Clinica Chimica Acta, 57 (1974) 55—61

CCA 6670

CARNITINE LEVELS IN HUMAN SERUM IN HEALTH AND DISEASE

THOMAS BÖHMER, ANDREAS RYDNING and HELGE ERIK SOLBERG

Department of Medicine B, and Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Oslo (Norway)

(Received June 11, 1974)

Summary

The level of carnitine in serum was examined in a control group and in different patient groups. A radiometric assay of carnitine with carnitine acetyltransferase and radioactively labeled acetyl-CoA was used. By the addition of dithionitrobenzoic acid (DTNB) to the incubation mixture to trap the CoASH released, the formation of radioactive acetylcarnitine was proportional to the carnitine concentration over a wide range. Carnitine was extracted from plasma after ethanol addition and the recovery of [*Me*-^{3}H]carnitine added to serum was 102%. The serum level of carnitine was reduced in patients with myotonia congenita, Crohn's disease in some patients with malabsorption and in cases of anorexia nervosa. During hemodialysis the carnitine concentration was reduced to 25% of the value occurring before dialysis. Normal levels of carnitine were found in ulcerative colitis patients, most patients with malabsorption, in liver cirrhosis, in right-sided heart failure with liver stasis, in juvenile and adult onset diabetes, and in cardiomyopathies. Two patients with progressive cardiac failure with icterus and oliguria showed an eight-fold increase in serum carnitine levels.

Introduction

L-Carnitine (L-3-hydroxy-4-*N*-trimethylaminobutyrate) facilitates the transfer of activated long chain fatty acids into mitochondria and is probably present in most tissues. It is synthesized from butyrobetaine, mainly in the liver [1,2], and is rapidly transferred via the plasma to other tissues [1]. It is secreted in the urine and only to a minor extent degraded to methylcholine [3].

The level of carnitine in plasma has been determined in normal humans [4]. It is unknown whether a primary change in carnitine metabolism or tissue levels might either contribute to human disease or be altered by disease. Some results obtained with laboratory animals, however, indicate that the carnitine

content of tissue may also be related to human disease. Thus the carnitine levels are reduced in the tissues of rats deficient in lysine [5], in the alloxan diabetic rat [6], in the heart of the guinea-pig infected with diphtheria [7], and in the heart of dogs with coronary ischemia [8].

We have therefore studied, using an improved method, the serum levels of carnitine in a reference group and in groups of patients with diseases of the gastrointestinal tract, liver, kidney, heart and skeletal muscle.

Materials and Methods

Reagents

Acetyl-coenzyme A (lithium salt, approximately 70% pure) and dithionitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co., St. Louis, Mo. Carnitine acetyltransferase (EC 2.3.1.7), activity 80 U/mg, was obtained from Boehringer and Soehne GmbH, Mannheim, G.F.R. L-Carnitine chloride was a gift from Otsuka Pharmaceutical Company, Osaka, Japan. Dowex-2X (50—100 mesh, Cl^- form) was bought from J.T. Baker Chemicals, Deventer, The Netherlands. [^{14}C]Acetyl-coenzyme A (specific activity 46.4 Ci/mole) was obtained as an aqueous solution from New England Nuclear Corporation, Boston, Mass. [*Me*-^{3}H]Carnitine (specific activity 80 Ci/mole) was a gift from Professor J. Bremer.

Extraction procedure

To 250 µl of serum were added 250 µl of water followed by thorough mixing. For recovery studies 0.78 nmole of L-[*Me*-^{3}H]carnitine (56 000 cpm) was included in the water. One ml of absolute ethanol was subsequently added, mixed, and left for a few minutes. The mixture was then centrifuged at 800 × *g* for 10 min, and the supernatant was filtered through a glass filter. A 750 µl aliquot of the filtrate was evaporated to dryness under an air current and subsequently dissolved in 250 µl of water; 50 µl were then removed for recovery studies together with duplicate 50 µl samples for carnitine assay as described below. Using this procedure, the mean recovery was 102 ± 4% (S.D.) in 96 consecutive samples. No differences were seen in carnitine content between heparin- or EDTA-plasma and serum in the 10 samples tested. Serum was therefore used throughout.

