after the treatment. However, we does not observe differential effects of treatment between diabetics and non-diabetics subjects.

Table V - Percent change of plasma amino acid levels after hemodialysis as compared to predialysis*.

Amino acids	Non diabetics (n=13)	p value	Diabetics (n=15)	p value	p value for differences of effects°
Glycine	–31.21 –49.44; 111.73	n.s.	12.12 –20.20; 65.58	n.s.	n.s.
Alanine	–26.53 –48.44; –14.01	0.048	–25.45 –50.00; –1.23	0.041	n.s.
Proline	–33.61 –47.08; –23.36	0.0005	–37.31 –41.54; –18.11	0.0006	n.s.
Valine	–33.71 –40.20; –11.74	0.0034	–24.76 –35.13; –4.54	0.0043	n.s.
Threonine	–20.43 –28.66; 1.85	0.0105	–26.47 –31.31; –0.54	0.0054	n.s.
Leucine/Isoleucine/ HydroxyProline	–14.89 –24.61; 1.06	n.s.	–9.39 –19.21; 9.54	n.s.	n.s.
Ornithine	–37.62 –46.50; –32.41	0.0005	–33.14 –44.03; –18.14	0.0020	n.s.
Methionine	–22.51 –25.78; –2.44	0.040	–15.22 –29.21; –2.24	0.0073	n.s.
Histidine	–23.65 –37.27; –19.33	0.0007	–23.32 –35.57; –5.20	0.0026	n.s.
Phenylalanine	–13.65 –26.81; 16.19	n.s.	–1.93 –15.60; 2.98	n.s.	n.s.
Arginine	–32.02 –43.08; –17.65	0.006	–11.53 –24.13; 6.46	n.s.	n.s.
Citrulline	–51.93 –59.15; –40.88	0.0005	–43.89 –56.64; –20.65	0.0084	n.s.
Tyrosine	–24.64 –33.54; 4.10	0.013	–9.27 –24.24; –3.40	0.018	n.s.
Serine	–43.58 –55.45; –32.55	0.0005	–29.04 –37.90; –19.02	0.015	0.040
Asparagine	–32.95 –47.23; 0.35	0.022	–27.50 –42.63; 1.00	0.015	n.s.
Aspartic acid	–26.69 –36.10; –6.64	0.0024	–18.34 –23.42; –9.24	0.0084	n.s.
Lysine-Glutamine	–23.36 –28.26 –17.09	0.0002	–24.93 –29.38; –12.02	0.0003	n.s.
Glutamic acid	–11.34 –27.61; 13.09	n.s.	–3.40 –11.93; 16.15	n.s.	n.s.

*Ratio post- to pre-dialysis (%) was calculated as follows: 100 x (post-pre)/pre. °Comparison between the two patient groups. n.s.: not significant.

