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Plasma Carnitines: Reference Values in an Ambulatory Population

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Summary: Carnitine was determined radioenzymatically in the plasma of 415 hospital employees involved in a screening programme for prevention of major cardiovascular risks. A reference population (N = 340) was extracted after excluding subjects with hypertension, diabetes mellitus or treatment for hypercholesterolaemia. This population showed a *Gauss*ian distribution for total and free carnitine concentrations both in females and males but not for acyl carnitine or the acyl/free ratio. Females had lower total and free carnitine concentrations but a higher ratio of acyl/free carnitine than males. These differences were not detectable in older subjects (35 years for the acyl/free ratio, 45 years for total and free carnitine concentrations). Females with a body mass index > 28 had a lower acyl/free ratio than their respective controls. The differences in carnitine concentrations indicate that sex and age should be matched in patients or experimental groups and controls in studies involving carnitine plasma concentrations.

Introduction

Carnitine (3-hydroxy-4-trimethylaminobutanoate) is required for the transport of long chain acyl moieties into the mitochondria, and for buffering the intramitochondrial acyl-CoA/CoA ratio (1). Carnitine deficiency either results from primary genetic defects or from other genetic or acquired disorders, drug treatments (including diet) or treatment leading to carnitine loss (2–7). Severe carnitine deficiency may result in myopathy (including cardiomyopathy), hepatic encephalopathy and lipid storage in liver and muscle. Hypercarnitinaemia has been described in some patients with liver cirrhosis (8–10), in undialysed patients with chronic renal insufficiency (11) and in patients treated with lipid lowering drugs (12), or in rare congenital disorders like carnitine palmitoyl transferase[1]) deficiency (13).

Published data on carnitine concentrations in normal adult subjects comprise only small sample populations (14). We therefore took the opportunity to study the plasma carnitine concentrations in a cohort of 415 female and male hospital employees freely attending the hospital prevention centre during a screening programme for the detection of the major risk factors for cardiovascular disease. The aim of this study was to determine carnitine concentrations in a reference population for ambulatory patients in dependence on age and sex.

Materials

L-Carnitine-HCl and acetyl-*DL*-carnitine-HCl were from Sigma (Buchs, Switzerland); acetyl-CoA (trilithium salt) and carnitine acetyl transferase (EC 2.3.1.7) from Boehringer Mannheim (Rotkreuz, Switzerland). N-Ethylmaleimide, HEPES (2-[4-(hydroxyethyl)-piperazinyl]-(1)-ethansulphonic acid) and the ion exchange resin DOWEX 2X8, 200–400 mesh, in the Cl^- form were from Merck (Zürich Switzerland).

[Acetyl-1-^{14}C] coenzyme A specific activity 1.92 MBq/mmol was obtained from Dupont (Geneva, Switzerland).

[1]) Enzymes
Carnitine acetyl-transferase (EC 2.3.1.7)
Carnitine palmitoyl-transferase (EC 2.3.1.21)

Methods

Subjects

Subjects were hospital employees participating in a screening programme for the detection of major risk factors for ischaemic heart diseases (Lausanne, CHUV, Switzerland, 8–28 November 1990) organized in our university hospital. They arrived independently at the centre between 11 am and 8 pm. Fasting was not a prerequisite.

Informed consent was obtained from each subject before blood sampling. The study was approved by the local ethical committee.

Collection of specimens

Blood samples (2.7 ml) were drawn from an antecubital vein after a 10 minutes rest, into lithium heparinate tubes (Sarstedt AG, Sevelen, Switzerland) in the sitting position. Blood was centrifuged, and the plasma was frozen at −80 °C within 4 hours after sampling.

Samples (415 out of 1397) were randomly assigned for carnitine determination.

Assays and statistics

Free carnitine was determined in the thawed plasma according to a modified radioenzymatic method (15) without deproteinisation. The reaction mixture contained, in a final volume of 220 µl, 2.3 MBq/l [^{14}C-acetyl]CoA in 0.31 mmol/l acetyl-CoA, 500 µmol N-ethylmaleimide (16), 103 mmol/l HEPES pH 6.7, 1.45 kU/l carnitine acetyl-transferase and 100 µl of prediluted sample (1/10 with H_2O, by vol.) or standards of *L*-carnitine. A suspension of Dowex anion exchange resin (300 µl; 1 + 1 with H_2O by weight) was then added, the mixture vortexed 20 seconds and placed on ice to improve reproducibility. Mixing was repeated twice at 5 min intervals. After centrifugation (4 °C, 10 min, 8000 *g*), 300 µl of the supernatant was added to 10 ml of Aquasol (Dupont International S.A) and assayed for radioactivity in a liquid scintillation counter (Tricarb 2500, Canberra Packard, Switzerland). A blank was run in parallel for correction of non-specific supernatant radioactivity.

