# Serum and urinary carnitine in children with cystic fibrosis

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## ABSTRACT

**Purpose:** Cystic fibrosis (CF) is inherited, congenital disease of multi-organ expression. Carnitine play a role as a lipid acid transporter to mitochondrium for beta-oxydation. Acylation of carnitine is inevitable for detoxication processes in cells. Low lean body mass In CF patients can lead to decreased levels. The aim of the study was the evaluation of free carnitine, acylcarnitine and acylcarnitine/free carnitine ratio in serum and urine of children with cystic fibrosis.

**Material and methods:** The study was conducted in a group of 15 CF children (4 F, 11 M), aged 12.6 ±5.4 years. The serum for a control group was collected from 32 healthy children. Urine samples for control group was collected from 62 health children. Free carnitine and total carnitine was assessed using spectrophotomeric method in which acyl group is transferred from acetyl-CoA to carnitine by carnitine acetyltransferase (CAT). Acyl carnitine concentration and acylcarnitine/free carnitine ratio was counted using Schmidt-Sommerfeld and Seccombe equation.

**Results**: In 12 CF patients (80%) free carnitine and total carnitine was below lower limit of normal ($p<0.001$). In 9 patients (60%) free carnitine level was $\leq$ 20 μmol/L, which can be clinically diagnosed. Acylcarnitine levels were also statistically lower in CF group ($p<0.01$). Acylcarinitie/free carnitine ratio did not differ between the groups ($p=0.05$). Urine excretion of free carnitine, total carnitine and acylcarnitine was lower in CF group ($p<0.001$).

**Conclusions:** In CF pediatric patients statistically significant lower levels of free carnitine, total carnitine and acylcarnitine were observed in comparison to controls. Low urine excretion of free carnitine, total carnitine and acylcarnitine was lower in CF group. No correlation between serum and urine levels of free carnitine, total carnitine and acylcarnitine were observed.

**Key words:** cystic fibrosis, free carnitine, total carnitine, acyl carnitine

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## INTRODUCTION

Cystic fibrosis (CF) is inherited, metabolic, multisystem disease with various clinical symptoms [1]. The cause of the disease is the mutation of *CFTR* gene, which encodes CFTR protein - a intracellular regulator of ion transport across apical part of bronchial epithelium. Pancreatic and liver insufficiency in CF often disturbs proper energetic balance and results in malnutrition [2 - 4].

Malabsorption causes the nutrient insufficiency including carnitine deficit. Carnitine (3-hydroxy-4-N-trimethyloamoniobutrate) is necessary for mitochondrial transport of very long-chain fatty acids (VLCFA) in liver, skeletal muscles and heart muscle. VLCFA after binding with carnitine (acylcarnitine) are transported to mitochondrion for further oxidation [5].

Carnitine prevents accumulation of acetyl and acyl groups which inhibit the β-oxidation. Overproduction of acetyl-coA during extensive β-oxidation causes the blockage of pyruvate dehydrogenase. This leads to decreased glucose oxidation in mitochondria and anaerobic glycolysis. Constant recreation of acetyl-coA in the presence of carnitine restores the free acetyl-coA/acetyl-coA balance and inhibits the anaerobic glycolysis [6-8].

Carnitine also restores the free, mitochondrial and cytostolic coenzyme A [9, 10]. In case of low carnitine concentration, short- and medium-chain fatty acids combined with overproduced acyl-coA can be toxic for the cell, and L-carnitine then plays an important role in cellular detoxification processes [7, 11].

Carnitine consumed with food (meat, poultry, fish, dairy products) is actively absorbed through the enterocytes [12]. Functional changes of mucous membrane in digestive tract (enteritis, hemorrhage, malabsorption syndromes, impaired intestinal passage and absorption, bacterial overgrowth syndrome) lead to impaired digestion and secondary carnitine insufficiency.

Carnitine insufficiency can be also observed in chronic liver and kidney diseases due to increased excretion of carnitine with urine and bile.

The aim of this work was the assessment of free carnitine, total carnitine, acyl carnitine and acylcarnitine/free carnitine ratio in serum and urine of children with cystic fibrosis.

## MATERIALS AND METHODS

The concentration of free and total carnitine was assessed in two groups of children: control group consisted of serum samples collected from 32 children aged 2-4 years and in urine samples collected from 62 children aged 2-14 years - chronic diseases of liver, kidneys and gastrointestinal conditions (GERD, malabsorption syndromes, acute and chronic diarrheas) were exclusion criteria in this group. Study group consisted of 15 CF patients (4F, 11M), aged 12,6 ±5,4 years, in a stable condition for at least 2 months - acute exacerbation, antibiotic therapy in the past 2 months CF related liver diseases and chronic diarrhea were exclusion criteria for this group.