Assay

Carnitine was assayed according to Cederblad and Lindstedt [4] with certain modifications. A 275 µl incubation mixture containing acetyl-CoA, 10 nmoles; [^{14}C]acetyl-CoA, 0.33 nmole (15 000 cpm); Tris—HCl (pH 7.3), 10 µmoles; DTNB, 0.5 µmole; carnitine acetyltransferase, 1 unit; unknown samples or L-carnitine standards, 0—2.0 nmoles; was incubated for 20 min at 37°. After incubation, a 225 µl aliquot was applied to small columns made of plastic syringes (0.6 cm × 6 cm) containing 0.6 ml Dowex 2x[Cl^-] suspended in water just before use. The columns were placed on top of counting vials and kept in an upright position by means of a special plastic stopper fitted to the vials. Radioactively labeled acetylcarnitine was eluted with two 500 µl volumes of water.

Isotope counting

All radioactive counting was done by means of a Packard TriCarb liquid scintillation spectrometer. For control of recoveries, triplicates of [^{3}H]carnitine standards in 50 µl water and 50 µl aliquots were counted in a scintillation solution containing xylol, 1925 ml; dioxane, 1925 ml; ethanol, 1150 ml; naphthalene, 80 g; PPO, 5 g; POPOP, 50 mg; and 0.25 ml conc. HCl per litre. Tritium was counted with an efficiency of 32%. [^{14}C]Acetylcarnitine formed in the assay was counted with an efficiency of 40%. The [^{3}H]carnitine present contributed to less than 10 cpm in the counting of ^{14}C.

Statistics

The *F*-test was used for testing the variance between the malabsorption group and the reference group. Student's *t*-test was used for testing differences between means, and the paired *t*-test was used for differences between serum carnitine levels before and after hemodialysis [9].

Patient groups

The reference group consisted of 30 normal individuals without any known disease. All had normal blood pressure, normal liver function tests, serum creatinine and blood urea.

Liver cirrhosis. Patients with characteristic results of liver function tests and findings in needle biopsy. Two patients had primary biliary cirrhosis. (For details of criteria see ref. 10.)

Right-sided heart failure. Patients with rheumatic valvular heart disease with peripheral edema, dilated neck veins and enlarged liver without any known primary liver disease.

Kidney disease, severe. Patients with plasma creatinine levels above 10 mg/dl.

Hemodialysis. Patients on chronic hemodialysis, dialysed every other day with a single-pass kidney for 8 h (mean flow in the kidney was 500 ml per min). The samples were taken just before the beginning of the dialysis and immediately afterwards.

Patients O.S. and E.T., with progressive rheumatic heart failure, icterus and oliguria before death.

Crohn's disease. Patients with characteristic symptoms and X-ray findings. Some cases were proven with operation biopsies.

Malabsorption. Patients with a history of malabsorption and villous atrophy at intestinal biopsy. A few of them also had immunological defects.

Ulcerative colitis. Patients with a history of numerous bloody stools per day and a positive biopsy from the rectum.

Anorexia nervosa. Patients with the psychiatric disorder of anorexia nervosa and extensive weight loss without any somatic disease.

Diabetes mellitus. Patients with a fasting blood sugar above 130 mg/dl and polyuria treated with insulin (a), or with diet and hypoglycemic agents only (b).

Cardiomyopathies. Patients with general enlargements of the heart without hemodynamic or angiographic (included coronary angiography) evidence of valvular or coronary heart disease by right and left heart catheterization.

Myotonia congenita. Patients with a recessive neurological disorder with decreased muscular force of contraction without atrophies.

Unspecified neurological diseases. Patients with muscle disease with or without muscular dystrophy, as well as a few with localized paralysis or paresis.

