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Renal carnitine excretion following abstinence after chronic drinking

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ABSTRACT

Purpose: Carnitine participates in the metabolism of lipids and cognitive activity. Excessive consumption of alcohol disturbs renal tubular canalicules, that increases urinary excretion of carnitine and its esters.

The study evaluates restoration of the urinary free- and total carnitine as well as acylcarnitine excretion after chronic drinking and during the 49-days of controlled abstinence.

Materials/methods: In 32 patients (6♀; 26♂), 26–60 years old, 2–30 years of alcohol dependence: 75–700 g of pure alcohol (166 ± 94 g) of alcohol daily consumption, 2–360 (35 ± 67) days of intoxication and 1.25 ± 0.8 days of abstinence at admission, we determined urinary free (FC) and total carnitine (TC) as well as acylcarnitine (AC) and acylcarnitine/free carnitine ratio (AC/FC) at admission (T0), after 30 (T30) and 49 (T49) days of the controlled abstinence.

Results: At T0 excretion of FC, TC and AC as well as AC/FC ratio were significantly higher as compared to the control group. After 30- and 49-days of abstinence, excretion of FC and TC decreased to the level of control group with an exception of the AC and AC/FC ratio at T30 that remained significantly increased.

Conclusion: 30 days for the FC and TC and 49 days of abstinence for the AC and AC/FC ratio was sufficient to normalize urinary excretion of the carnitines.

1. Introduction

World Health Organization estimates that 140 million people worldwide suffer from the alcoholism. Alcoholic disturbance of the body metabolism [1–3] is increased due to malnutrition that is a common finding in chronic alcoholics [4,5]. Normally, free fatty acids are re-esterified with glycerol to form triacylglycerols or they enter mitochondria for β-oxidation [6]. One of the major consequences of alcohol ingestion is an excessive production of NADH, which reduces glucose synthesis and fatty acids oxidation [7,8]. Alcoholism causes structural and functional damage of many body organs including kidney [9]. Renal injury may be associated with ethanol-induced changes in the membrane composition of renal tubules and lipid peroxidation in epithelial cell membranes [10–12].

Chronic alcohol dependence is associated with an excessive excretion of the L-carnitine and its fatty acid esters into the urine [11,13]. L-carnitine (2-hydroxy-4-trimethylammonium butyrate) is a small hydrophilic molecule synthesized in the liver, brain and kidneys from protein-bound lysine and methionine. L-carnitine participates in the transport of long chain fatty acids from cytoplasm into the mitochondrial matrix for their oxidation. It takes part in the intracellular decomposition and excretion of branched-chain ketoacids, improves cognitive abilities in neuro-degenerative diseases and provides acetyl groups for the acetylcholine synthesis [10,14,15]. Demand for the carnitine is covered by endogenous synthesis (25%) and by diet (75%) [14,16]. Carnitine is not degraded in the human body but it is excreted with urine. Kidney plays a major role in the homeostasis of carnitine [13,17,18]. Proximal renal tubules are the intra-renal site of carnitine acylation and regulators of the blood and/or urinary carnitine acylation [19]. In health, tubular reabsorption of free carnitine, a process facilitated by the active transport of carnitine and its short-chain esters by the OCTN2 transporter on the renal brush border membranes, reaches 96–99% [17,20] and is higher than that for carnitine esters [20,21].

An important question is if and when the renal carnitine excretion recovery is possible after heavy drinking period. Therefore, the aim of this study was to evaluate urinary excretion

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of the free-(FC), total-(TC) and acyl-(AC) carnitine in persons chronically addicted to alcohol before and during controlled abstinence.