Total carnitine (free and esterified) was determined as described above except that 500 µl of the prediluted sample (1/10, by vol.) was added to 100 µl 0.6 mol/l KOH and allowed to stand at 56 °C for 1 hour. pH was adjusted to 6.7 with 0.5 mmol/l HCl and HEPES 2.5 mol/l pH 6.7. The total carnitine concentration of the sample was calculated from the standard curve of free carnitine, taking into account the dilution. Acylcarnitine concentration was calculated from the difference of total and free carnitine. The acyl to free ratio (acyl/free) was calculated as mol/mol.

Intraserial imprecision (N = 8) was 2.7% and 1.8% for total (57 µmol/l) and free (43 µmol/l) carnitine, respectively. Coefficients of variation of the day to day imprecision were assessed with pools of serum and were less than 7% for total and free carnitine.

Other clinical chemistry quantities were assayed on a Hitachi 717 (Boehringer, Rotkreuz, Switzerland) according to the recommendations of the Swiss Society of Clinical Chemistry or IFCC, the enzyme activities being measured at 37 °C.

The body mass index was calculated as weight(kg)/[height(m)]^2.

The distribution pattern was assessed by comparing the group frequencies with the theoretical *Gauss*ian or log-normal distribution with the *Kolmogorov Smirnov* and the χ^2 tests. Comparisons between groups were made with the *Mann-Whitney* test, and correlations were tested with the *Spearman* rank correlation test.

Results

Samples were analysed from 180 females and 160 males; none was hypertensive or diabetic, and none was receiving treatment for hypercholesterolaemia.

In both sexes, the total and free carnitine concentrations were compatible with a *Gauss*ian distribution (figs. 1 and 2); acyl carnitine and the acyl/free ratio differed significantly from this distribution. The acyl/free ratio could be normalised by the log transformation in the males only. Thus percentiles and non-parametric tests were used for evaluating these quantities.

Mean values for total and free carnitine were significantly higher in the males than in the females. Females had significantly higher median values for acyl carnitine and acyl/free ratio than males (tab. 1). Total and free carnitine tended to increase with age in both sexes. No age effect was observed on acyl carnitine or on the acyl/free ratio in both sexes (tab. 2). Before 45 years of age total carnitine levels were found to be lower in females than in males (tab. 2). The same is true for free carnitine. No more differences were observed after this age. The acyl/free ratio was higher in females than in males whatever the age, but the differences were statistically significant only before the age of 35 years.

In the females, total and free carnitine was lower in the group with a body mass index < 28 [M1] (N = 157, 34% $>$ 44 years of age) than in the group with a body mass index > 28 [M2] (N = 23, 43% $>$ 44 years of age): median total carnitine, M1 = 38 and M2 = 42 µmol/l, $p < 0.01$; median free carnitine, M1 = 30 and M2 = 37 µmol/l, $p < 0.001$. The median acyl/free ratio was lower in the group with a body mass index > 28 (0.15) than in the group with a body mass index < 28 (0.20), $p < 0.005$. These differences cannot be explained by difference in group age. No such differences were seen in the male groups.

Among the frequently assayed clinical chemistry components (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, γ-glutamyl-transferase, alkaline phosphatase, bilirubin (direct and total), lipase, amylase, albumin, iron, uric acid, urea and creatinine) only a few significant correlations with the carnitine concentrations were found. Significant *Spearman* rank correlation coefficients did not exceed 0.24 except for the correlation of uric acid with free (0.41) and total (0.41) carnitine in normouricaemic females.

Female participants with cholesterol values below 6.5 mmol/l [FC1] (N = 147, 26% over 44 years old) had lower total carnitine and free carnitine concentrations