Results

The assay of L-carnitine

In the assay of L-carnitine as described by Cederblad and Lindstedt [4], the formation of radioactively labeled acetylcarnitine was not proportional to increasing L-carnitine concentration. The reaction:

$$\text{L-carnitine} + [^{14}\text{C}]\text{acetyl-CoA} \overset{\text{carnitine acetyltransferase}}{\rightleftharpoons} [^{14}\text{C}]\text{acetylcarnitine} + \text{CoASH}$$

used for the assay will rapidly attain chemical equilibration. However, if DTNB is included in the reaction mixture, almost all the L-carnitine will react because DTNB traps the CoASH formed [11], and proportional formation of [^{14}C]-acetylcarnitine with increasing amounts of L-carnitine will then be obtained (Fig. 1).

An additional requirement for a linear relationship between L-carnitine and acetylcarnitine formation (Fig. 2) was that L-carnitine in the assay was less than 20% of the acetyl-CoA initially present.

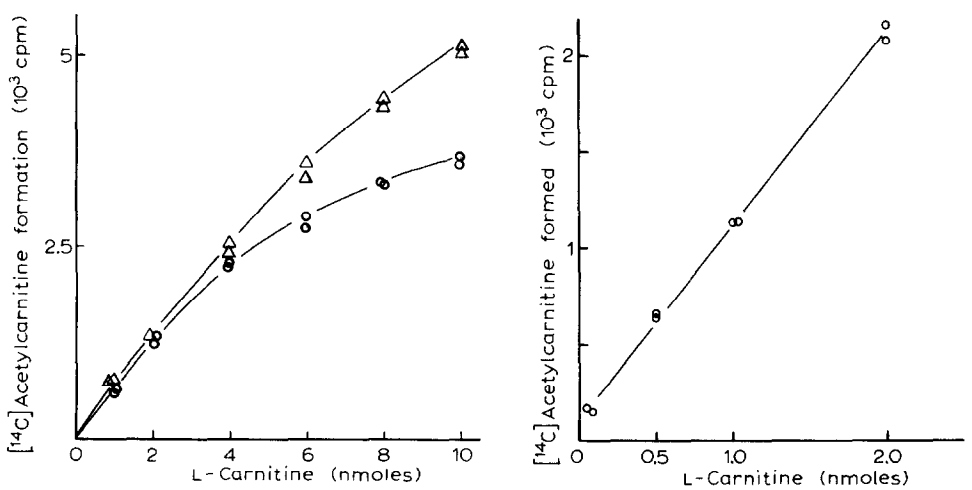

Fig. 1. The effect of Tris—HCl/dithionitrobenzoic acid (DTNB) and phosphate buffer on the carnitine assay. The incubation mixture contained acetyl-CoA, 20 nmoles; [^{14}C]acetyl-CoA, 0.33 nmole (15 000 cpm); L-carnitine, 10 nmoles; carnitine acetyltransferase, 1 unit; ○——○, phosphate buffer (pH 7.3), 50 μmoles; or △——△, Tris—HCl (pH 7.3) 50 μmoles and DTNB, 0.5 μmoles. The total volume was 275 μl. The incubation was performed for 20 min at 37°.

Fig. 2. [^{14}C]Acetylcarnitine formation from L-carnitine. The incubation mixture contained acetyl-CoA, 10 nmoles; [^{14}C]acetyl-CoA, 0.33 nmole (15 000 cpm); L-carnitine, 0—2 nmoles; carnitine acetyltransferase, 1 unit; Tris—HCl (pH 7.3), 50 μmoles; DTNB, 0.5 μmole. The total volume was 275 μl. The incubation was performed for 20 min at 37°.

TABLE I

CARNITINE LEVELS IN HUMAN SERUM IN HEALTH AND DISEASE

The serum carnitine level was determined in serum of fasting humans. The assay and the statistical methods are described in the Methods section. The groups are tested against the reference group, except in the paired tests where results before and after dialysis have been compared.