**Blood samples.** 2 ml of peripheral blood was collected to coagulation-activator test-tube and centrifuged at 2000g for 40 min in room temperature, using Eppendorf AG Centrifuge 5702R (Hamburg, Germany) on Centricon YM-30 (Millipore, Bedford, MA, U.S.A.) filters.

**Urine samples.** Midstream urine samples were collected into sterile containers. The samples were stored at -86$^{o}$C until processing. Before analysis the samples were centrifuged for 10 min. at 2000g in Eppendorf AG Centrifuge 5702R (Hamburg, Germany).

**Serum carnitine concentration.** Free creatinine concentration was assessed in non-hydrolyzed samples. Total carnitine concentration was assessed after deproteination of plasma and hydrolysis of carnitine esters in incubation with 1 mol/L KOH at 56$^{o}$C for 60 min. Hydrolyzate was neutralized with 5 mol/L HCl. Free and total carnitine was assessed using spectrophotometric method by Cederblad et al [13] - transferring of acetyl rest from acetyl-coA is catalyzed by carnitine acetyltransferase (CAT). Released from coenzyme A -SH group reacts in quantitative matter with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). Created DTNB anion exhibit the maximum absorbance at wave length of 405 nm. The absorbance was measured using Hitachi UV/VIS Spectrophotometer (Model U-2900, Tokyo, Japan).

**Serum acetylcarnitine concentration.** Serum acetylcarnitine concentration and acetylcarnitine/free carnitine ration was counted according to equations by Schmidt-Sommerfeld [14] and Seccombe [15].

**Urine carnitine concentration.** Urine creatinine was measured using Jaffe method in Larsen modification [16]. Creatinine with picric acid in alkaline environment (NaOH) creates the chromogen. Chromogen absorbance was measured at wave length of 450 nm using Creatinine Analyzer 2 (Beckman, Munich, Germany).

**Statistical analysis.** The data were analyzed using Statistica 7 (StatSoft, Cracow, Poland). All results were checked for normality and expressed as mean ± SD In case of normal distribution or median in case of other-than-normal distribution. For variable with normal distribution student t test was used to test the differences between the groups, in other cases U Mann-

Whitney test was used. Statistical significance was achieved if $p \leq 0.05$.

**Ethical issues.** Written, informed consent was collected from every patient or legal guardian and aims and purpose of this study were explained thoroughly. The study has been approved by the Local Bioethical Committee of Medical University of Bialystok (agreement no. R-I-002/275/2013).

## RESULTS

Free carnitine concentration in control group (n=32) was 37.1 ± 6.1 µmol/l, total 50,0 ± 10.3 µmol/l, acylcarnitine 12.9 ± 6.8 µmol/l and acylcarnitine/free carnitine ratio was 0.35 ± 0,18.

In urine in control group (n=62) creatine excretion counted per one gram of creatinine 111.3 ± 88.3µmol/g of creatinine, total carnitine concentration was 187.3 ± 126.6 µmol/g of creatinine, acylcarnitine was 76 ± 38 µmol/g of creatinine. acylcarnitine/free carnitine ratio was 0.68 ± 0.43.

In 12 CF patient (80% of studied group) free serum carnitine (ref. values 35-75 µmol/L) and total serum carnitine (ref. values 42-80 µmol/L) was lower limit of normal range.

In studied group of patients significant decrease of free and total carnitine concentration was observed ($p<0.001$), concentration of acylcarnitine was also lower in comparison to controls ($p<0.01$) (Fig. 1).

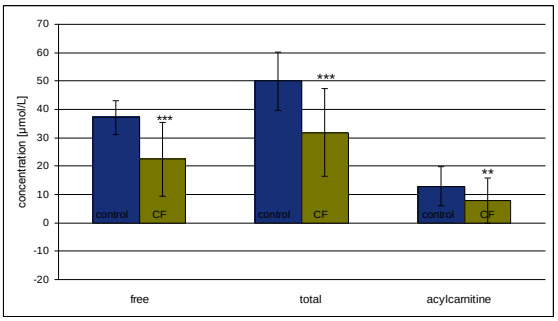


**Fig. 1.** The concentration of free carnitine, Total carnitine and acylcarnitine in serum of CF patients. Results are presented as mean ±SD. Difference between groups is analyzed by one-way t-Student's t-test, where *** statistically significant different from control group at $p<0.001$; ** statistically significant different from control group at $p<0.01$.