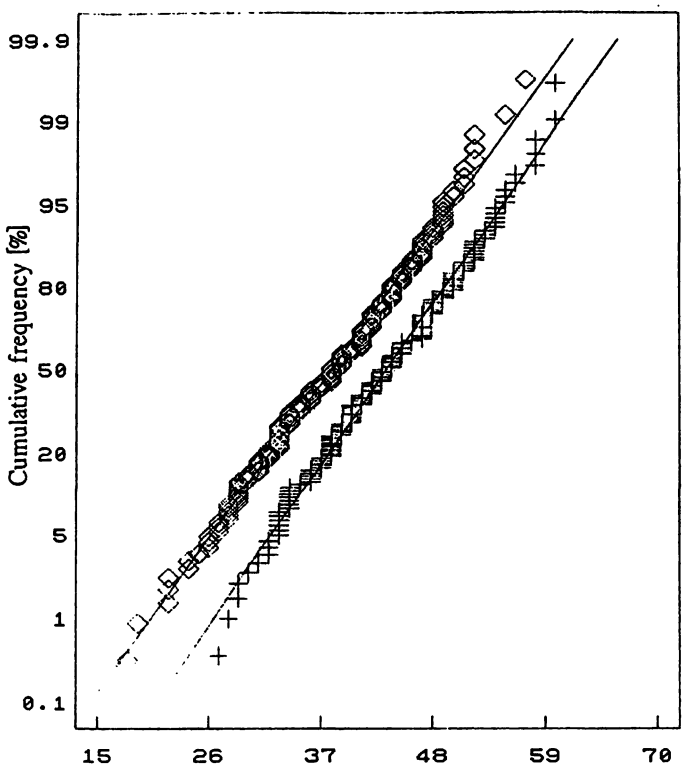

Fig. 1. Total carnitine: Normal probability plot for total carnitine in the reference population according to sex. The distribution fits the *Gaussian* model (*Kolmogorov-Smirnoff* and χ^2 tests).
◇: females, +: males.

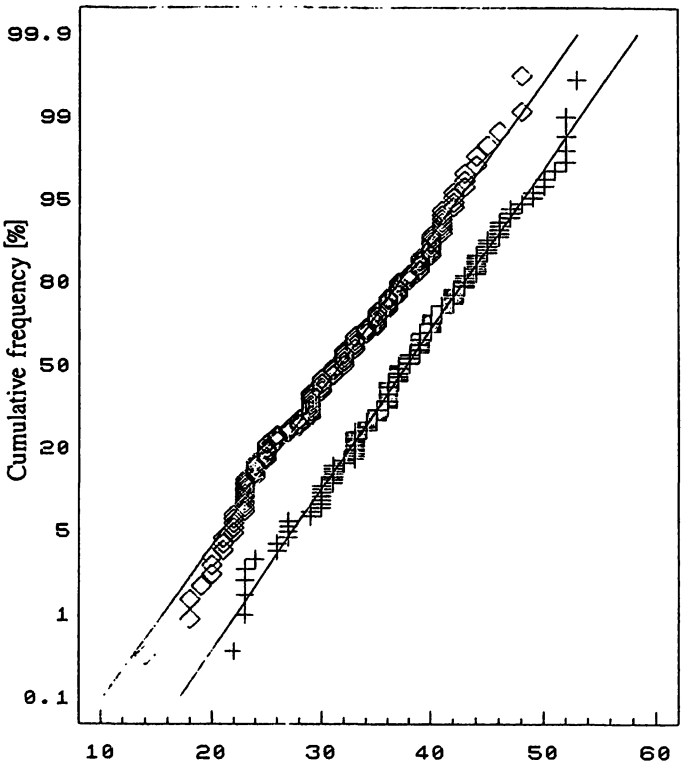

Fig. 2. Free carnitine: Normal probability plot for free carnitine in the reference population according to sex. The distribution fits the *Gaussian* model (*Kolmogorov-Smirnoff* and χ^2 tests).
◇: females, +: males.

Tab. 1. Reference values of plasma carnitine concentration: Concentrations of carnitine (µmol/l) in females and males and acyl/free ratio (mol/mol) in the reference population.
N = number of specimens; Min = minimal value; Max = maximal value; P2.5 = 2.5th percentile; P97.5 = 97.5th percentile; p = significance of the *Mann-Whitney* test between corresponding female and male groups; SD = standard deviation.

	N	Mean	SD	Median value	Min	Max	P2.5	P97.5
Females								
Total	180	38.1	7.6	38	18	57	22	52
Free	180	31.7	6.9	32	14	48	20	44
Acyl	180	6.47	3.3	6	1	18	1	14
Acyl/Free	180	0.214	1.12	0.18	0.02	0.65	0.03	0.41
Males								
Total	160	43.6	7.3	43.5	27	60	29.5	58
Free	160	37.8	6.6	37.5	22	53	23	52
Acyl	160	5.8	4.9	5	1	21	1	13.5
Acyl/Free	160	0.159	0.1	0.15	0.02	0.54	0.03	0.56

than female participants with cholesterol above 6.5 mmol/l [FC2] (N = 71, 75% over 44 years old): median total carnitine, FC1 = 38 and FC2 = 42 µmol/l, $p < 0.002$; median free carnitine FC1 = 31 and FC2 = 35 µmol/l, $p < 0.001$. In the group of females with cholesterol values above 6.5 mmol/l, acyl/free ratio was higher when the cholesterol/HDL-cholesterol ratio was below 5 (N = 44, acyl/free median 0.2) than when the cholesterol/HDL-cholesterol exceeded 5 (N = 23, acyl/free median 0.15, $p < 0.05$, 91% over 44 years old).

