Increased Serum Carnitine Concentration in Renal Insufficiency

Shi-Hua Chen¹ and Susan D. Lincoln

We measured serum carnitine in groups of patients with various diseases. The concentration was frequently above normal in patients with renal insufficiency. The clinical significance of such an increase in renal insufficiency may be analogous to that of increases in urea nitrogen, creatinine, or uric acid in the serum.

Additional Keyphrases: normal values · lipid metabolism · muscle necrosis · myocardial infarction · enzymatic methods · liver cirrhosis · cancer · coronary insufficiency · hemodialysis · correlation with blood urea nitrogen · kidney disease · diagnostic aids

L-Carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) plays an important role in lipid metabolism (1) and is a normal constituent of human serum. A few investigators have reported alterations in the concentration of carnitine in serum of patients with acute muscle necrosis (2), renal diseases (3), and systemic carnitine deficiency (4). Thus, such data may be clinically useful in diagnosis and management of certain diseases.

Some convenient and specific techniques have been developed for measuring serum carnitine concentration (5,6). The enzymatic assay procedure can easily be performed in most clinical chemistry laboratories. Attempting to explore the possibility of using this as a laboratory test, we applied the procedure (6) in several groups of patients, and describe the results here.

We were particularly interested in the concentration of carnitine in the serum in cases of myocardial infarction. Carnitine is widely distributed in many mammalian tissues, the highest concentrations being found in skeletal and heart muscle (7). Carnitine increases during attacks of myoglobinuria (2). Because myocardial infarction is known to give rise to transient myoglobinuria (8), we wondered if serum carnitine might be a useful index of muscle destruction in myocardial infarction. Our results for 16 such patients, several of whom had daily carnitine determination, indicated that their serum carnitine concentration was not significantly altered.

Liver and kidney are respectively the sites of biosynthesis and excretion of carnitine. For this reason, we also measured serum carnitine in patients with liver cirrhosis or chronic renal failure, or both. Most of these patients showed an increase, which suggests that measurement of serum carnitine may be useful in evaluating kidney function.

Materials and Methods

Assay of Carnitine

We purchased acetyl coenzyme A, carnitine acetyltransferase (EC 2.3.1.7), and D,L-carnitine HCl from Sigma Chemical Co., St. Louis, Mo. 63178. Dithionitrobenzoic acid was obtained from Aldrich Chemical Co., Milwaukee, Wis. 53233.

Patients fasted overnight and venous blood was sampled early in the morning. The blood was allowed to clot, centrifuged, and serum collected. Occasionally plasma was collected from blood drawn in ethylenediaminetetraacetic-coated tubes. The anticoagulant had no apparent effect on results for carnitine. Serum or plasma was stored at −20°C for various intervals. Immediately before carnitine was determined, the serum or plasma samples were thawed and deproteinized as described by DiMauro et al. (2). Carnitine was measured by the enzymatic method of Pearson et al. (6). The reaction mixture contained the following in a final volume of 1 ml: 0.15 mmol of acetyl CoA, 0.125 mmol of dithionitrobenzoic acid, 100 mmol of tris(hydroxymethyl)aminomethane HCl (pH 7.8), 1.25 mmol of ethylenediaminetetraacetate and 0.2 ml of deproteinized serum. The reaction was started by adding 10 µl of carnitine acetyltransferase (1 g/liter). A standard solution of D,L-carnitine HCl was run with each set of assays.
Patient Groups

Control group: This group consisted of 53 apparently healthy adults, volunteer donors at the local blood bank.

Myocardial infarct: Patients were classified as having suffered an acute myocardial infarct when the clinical presentation, electrocardiographic criteria, and serum enzyme activity were all consistent with the diagnosis.

Coronary insufficiency: Patients were admitted to the coronary care unit for observation because of chest pain. However, there was no electrocardiographic evidence of infarction, and serial determinations of serum did not show that activities of the appropriate enzymes were above normal.

Liver cirrhosis: Eleven patients were classified into this group, based on their history of alcohol intake, liver-function tests, liver biopsy, and clinical presentations, such as ascites and bleeding esophageal varices. Autopsy was done on two patients.

Renal insufficiency: These patients had increased values for blood urea nitrogen and creatinine. The group included various kidney diseases, such as arterionephrosclerosis, chronic pyelonephritis, and polycystic kidney.

Neoplasia: There were four cases of malignant lymphoma, four cases of acute leukemia, and one case each of esophageal carcinoma, chromophobe adenoma, neurilemoma, and medulloblastoma.

Results

Figure 1 shows our results. The normal range obtained for 53 healthy adults is 38 to 70 μmol/liter (mean ± 2 SD = 54 ± 16). This agrees well with values reported by others (2-5).

To evaluate whether data on serum carnitine are useful as an index of muscle necrosis in myocardial infarct, we investigated patients admitted to the coronary care unit for observation because of chest pain. Serum enzyme and carnitine values for most of these patients were measured daily, starting on the day of admission. On discharge, patients were classified by attending physicians as either having or not having suffered an acute myocardial infarct on the basis of clinical, electrocardiographic, and serum criteria (9). In the same patient the variation in serum carnitine concentration from day to day was usually small. There was no significant difference in carnitine concentration between the group of patients with myocardial infarction and the group of patients without infarction (coronary insufficiency). Only four of 16 patients with myocardial infarction had values that were slightly above the upper normal limit, which is 70 μmol/liter.