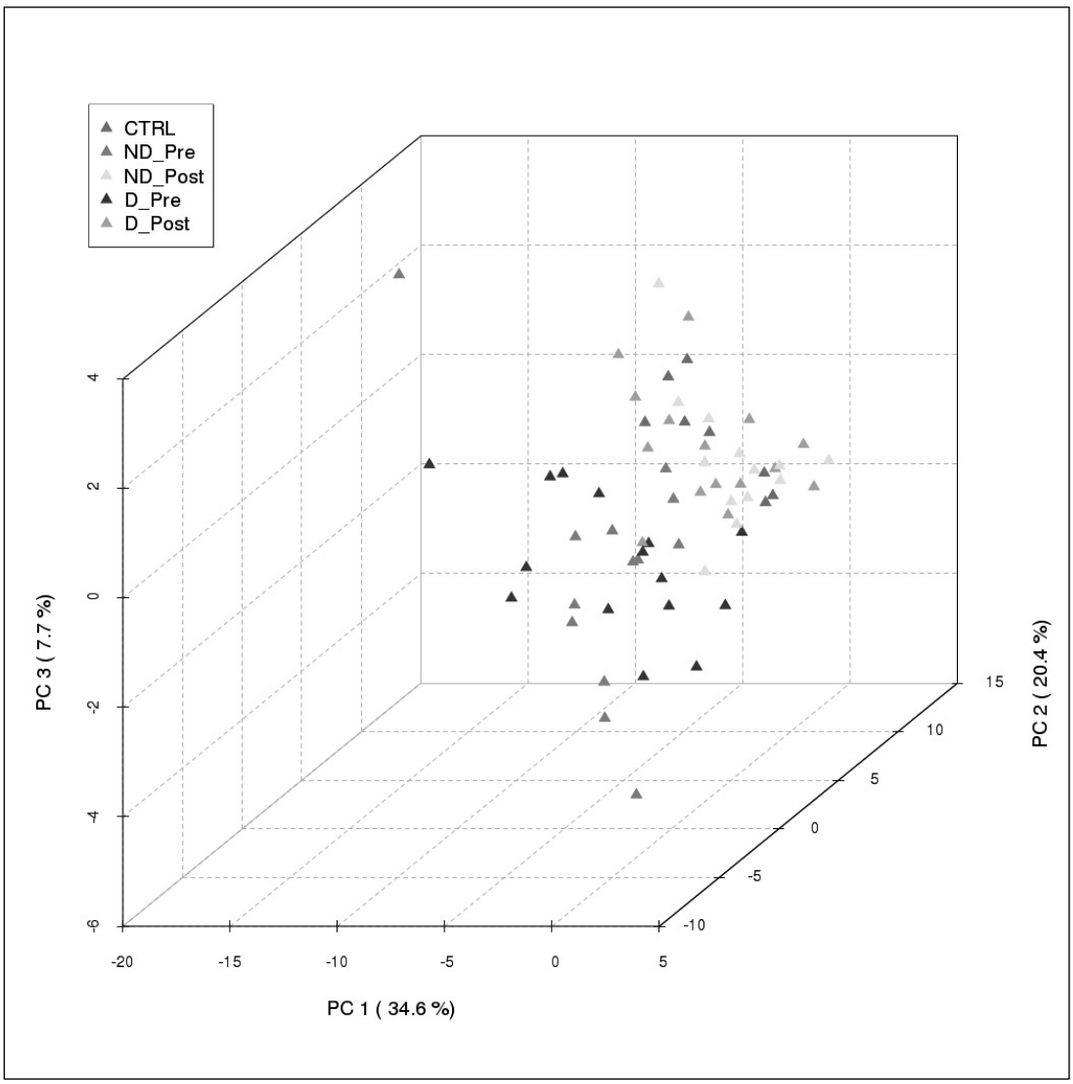

Figure 2 - Three-dimensional score plot from principal component analysis (PCA). The amounts of variance explained by each principal component (PC1, PC2, and PC3) are shown in parentheses. CTRL: control subjects; ND_Pre: non diabetics pre-dialysis; ND_Post: non diabetics post-dialysis; D_Pre: diabetics pre-dialysis; D_Post: diabetics post dialysis.

Discussion and conclusions

Coenzyme A is an essential metabolic cofactor, acting as an acyl group carrier and carbonyl-activating group in crucial biochemical reactions. It is therefore not surprising that specific acyl-CoA esters lie at important crossroads between metabolic pathways, where they may not only exert significant metabolic control through direct modulation of specific key enzymes (i.e. pyruvate carboxylase, pyruvate dehydrogenase, etc), but also affect gene expression, membrane trafficking and ion-channel activity[9]. Important components of the metabolic CoA network are L-carnitine, and its carnitine acyltransferases and translocases. Indeed, due to the activities of the various carnitine acyltransferases, which catalyze a reversibly transfer of an activated acyl unit from CoA to L-carnitine, changes in the availability of L-carnitine in the cell affect acyl-CoA pools rapidly[10]. This rapid equilibrium can also serve to mediate the efflux of acylcarnitine esters from different subcellular compartment, since free CoA and its esters are highly compartmentalized

and unable to cross biological membranes. Thus, the carnitine acylation state in the plasma reflects the cytosolic acylcarnitine pool and may serve as a diagnostic marker for the altered equilibria between acyl-CoA and acylcarnitine species. In particular, it is worth noting that, along the well-known alteration of the ratio between free carnitine and its acyl-esters, a marked increased of a subset of short-chain dicarboxylic acylcarnitine esters has been observed in our study. This is reminiscent of the metabolic derangement occurring in a discrete number of organic aciduria, inherited disorders of the metabolism of certain amino acids[11]. For example, glutaric aciduria type 1 is an inborn error characterized by the inability to catabolize lysine (Lys), hydroxylysine and tryptophan (Trp), and by plasma elevation of, among the other metabolites, glutarylcarnitine (indicated in the text and table as C5DC), which seems to be extremely elevated in our hemodialysis patients. It would be very important to verify if in hemodialysis patients the elevation of glutarylcarnitine somehow associated with an alteration of amino acid metabolism. Intriguingly, it has been demonstrated that a major subset of patients affected by organic aciduria develops chronic renal failure[12]. In keeping with this observation, in the course of a newborn screening for glutaric aciduria type I in infants, glutarylcarnitine was found to be significantly associated with renal failure and was significantly higher in infants with congenital renal insufficiency than in those with acquired renal insufficiency[13]. Finally, since some of the metabolites of glutaric aciduria are known to be neurotoxic (i.e., glutaric acid and 2-hydroxyglutaric), it would be interesting to evaluate if carnitine treatment may facilitate their removal.