Tab. 2. Carnitine concentrations and age: Percentiles for carnitine concentrations and acyl/free ratio in females and males according to age.
N = number of specimens; Min = minimal value; Max = maximal value; P2.5 = 2.5th percentile; P97.5 = 97.5th percentile; p = significance of the *Mann-Whitney* test between corresponding female and male groups; SD = standard deviation.

Total carnitine								Free carnitine						
	Females			Males				Females			Males			
Age (a)	N	P2.5	P97.5	N	P2.5	P97.5	p	N	P2.5	P97.5	N	P2.5	P97.5	p
<24	24	25	49	16	33	52	NS	24	19	41	16	23	47	<0.04
25–34	52	24	49	61	31	51	<0.002	52	21	41	61	26	50	<0.001
35–44	41	22	52	43	32	58	<0.001	41	18	44	43	27	52	<0.001
45–54	45	28	52	29	27	60	NS	45	23	45	29	23	53	NS
55–64	17	29	52	11	37	53	NS	17	25	48	11	30	46	NS

Acyl carnitine								Acyl/Free ratio						
	Females			Males				Females			Males			
Age	N	P2.5	P97.5	N	P2.5	P97.5	p	N	P2.5	P97.5	N	P2.5	P97.5	p
<24	24	1	13	16	2	12	NS	24	0.03	0.52	16	0.05	0.52	<0.007
25–34	52	1	14	61	1	11	<0.03	52	0.03	0.47	61	0.03	0.39	<0.001
35–44	41	1	13	43	2	17	NS	41	0.03	0.57	43	0.04	0.46	NS
45–54	45	2	14	29	1	17	NS	45	0.06	0.56	29	0.03	0.4	<0.03
55–64	17	2	14	11	2	11	NS	17	0.06	0.48	11	0.04	0.27	NS

Male participants with cholesterol values below 6.5 mmol/l (N = 138, 24% over 44 years old) had lower values of total carnitine (median value 43 µmol/l) than male participants with cholesterol above 6.5 mmol/l (N = 59 median value total carnitine: 47 µmol/l, $p < 0.05$, 47% over 44 years old).

There was no linear correlation between carnitine and systolic or diastolic blood pressure. No difference of carnitine concentrations was observed between smokers (1 or more cigarettes per day) and non-smokers.

Discussion

Carnitine concentrations obtained in our reference population compared well with the data of most other authors, which were, however, obtained on smaller samples of males and females (8, 9, 14, 17, 18). Some authors (19–21) have reported higher total and free carnitine concentrations. As already discussed by *Schmidt-Sommerfeld & Penn* (19), the total carnitine concentration might be underestimated, because the calculation of total carnitine is based on free carnitine standards. The reasons for this effect are not clear. It can lead to calculate free carnitine concentrations that are higher than the total carnitine concentration in some samples. In our study, such rare cases were discarded. Total and free carnitine concentrations were higher in males than in females, confirming the previously reported observations (14, 18–21). In accordance with some authors (20) we found higher acyl/free ratio in females than in males. We are not aware of any endocrinological data that would explain the lower carnitine concentrations found by us in premenopausal women. This fact and the differences observed on carnitine concentrations with age suggest that sex and age must be considered in studies involving carnitine.

All correlations observed between carnitine fractions and other clinical chemistry quantities, although statistically significant, account only for up to 5% of the total variance and thus can be considered as not clinically relevant. With respect to uric acid, creatinine and albumin, the correlations were probably due to the nutritional state, as fasting was not a prerequisite. These correlations may also reflect a general dietary effect in a well nourished population (22–24). Such factors have however been shown not to affect the intraindividual variation of carnitine concentrations (14). The correlation between total and free carnitine and uric acid has already been observed in undialysed patients with chronic renal insufficiency (11).

In contrast to data of patients with liver cirrhosis (9), the γ-glutamyl transferase was inversely correlated with the acyl/free ratio in females (not in males) in our healthy ambulatory population.

Our results clearly indicate that when plasma carnitine concentrations are compared in adults, the groups have to be matched at least for sex and, particularly in females, for age.

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