2. Material and methods

2.1. Patients

The study group consisted of 32 patients from the Department of Detoxification and Therapy for Alcohol Dependence in Choroszcz Psychiatric Hospital, Poland, 6 women and 26 men, aged from 26 to 60 years (44.6 ± 8.9 years). Alcohol dependence ranged from 2 to 30 years (14.5 ± 7.9 years). Amount of alcohol consumed ranged from 75 to 700 g/day of pure alcohol (166.7 ± 94.3 g/day). Intoxication lasted 3–360 (35.6 ± 67.1) days. At admission, abstinence from alcohol lasted 1.25 ± 0.8 days. Patients with alcoholic liver disease (steatosis, inflammation, fibrosis and cirrhosis) were excluded from the study. During abstinence the patients were hydrated, balanced with electrolytes and glucose for 2.4 ± 0.6 days and treated with the following drugs: diazepam for 8.6 ± 4.6 days, hydroxyzinum for 3.6 ± 2.7 days, haloperidolum for 4.8 ± 2.5 days, carbamazepine for 14.7 ± 5.4 days, promazine for 11.4 ± 10.8 days, tiazolidyncarboxylic acid for 5–36 days and NSAIDs (mostly ketoprofen) for 2–8 days.

The control group consisted of 18 healthy adult volunteers, aged from 22 to 60 years (8 women and 10 men) remaining on the overall diet with an occasional drinking. Prior to collection of the urine samples all persons from the control group did not consume alcohol for at least one week.

2.2. Carnitine determination

In the group of alcoholics urinary FC and TC were determined at admission to hospital (T0), after 30 (T30) and 49 days (T49) of the abstinence. Carnitine excretion was expressed as μmol/g creatinine [18].

Samples of the urine collected during 12-h were centrifuged for 10 min at 2000 × *g* using 5702R Eppendorf AG centrifuge, Hamburg, Germany, and kept at −86 °C until measurements. Urinary free carnitine was determined by enzymatic method of Cederblad et al. [22], which is based on the reaction of free carnitine with acetyl-CoA catalyzed by CAT (carnitine acetyltransferase). Free carnitine reacts with acetyl moiety of acetyl-CoA releasing CoA-SH, which is determined by reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Increase of the absorbance at 412 nm was measured using Hitachi UV/VIS Spectrophotometer – Model U-2900, Tokio, Japan. For the determination of total carnitine, 100 μL of centrifuged urine was added to 10 μL 1 mol/L KOH solution, mixed and incubated at 56 °C for 1 h (for hydrolysis of the carnitine esters) and finally neutralized to pH ∼ 7.0 with 2 μL 5 mol/L HCl, and assayed for the free carnitine as described above. Acylcarnitine (AC) level was calculated by subtracting the concentration of free carnitine from the total carnitine. Acylcarnitine/free carnitine ratio (AC/FC) was calculated as total carnitine minus free carnitine/free carnitine, according to Schmidt-Sommerfeld et al. [23] and Seccombe et al. [24].

Creatinine in the urine was measured by Jaffe's method in modification of Larsen [25]. The absorbance of chromogen was measured at a 450 nm on Creatinine Analyzer 2 (Beckman, Munich, Germany).

2.3. Statistical analysis

The data are expressed as a mean ± SD and were analyzed by the Statistica version 10.0 (Statsoft, Cracow, Poland). Independent groups were compared using Student's *t*-test or Mann–Whitney *U*-test depending on their distribution, that was assessed with Shapiro–Wilk test for normality. Dependent groups were evaluated using Wilcoxon signed-rank test. A p value less than 0.05 was considered to be significant.

3. Results

Urinary excretion of the FC, TC and AC during abstinence after a period of heavy drinking is shown in Fig. 1.

Urinary excretion of the FC in healthy subjects was 88.6 ± 54.1 μmol/g creatinine, TC excretion was 138.1 ± 94.5 μmol/g creatinine, and the excretion of AC was 49.4 ± 48.4 μmol/g creatinine. Fig. 1 shows significantly increased excretion of the FC, TC ($p < 0.05$) and AC ($p < 0.001$) at the first day (T0) of hospitalization as compared to the control group. Abstinence for 30 days (T30) significantly decreased excretion of the FC ($p < 0.001$), TC ($p < 0.001$) and AC ($p < 0.05$) as compared to the carnitine excretion at admission (T0). After 30 days of abstinence, excretion of FC and TC did not differ significantly from excretion in the control group, whereas excretion of AC remained significantly increased ($p < 0.001$). After 49 days of hospitalization (T49), urinary FC, TC and AC levels were not significantly different from those in the T30 and in control group.