Serum acylcarnitine/free carnitine ratio did not differ between the groups ($p=0.05$) (Fig. 2), tendency toward increased values was observed.

In 8 subjects (53% of studied group) urine excretion of free carnitine (ref. values 25-340 µmol/g of creatinine) and total carnitine (ref. values 40-440 µmol/g of creatinine) was below the lower limit of normal range.

Urine excretion of free carnitine, total carnitine and acylcarnitine was significantly lower in comparison to controls ($p<0.001$) (Fig. 3).

Urine acylcarnitine/free carnitine did not differ between the groups ($p=0.05$) (Fig. 4), tendency toward increased values was observed.

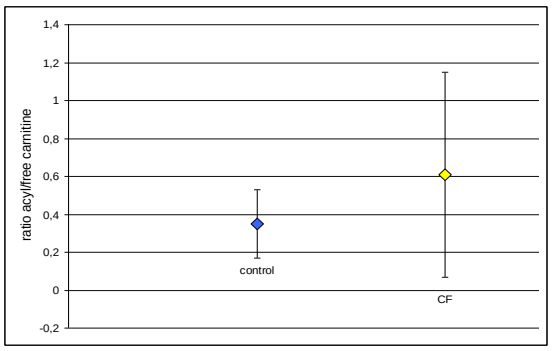


**Fig. 2.** Acylcarnitine/free carnitine ratio in serum of CF patients. Results are presented as mean ±SD. No statistically significant differences were observed between groups in one-way t-Student's t-test.

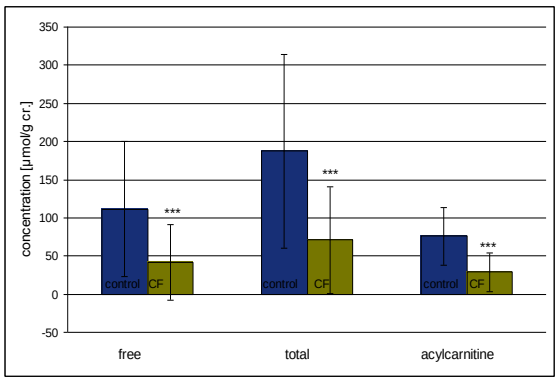


**Fig. 3.** Concentration of free carnitine, total carnitine and acylcarnitine in urine of CF patients. Results are presented as mean ±SD. Difference between groups is analyzed by one-way t-Student's t-test, where *** statistically significant different from control group at $p< 0.001$.

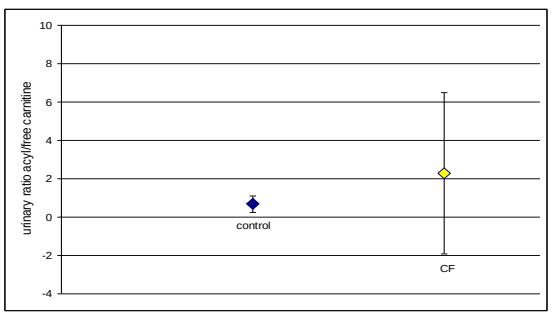


**Fig. 4.** Acylcarnitine/free carnitine ratio if urine of CF patients. Results are presented as mean ±SD. No statistically significant differences were observed between groups in one-way t-Student's t-test.

## DISCUSSION

The distribution of free carnitine and acylcarnitine is essential for proper ATP production. In the lack of free carnitine accumulation of acylcarnitine is observed. Acylcarnitine, main short-chained carnitine ester, is a donor of acyl rest. In mitochondrion carnitine reacts with coenzyme

A catalyzed by carnitine palmitoyl-transferase (CPT). In mitochondrial matrix acyl-coA is regenerated and carnitine is released [5]. Lack of carnitine leads to toxic accumulation of long-chain fatty acids in cytoplasm and acyl-coA in mitochondrion.

Abnormal carnitine metabolism in cystic fibrosis was previously described in the literature [17-22]. Clinical symptoms of carnitine deficiency include cachexia, glucose intolerance, anemia, heart arrhythmia, liver steatosis, growth retardation and skeletal muscles weakness [23 - 25]. Biochemical consequences of carnitine deficiency are caused by accumulation of free fatty acids, impaired transport of organic acids, inhibition of basic oxidative enzymes secondary to increased acetyl-coA/pyruvate coA-carboxylaze ratio and citrate synthase.

