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

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Introduction

End-Stage Renal Disease (ESRD) on chronic hemodialysis is generally associated with carnitine deficiency, defined by subnormal plasma and tissue free carnitine or elevated acylcarnitine concentrations [1]. L-Carnitine (LC) is obtained primarly from the diet and plays an essential role in multiple primary function such as the release of mitocondrial Coenzyme A (CoA) from acyl-CoA when free CoA supply is limited [2]. Several studies gave prominence to the correletion between patients with type II diabetes on hemodialysis and the reduction of plasma levels of propionyl-L-carnitine (whereas acetyl-L-carnitine levels proved to be elevated) [1,3]. 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 have been quantified in uremic patients, diabetics and non diabetics on chronic hemodialysis treatment.

Materials and Methods

We enrolled 28 patients (15 diabetics and 13 non diabetics), on chronic hemodialysis since at least six months, followed for two weeks (screening time) and then observed for a week in which two blood samples were withdrawn, before and after the first dialysis treatment of the week. We pursued a target metabonomics investigation on plasma levels of all carnitine esters and of several amino acids. Multiplex data were collected in LC-MRM (Multiple Reaction Monitoring) and analysed by unsupervised multivariate analysis.

	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.

Results

Basal values of some plasma carnitine species were significatly increased in both diabetic and non diabetic patients as compared to control (Table II, Figure 1A).

Basal plasma levels of amino acids Proline, Ornithine, Citrulline and Serine were significatly elevated in uremic patients; no differences were observed for the other amino acids tested (Table III, Figure 1B).

Table II

Carnitine species	Controls (n=10)	Non diabetics (n=13)	Diabetics (n=15)	p VALUE*
Short-chain acylcarnitines				
Free carnitine	42.81 35.94-47.63	40.91 33.88-48.23	36.24 28.08-50.57	n.s.
C2 carnitine	6.66 3.28-8.35	12.68 8.64-16.30	12.05 7.11-25.04	0.012[a]
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 0.69-1.00	0.56 0.50-1.36	0.0007[a]
C5:1 carnitine	0.03 0.03-0.04	0.08 0.06-0.09	0.06 0.04-0.05	0.0005[a]
C5 carnitine	0.13 0.12-0.14	0.32 0.23-0.38	0.33 0.16-0.55	0.0011[a]
Medium-chain acylcarnitines				
C6 carnitine	0.01 0.02-0.06	0.10 0.09-0.14	0.10 0.05-0.17	0.0027[a]
C8:1 carnitine	0.04 0.03-0.05	0.11 0.10-0.20	0.10 0.08-0.14	<0.0001[a]
C8 carnitine	0.14 0.07-0.24	0.35 0.28-0.41	0.34 0.19-0.66	0.0054[a]
C10:2 carnitine	0.01 0.01-0.02	0.05 0.03-0.06	0.04 0.02-0.06	0.0041[a]
C10:1 carnitine	0.09 0.03-0.17	0.22 0.16-0.28	0.15 0.11-0.27	0.0145[a]
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 0.05-0.14	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 0.06-0.11	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[a]
C4DC/C5OH	0.04 0.04-0.06	0.15 0.10-0.20	0.14 0.09-0.18	<0.0001[a]
C5DC/C6OH	0.13 0.10-0.15	2.35 1.40-3.00	1.85 1.05-2.03	<0.0001[a]
C5DC	0.13 0.13-0.14	2.39 1.51-3.47	1.84 1.37-2.27	<0.0001[a]

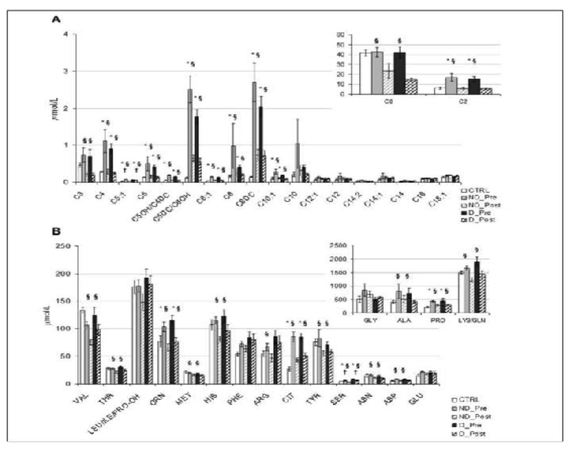
*Comparison between the three study groups. °Controls versus both patient groups. n.s.: not significant.