Group	No.	Carnitine μmoles/l	
		Mean	S.D.
Control	30	43.1	8.9
Liver cirrhosis	7	38.4	10.7
Right-sided heart failure liver stasis	11	47.1	12.6
Kidney disease, severe	5	73.5	4.7*
Hemodialysis, before	6	33.5	13.1
Hemodialysis, after	6	11.4	4.7*
Patients O.S. and E.T.		323 and 273	
Crohn's disease	9	30.9	9.6*
Malabsorption	16	43.9	17.2*
Ulcerative colitis	4	36.1	8.2
Anorexia nervosa	3	16.1—34.1—45.7	
Diabetes mellitus, insulin treated	16	44.3	10.9
Diabetes mellitus, diet/tablets treated	6	52.5	10.7
Cardiomyopathies	9	47.7	6.4
Myotonia congenita	6	34.0	6.6*
Unspecified neurological diseases	5	48.1	6.4

* $P \leqslant 0.02$.

Serum levels of L-carnitine

The mean level in the reference groups of 43.1 μmoles/l (Table I) is in close agreement with the result of 51 μmoles/l (also including some short-chain acylcarnitines) reported by Cederblad and Lindstedt [4].

A reduced level of carnitine in serum was observed in patients on chronic hemodialysis, both before and after the dialysis, in Crohn's disease, in some patients with malabsorption, in one patient with anorexia nervosa, and in myotonia congenita.

The carnitine level in serum was normal in liver cirrhosis, in right-sided heart failure with liver stasis, in some patients with malabsorption, in ulcerative colitis, in diabetes mellitus, in cardiomyopathies and in some different neurological disorders.

The serum carnitine increased moderately in severe kidney disease and extensively in two patients with severe progressive heart failure.

Discussion

Carnitine has an important function in intermediary metabolism, especially in the heart and the muscle which depend heavily on fatty acid oxidation for their performance. The fact that nutritional deficiency [5], diphtheria toxin [7], and alloxan diabetes [6] influence carnitine metabolism in animals, and

that carnitine is mainly synthesized in the liver [1,2], may suggest a causal relationship between human disease and carnitine metabolism.

In the animal studies where the tissue levels of carnitine have been studied, plasma carnitine has not been determined. The carnitine uptake both in sperm [12] and in isolated heart cells (T. Böhmer and J. Jonssen, unpublished observations) increases with increasing concentration of carnitine in the surrounding medium, indicating that at least under certain conditions, the tissue carnitine level parallels the plasma level of carnitine. A localized defect in the uptake of carnitine in the ischemic dog heart (due to a membrane defect?) will probably not be overcome by increasing the plasma carnitine level. It is likely, therefore, that under certain conditions tissue carnitine parallels the plasma carnitine while under other conditions such a relationship will not prevail.

The tissue levels of carnitine have been studied in animals but this has not been possible in large scale studies with humans. Therefore, a study of human serum carnitine levels was undertaken. A localized defect in the uptake of carnitine in one organ (as seen in an ischemic heart [8]) will not be revealed by our studies.

Disturbances in carnitine metabolism were expected in disorders of the organs responsible for the synthesis and excretion of carnitine, i.e. liver and kidney. We were unable to discover any liver disorders with a reduced carnitine level. This indicated that the liver has a high reserve capacity for the hydroxylation of butyrobetaine to carnitine. Patients with right-sided heart failure had a low cardiac output but this did not reduce the oxygen transport to the liver to such an extent as to interfere with the synthesis of carnitine.

The increase in the serum carnitine level in severe kidney disease is compatible with the idea that carnitine is normally excreted in the urine. The chronic hemodialysis patients have striking reductions in their serum carnitine level. Their pre-dialysis values were lower than those of the reference group. This may indicate either a deficiency in carnitine precursors or an inability to cope with the increased needs caused by loss of carnitine in the dialysis fluid.