In CF patients impaired (n-3) and (n-6) fatty acids metabolism has been described. According to Lloyd-Still et al. [16] primary carnitine deficiency are caused by improper energetic balance due to genetically determined flaws in fatty acid metabolism. They also found that in cord blood of CF infants there is a low acylcarnitine concentration. This led to the conclusion that in CF impaired fatty metabolism is already present during fetal life [17]. No differences in concentration of free carnitine and total carnitine were observed between healthy and CF children, although a tendency toward lower values was observed in CF children and carnitine supplementation has normalized the carnitine levels in serum [18]. Treem and Stanley concluded that secondary carnitine deficiency in cystic fibrosis may be caused by chronic malnutrition, feeding infants with low-carnitine baby milk, loss of appetite, vomiting due to frequent lower respiratory tract infections, vitamin D deficiency, secondary hyperparathyroidism followed by renal tubule defect with hyperphosphaturia, aminoaciduria and tubule acidosis [19].

Another of carnitine deficiency in cystic fibrosis may be an increased demand for this compound - increased respiratory muscle workload is over 25% higher in CF patients [26]. Carnitine deficiency can be also caused by long-term antibiotic therapy that lead to free carnitine reserve depletion by creating a valproilcarnitine or pivaloylcarnitine which are excreted with urine [27].

In our study we have observed low concentration of free and total carnitine in CF patients. IN 80% of patient free and total carnitine concentration was below lower limit of normal range. This difference was statistically significant ($p<0.001$) (Fig.1). Similar results were observed by Woś et al. [20, 21]. Contrary, Kovesi et al. did not observed decreased concentration of carnitine if serum and urine [22]. In this study conducted in a group of 43 infants, adolescents and adults free carnitine levels was slightly elevated in comparison to controls - this difference was statistically significant. Only in 2 patient (4.5% of studied group) they observed low values of serum free carnitine. No differences were observed in urine acylcarnitine excretion as well. Acyl carnitine/free carnitine ratio was significantly lower in comparison to controls [22]. In our study we did not observe the urine carnitine excretion. In CF patients we observed low free, total ($p<0.001$) (Fig. 3) and acylcarnitine concentration ($p<0.001$) (Fig.4).

Carnitine deficiency is defined as serum free carnitine concentration <20 μmol/l (below this value clinical symptoms may be present) and low total carnitine concentration [28]. High acylcarnitine to free carnitine concentration ratio indicate low free carnitine. High acylcarnitine/free carnitine ratio ≥0.4 according to Suahail [29] or ≥0,6 according to Calvani et al [30] might block the key enzymes in mitochondria. In our studied group in 9 cases (60%) free carnitine concentration was ≤ 20 mmol/l and in 10 cases (66%) acylcarnitine/free carnitine ratio was elevated ≥0,4, but no significant differences were observed in comparison to controls ($p=0.05$) (Fig. 2).

Secondary carnitine deficiency is present in case of kidney diseases. In normal conditions carnitine is excreted into urine as free carnitine and estrified form (mostly acylcarnitine). Acylcarnitine in urine comprise ca. 56%, in serum ca. 22% [30, 31]. In our study, acylcarnitine in urine comprised only 39% of total carnitine. CF-related kidney disease traditionally, the kidney has been thought to be one of the organs not affected by the CF condition, despite the fact that the CFTR protein abnormality is expressed in renal tubule cells. More recently, kidney disease has come to light in CF, not as a primary manifestation of the condition, but as a result of treatment. Some groups of antibiotics are also known to have an acute toxic effect on the kidney, particularly aminoglycosides (such as tobramycin), and also colomycin [32]. Whilst this acute damage is usually reversible, long term use may, over time, cause clinical disease by diminishing the number of functioning nephrons [33]. Treem et al. described high urine carnitine concentration, aminoacyduria and hyper-phosphaturia in CF patient with coexisting renal tubules defect [19]. Loyd-Still et al. showed that in urine of 23 CF-children increased excretion of free

carnitine, total carnitine and acylcarnitine is present [17]. They also concluded that, increased acylcarnitine excretion depends on presence of middchain-long fatty acids in diet. Contrary, in our study significant ($p<0,001$) low concentrations of free carnitine, total carnitine and acylcarnitine in urine in CF patients were observed (Fig.3). In our patients no chronic kidney disease or nephrolithiasis was diagnosed. We think that low carnitine concentration in urine is due to low serum carnitine concentration not due to kidney disease.

Carnitine deficiency in children and adolescents appears to be an important clinical problem, as fatty acid metabolism is essential for some features of cystic fibrosis. Nutrition is one of the keypoints in modern CF therapy, but prevention of impaired absorption of nutrients, including carnitine, should be one of the therapy objectives. Taking into account the results of the presented study, minding the limitations of the small study group, it would be necessary to expand the studied CF population in future projects.

**Conflict of interest:** none declared.

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