Of 26 cases of renal insufficiency, 17 had values for carnitine that exceeded the normal limit. Eight of 11 cases of liver cirrhosis also had increased values. The most nearly constant and striking increase was seen in cases of liver cirrhosis complicated by renal failure, the so-called hepatorenal syndrome. All four such cases in the study showed increases.

The value was normal in patients with lymphoma or leukemia.

Discussion

L-Carnitine enables coenzyme A esters of long-chain fatty acids to enter the inner mitochondrial compartment for beta oxidation. Although carnitine is present in many food items, the dietary intake is not sufficient for the need of the entire body. Liver has been identified as the major organ actively synthesizing carnitine from γ-butyrobetaine to meet the requirement. Carnitine is transferred from the liver into the blood and subsequently taken up primarily by skeletal and cardiac muscles, which form the major body pool. The turnover of carnitine in the body is relatively slow. It is excreted in the urine and is only to a minor extent degraded to β-methylcholine. In view of these metabolic pathways, the serum carnitine concentration probably represents an equilibrium between intestinal absorption, biosynthetic activity of the liver, uptake and release by skeletal and cardiac muscles, and renal clearance (1).

The results of this study and those of others (2-4) indicate that its concentration was increased in muscle necrosis, renal failure, and liver cirrhosis, and decreased in systemic carnitine deficiency and after hemodialysis. In our myocardial infarct patients, serial determinations did not demonstrate a significant increase. Compared to necrosis involving skeletal muscles, the amount of carnitine released from infarcted cardiac muscle is probably small. Thus the value is not increased be-
cause either the increased portion is shuttled into the massive pool of the skeletal muscle or the increase does not exceed clearance limits and is rapidly cleared.

Serum carnitine was above normal in most of our patients with renal insufficiency. In the study reported by Bohmer et al. (9), two patients with oliguria also showed a large increase in serum carnitine. Impaired renal clearance might also be responsible for the increase in serum carnitine in liver cirrhosis. Thus, the clinical significance of increased serum carnitine in renal insufficiency might be analogous to that of blood urea nitrogen, serum creatinine, or serum uric acid. For this reason we have analyzed the correlation between blood urea nitrogen and carnitine values (Figure 2). Many of our patients with renal insufficiency were undergoing periodic hemodialysis at the time of the carnitine study, and this can decrease serum carnitine (3). The extent and the rate of carnitine decrease induced by hemodialysis might not be the same as for urea nitrogen. This might explain the discrepancy between urea nitrogen and serum carnitine values found in some of our patients.

We conclude that data on serum carnitine are not a useful index of muscle destruction in myocardial infarction. Because it is increased in renal insufficiency, such data might be of value in evaluating kidney function. However, more clinical and animal studies on factors controlling serum carnitine concentration are needed in order to establish its usefulness as an aid to diagnosis and management of kidney diseases.

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References

Enzymic Measurement of Cholesterol in Serum with the CentrifiiChem Centrifugal Analyzer

Michael A. Pesce and Selma H. Bodourian

Cholesterol is measured by mixing 5 μl of sample with 350 μl of a reagent consisting of phenol, 4-aminopyrrole, and the enzymes cholesterol oxidase, cholesterol esterase, and peroxidase. After 12 min, the resulting quinoneimine is measured at 520 nm. Readings and cholesterol concentrations are linearly related up to 4.0 g/liter. Lipemic sera and samples containing uric acid (up to 200 mg/liter), hemoglobin (up to 1 g/liter), and certain drugs (clofibrate, phenobarbital, nicotinic acid, Kethocil, Ovral-28), gave no interference. Abnormally high concentrations of bilirubin and ascorbic acid in serum lowered the cholesterol values. This enzymatic assay, compared with the method of Abell and with a rate method that uses the Hantzsch reaction, gave correlation coefficients of 0.987 and 0.989, respectively.

Recently, an enzymic system for measuring cholesterol in serum and with the CentrifiiChem centrifugal analyzer was introduced by Union Carbide. This assay is based on hydrolysis of cholesterol esters by cholesterol esterase (EC 3.1.1.3), oxidation of cholesterol by cholesterol oxidase (EC 1.1.3.6), and coupling of the hydrogen peroxide with phenol and 4-aminopyrrole in the presence of horseradish peroxidase (EC 1.1.1.7) to form a colored product, which is measured at a wavelength of 520 nm.

We have evaluated this system with the CentrifiiChem analyzer for measuring cholesterol in serum and present our results here.

Materials and Methods

We used a CentrifiiChem centrifugal analyzer (Union Carbide Corp., Rye, N. Y. 10580).

Reagents

Cholesterol reagents, obtained in lyophilized vials from Union Carbide, were reconstituted by adding 12 ml of distilled water. The final concentrations, per liter, of the substrates in the working reagent were: sodium cholate, 12 mmol; phenol,