Table III

Amino acids	Controls (n=10)	Non diabetics (n=13)	Diabetics (n=15)	p value*
Glycine	423.57 318.09-784.53	521.54 507.95-1006.83	500.79 363.08-634.45	n.s.
Alanine	338.41 302.87-501.04	522.58 353.19-762.94	428.11 282.47-891.69	n.s.
Proline	200.31 190.42-222.17	377.81 333.91-464.54	413.34 307.21-629.73	0.2053[a]
Valine	130.28 122.78-145.96	97.83 84.46-127.68	107.50 88.59-145.87	n.s.
Threonine	27.89 24.43-33.05	29.08 20.93-37.69	30.03 27.78-35.38	n.s.
Leucine/Isoleucine/HydroxyProline	169.68 146.38-207.89	170.84 162.88-186.18	186.28 134.52-241.45	n.s.
Ornithine	67.10 50.76-92.22	96.35 87.43-114.87	118.14 99.90-138.22	0.0177[a]
Methionine	22.92 16.29-26.95	19.42 16.41-22.10	18.17 14.83-19.20	n.s.
Histicine	104.27 93.78-121.50	118.54 97.67-124.71	132.31 83.32-159.82	n.s.
Phenylalanine	55.33 45.52-59.77	70.44 60.55-79.68	62.90 51.14-125.79	n.s.
Arginine	52.51 42.06-57.90	66.04 49.42-77.98	62.11 52.94-153.53	n.s.
Citrulline	24.29 18.19-37.80	83.83 65.96-89.64	91.41 57.25-111.31	0.0002[a]
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[a]
Asparagine	14.77 9.58-18.53	15.93 11.82-18.69	14.51 11.53-17.72	n.s.
Aspartic acid	5.76 4.73-5.60	8.55 5.95-10.16	6.95 5.78-10.05	n.s.
Lysine-Glutamine	1,575.29 1,354.61-1635.44	1,629.40 1,517.93-1,780.31	1,873.13 1,384.94-2,566.55	n.s.
Glutamic acid	16.94 8.90-21.56	20.85 14.25-25.14	18.63 12.50-24.05	n.s.

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

Figure 1



After hemodialysis it was found a significant reduction, as compared to predialysis, for short-chain acylcarnitines (70%), medium-chain acylcarnitines (50%) and dicarboxylic acylcarnitines (70%); long-chain acylcarnitines were significantly modified after dialysis in diabetic patients only (Table IV, Figure 1A).

Table IV

Carnitines	Non diabetics (n=13)	p value	Diabetics (n=15)	p value	p value for differences of effects*
Short-chain acylcarnitines					
Free carnitine	-69.71 -69.63; -61.52	0.027	-65.62 -74.08; -62.26	<0.0001	n.s.
C2 carnitine	-68.83 -74.21; -36.46	0.0002	-61.54 -70.79; -48.52	<0.0001	n.s.
C3 carnitine	-70.00 -75.21; -63.57	0.0002	-71.43 -75.95; -53.14	<0.0001	n.s.
C4 carnitine	-75.00 -77.78; -72.63	0.0002	-70.66 80.00; -62.61	<0.0001	n.s.
C5:1 carnitine	-57.14 -65.67; -50.00	0.0002	-25.00 -40.00; -16.67	0.0169	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	-62.16 -65.67; -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; -42.45	<0.0001	n.s.
C8 carnitine	-51.72 -67.21; -40.00	0.0002	-45.83 -61.64; -17.79	0.0004	n.s.
C10:2 carnitine	-66.67 -71.73; -66.67	0.0005	-50.00 -66.67; -25.00	0.0190	n.s.
C10:1 carnitine	-61.51 -72.02; -40.91	0.0005	-51.52 -55.09; -28.57	0.0002	n.s.
C10 carnitine	-57.14 -67.61; -33.33	0.0005	-41.67 -52.53; -7.14	0.0010	n.s.
C12:1 carnitine	-33.33 -52.02; -12.50	n.s.	0 -25.00; 33.33	n.s.	n.s.
C12 carnitine	-37.5 -52.02; -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	0.2240	n.s.
C16 carnitine	0.00 -7.59; 8.33	0.82	16.67 0.00; 30.00	0.2220	n.s.
C18:1 carnitine	6.25 -7.63; 27.78	0.27	20.00 0.00; 45.67	0.2430	n.s.
Dicarboxylic acylcarnitines					
C3DC/C4OH	-71.43 -71.43; -66.67	0.0002	-65.67 -71.43; -50.00	0.2002	0.044
C4DC/C5OH	-68.75 -75.00; -64.29	0.0002	-64.71 -72.73; -55.56	0.2001	n.s.
C5DC/C6OH	-75.21 -78.02; -68.09	0.0002	-74.51 -75.53; -62.00	<0.0001	n.s.
C5DC	-72.51 -78.02; -70.42	0.0002	-67.5 -75.53; -58.75	0.2001	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.

To examine whether the plasma carnitine species and amino acids has been modified in relation to hemodialysis treatment in a different way in non diabetic as compared to diabetic subjects, an analysis for differences of effects was done.

A significant different was only found for short-chain C5:1 carnitine and for dicarboxylic C3DC/C4OH carnitine and for serine.

Discussion and Conclusions

Coenzyme A is a cofactor acting as an acyl group carrier and carbonyl-activating group. Important components of the metabolic CoA network are L-carnitine and its acyltransferases and translocases. Due to the activities of the various carnitine acyltransferases, which catalyze a reversibly transfer of an activated acyl unit from CoA to LC, changes in the availability of LC in the cell affect acyl-CoA pools rapidly [4]. This rapid equilibrium mediates the efflux of acylcarnitine esters from different subcellular compartement, 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 betweeen acyl-CoA and acylcarnitine species. Along the well-known alteration of the ratio between free carnitine and its acyl-esters, a marked increased of a subset of shorth-chain dicarboxylic acylcarnitine esters (glutarylcarnitine/C5DC) has been observed in our study. This is a reminescent of the metabolic derangement occurring in a discrete number of organic aciduria, disorder often associated with chronic renal failure [5]. Our study shows that abnormalities in plasma carnitine profile are common in ESRD patients on regular hemodialysis, regardeless of the diabetic state. Patient metabolic fingerprint may be a useful tool to drive supplementation therapies targeted to normalize the altered plasma carnitine composition of patients on the basis of a personalized approach.

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