The level of carnitine during hemodialysis was reduced to 25% of the predialysis values but not to zero. Carnitine is a hydrophilic substance with a molecular weight of 196 which suggests that it can easily be lost during hemodialysis. It is unknown how rapidly carnitine is equilibrated across the cellular membranes, whether it is bound to plasma proteins or what amounts are actually lost during hemodialysis. The reduced carnitine level in plasma might be accompanied by a similar reduction in the carnitine level in the tissues. A reduced level of carnitine may possibly interfere with fatty acid metabolism in the cells and contribute to the post-dialysis syndrome with weakness which patients experience within the first few hours after hemodialysis.

Two patients showed a great increase in the serum carnitine level. They both had extremely low cardiac outputs, kidney failure with rising creatinine in plasma and icterus due to secondary liver failure. Their kidney failure probably contributed to the increase in serum carnitine as seen in the other patients with severe kidney disease (Table I). The level of carnitine, however, was increased out of proportion to the reduction in the kidney function and another explanation should be sought. A generalized hypoxia in a number of tissues may

possibly reduce the normal uptake and removal of carnitine from plasma and thus give rise to an increased level of circulating carnitine.

The patients with Crohn's disease, some patients with malabsorption and one patient with anorexia nervosa showed reduced serum carnitine levels. This was probably due to malnutrition through a reduced uptake of carnitine or carnitine precursors from the gut.

Rats with alloxan diabetes show a decrease in their tissue carnitine levels and a reduced plasma turnover time for carnitine with only partial normalization after injection of insulin [6]. A normal serum carnitine level was found in our diabetic patients. This difference between diabetic patients and the alloxan diabetic rats raises the question whether the reduced carnitine level in the rat is due to a toxic effect of the alloxan.

Another explanation could be that a definite incompensated diabetes must be present to reveal the changes seen in the diabetic rat [6]. This explanation is also unlikely as even after insulin injection, carnitine metabolism in the rat is only partially normalized, while in the human diabetics at least the serum carnitine level returns(?) to normal (Table I). This does not exclude an increased carnitine turnover, however, in human diabetics.

We have looked for a carnitine-deficiency syndrome among the neuromuscular diseases of unknown etiology, without really finding one. The patients with myotonia congenita have a carnitine level in serum which is 70% of normal. It is unlikely that this small reduction could be of great importance for the development of their disease or their symptoms.

Acknowledgements

The collaboration of Dr S. Årseth, Diakonhjemmet who submitted samples from his diabetic patients, and by Dr O. Sjaastad, Department of Neurology, Rikshospitalet, who contributed samples from patients with neuromuscular diseases, is greatly appreciated. Excellent technical assistance was given by Anglia Solem.

This study was supported by Nordisk Insulinfond, Anders Jahres fond for Medisinsk Forskning, and the University of Oslo.

References

1 T. Böhmer, Biochim. Biophys. Acta, 343 (1974) 551
2 H.T. Haigler and H.P. Broquist, Biochem. Biophys. Res. Comm., 56 (1974) 676
3 E.A. Khairallah and G. Wolf, J. Biol. Chem., 243 (1967) 32
4 G. Cederblad and S. Lindstedt, Clin. Chim. Acta, 37 (1972) 235
5 V. Tanphaichitr, D.W. Horne and H.P. Broquist, J. Biol. Chem., 246 (1971) 6364
6 M.A. Mehlmann, M.M. Abdel Kader and D.G. Therriault, Life Sci., 8 (1969) 465
7 B. Wittels and R.J. Bressler, J. Clin. Invest., 43 (1964) 630
8 A. Schwartz, J.M. Wood, J.C. Allen, E.P. Bornet, M.L. Entman, Margaret A. Goldstein, L.A. Sordahl and M. Suzuki, Amer. J. Cardiol., 32 (1973) 46
9 G.W. Snedecor and W. Cochran, Statistical Methods, Iowa State Univ. Press, Ames, Iowa, 1967
10 S. Skrede, J.P. Blomhoff, K. Elgjo and E. Gjone, Scand. J. Clin. Lab. Invest., in press
11 N.R. Marquis and I.B. Fritz, J. Lipid Res., 5 (1964) 184
12 E.R. Casillas, J. Biol. Chem., 248 (1973) 8227