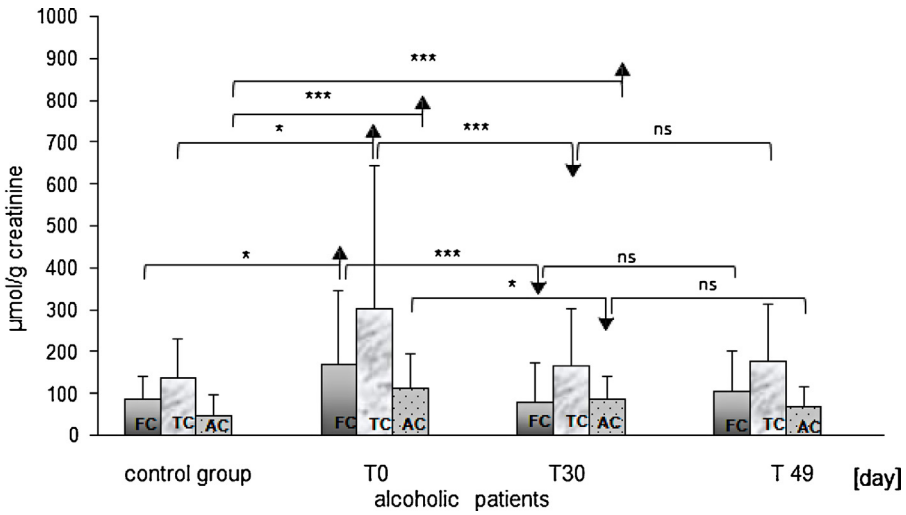

Fig. 1. Urinary excretion of free (FC), total (TC) and acylcarnitine (AC) during abstinence after chronic drinking of the persons dependent on alcohol. Abbreviations: T0 admission day, T30 and T49 days of abstinence; significantly different: * ($p < 0.05$); *** ($p < 0.001$); ns, not significant.

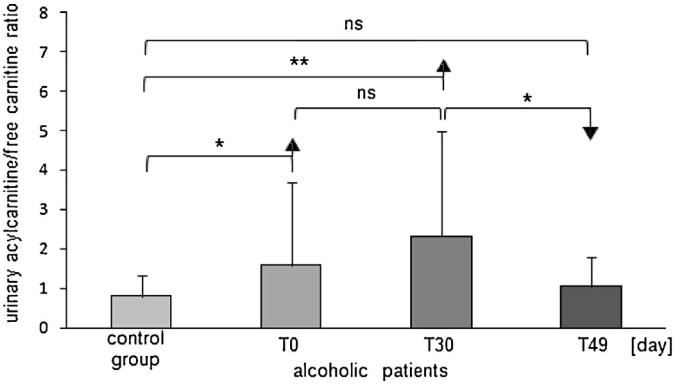

Fig. 2. Urinary acylcarnitine/free carnitine ratio during abstinence after chronic alcohol drinking. Abbreviations: T0 admission day, T30 and T49 days of abstinence; significantly different: * ($p < 0.05$); ** ($p < 0.01$); ns, not significant.

AC/FC ratio during abstinence after prolonged heavy drinking is shown in Fig. 2. In the control group the AC/FC ratio was 0.79 ± 0.55 (Fig. 2). A significantly higher AC/FC ratio at T0 ($p < 0.05$), and T30 ($p < 0.01$), was noted in the urine of alcoholics, as compared to the control group. At T49 the AC/FC ratio was significantly lowered ($p < 0.05$) in comparison to T30 and was near the same like in the control group (Fig. 2). Between T0 and T30 there were no significant differences in the AC/FC ratio.

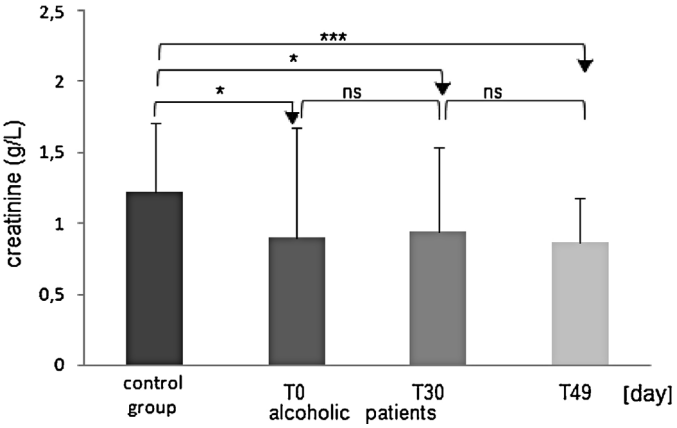

Fig. 3. Urinary excretion of creatinine during abstinence after chronic drinking of the persons dependent on alcohol. Abbreviations: T0 admission day, T30 and T49 days of abstinence; significantly different: * ($p < 0.05$); *** ($p < 0.001$); ns, not significant.