Previous studies have indicated that responsiveness to L-carnitine supplementation in hemodialysis patients, the subject of intensive debate[14,15], may be related to a person's carnitine profile, particularly abnormalities of endogenous carnitine esters[16,17]. Our study shows that abnormalities in plasma carnitine profile are common in ESRD patients on regular hemodialysis, regardless of the diabetic state. Patient metabolic fingerprint may be a convenient and useful tool to drive supplementation therapies targeted to normalize the altered plasma carnitine composition of patients on the basis of a personalized approach. This in turn might lead to the improvement of several hemodialysis-associated conditions, to the benefit of the patient. Whether this assumption holds true requires further investigation.

Acknowledgements

This work was supported by a grant from the University of Chieti-Pescara (ex 60%). Andrea Urbani was in part supported by the following grant: Fondazione Roma 2008.

Contributions

Vittorio Sirolli and Claudia Rossi equally contributed to this work.

Conflicts of interest disclosure

Arduino Arduini is an employee of CoreQuest. No other potential conflicts of interest relevant to this article were reported.

References

1) Evans A. Dialysis-related carnitine disorder and levocarnitine pharmacology. Am J Kidney Dis 2003; **41**: S13-26.
2) Guarnieri G, Situlin R, Biolo G. Carnitine metabolism in uremia. Am J Kidney Dis 2001; **38** Suppl 1: S63-7.
3) Ahmad S. L-carnitine in dialysis patients. Semin Dial 2001; **14**: 209-17.
4) Hoppel C. The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 2003; **41**: S4-12.
5) Evans AM, Faull R, Fornasini G, et al. Pharmacokinetics of L-carnitine in patients with end-stage renal disease undergoing long-term hemodialysis. Clin Pharmacol Ther 2000; **68**: 238-49.
6) Qureshi AR, Alvestrand A, Divino-Filho JC, et al. Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. J Am Soc Nephrol 2002; **13** Suppl 1: S28-36.
7) Ostrup J, Wallac O. Method and apparatus for handling samples and sample collection system. EP0583078 (A2), February 16, 1994.
8) Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolic data analysis and interpretation. Nucleic Acids Res 2009; **37** (Web Server issue): W652-60.
9) Arduini A, Bonomini M, Savica V, et al. Carnitine in metabolic disease: potential for pharmacological intervention. Pharmacol Ther 2008; **120**: 149-56.
10) Zammit VA. Carnitine acyltransferases: functional significance of subcellular distribution and membrane topology. Prog Lipid Res 1999; **38**: 199-224.
11) Wajner M, Goodman SI. Disruption of mitochondrial

homeostasis in organic acidurias: insights from human and animal studies. J Bioenerg Biomembr 2011; **43**: 31-8.
12) Hörster F, Hoffmann GF. Pathophysiology, diagnosis, and treatment of methylmalonic aciduria-recent advances and new challenges. Pediatr Nephrol 2004; **19**: 1071-4.
13) Hennermann JB, Roloff S, Gellermann J, et al. False-positive newborn screening mimicking glutaric aciduria type I in infants with renal insufficiency. J Inherit Metab Dis 2012; DOI 10.1007/s10545-009-9017-6.
14) Steinman TL. L-carnitine supplementation in dialysis patients: does the evidence justify its use? Semin Dial 2005; **18**: 1-2.
15) Schreiber B. Common misconceptions about levocarnitine and dialysis. Semin Dial 2005; **18**: 349-54.
16) Sakurauchi Y, Matsumoto Y, Shinzato T, et al. Effects of L-carnitine supplementation on muscular symptoms in hemodialyzed patients. Am J Kidney Dis 1998; **32**: 258-64.
17) Riley S, Rutherford S, Rutherford PA. Low carnitine levels in hemodialysis patients: relationship with functional activity status and intra-dialytic hypotension. Clin Nephrol 1997; **48**: 392-3.

Correspondence: Andrea Urbani
Department of Internal Medicine
University of Rome "Tor Vergata"
Via Montpellier 1
00133 Rome, Italy
e-mail: andrea.urbani@uniroma2.it