Mean concentrations of the urinary creatinine in the healthy controls was 1.22 ± 0.49 g/L but in our patients it was 0.90 ± 0.77 g/L, 0.94 ± 0.59 g/L and 0.87 ± 0.31 g/L at T0, T30 and T49, respectively.

Urinary excretion of the creatinine after chronic drinking was significantly lower at T0 and T30 ($p < 0.05$) as well as at T49 ($p < 0.001$), as compared to the controls (Fig. 3). There were no significant differences in urinary excretion of creatinine after chronic drinking at T0 vs T30 and T30 vs T49.

4. Discussion

There is consensus that the kidney is the major organ responsible for the homeostasis of carnitine and its esters [13,17]. Excretion of FC and AC in 12 h collected urine in our healthy subjects was 64% and 35%, respectively and it was comparable to that reported by Müller et al. [26]. They have shown that in healthy subjects on a standard diet the FC excreted in 24 h urine constituted 72% of the TC. Other studies on perfused rat kidney indicate that the kidney is involved in the metabolism of propionyl-L-carnitine to L-carnitine and acetyl-L-carnitine [27].

Our research on 12-h collected urine samples from the patients after chronic drinking showed a significant, 2.4 times higher urinary excretion of FC, 2.7 times higher excretion of TC and 2-fold higher concentration of AC at the first day (T0) as compared to the controls (Fig. 1). Our results are comparable with those in the study [28] on rats where one week administration of 20% ethanol at a dose of 2 g/kg body weight, increased urinary excretion of AC about 4.2 times in comparison to controls, whereas the increase of propionylcarnitine and butyrylcarnitine was 2.3 and 1.4 times higher respectively. It has been reported that chronic alcohol abuse damages renal tubules that manifestes by impaired reabsorption of various substances [29]. One third of chronic alcoholics show a complex renal tubular dysfunction [30].

It has been reported that most of the alcohol- derived disregulations of the electrolyte and water homeostasis was spaling after 2–4 weeks of abstinence [29]. However, neuronal and hormonal alterations persisted even for several months longer [31]. Answer to the question if, and eventually when, it is possible to recover renal carnitine metabolism and urinary excretion in alcoholics after heavy drinking period, is positive. We have observed recovery of the FC renal excretion after 30 days (T30) of abstinence (Fig. 1). However, after 30 days of abstinence, excretion of the AC still remained significantly increased ($p < 0.001$) in comparison to the control group. It was 1.5 times higher than in the control group. At the 49 day (T49) of abstinence the FC, TC and AC excretion did not differ significantly as compared to the control group ($p = 0.05$) (Fig. 1), that suggests substantial recovery of the renal AC metabolism.

Persistently increased excretion of the AC at T30 vs control group (Fig. 1) during abstinence may be caused by increased renal carnitine acylation in the recovering renal tubules, or by medications of our patients that could impair metabolism of carnitine. It has been reported that the L-carnitine reacts with the acyl groups of certain compounds, like xenobiotics, such as ampicillin, valproic and salicylic acids that are cleared from the body into the urine in a form of acyl-L-carnitine [32]. It was reported that pivalate-conjugated antibiotics create compounds with carnitine (pivaloyl-carnitine) that are excreted at 10 times higher amounts than cartitine derived from the diet leading to the lost of carnitine from the body [33]. Experiments on administration of therapeutic doses of valproic acid to mice reveal reduced reabsorption of acylcarnitine, and carbamazepine, phenytoin, whereas phenobarbital reduced the reabsorption of free carnitine [34].

Significant urinary decrease of the FC ($p < 0.001$), TC ($p < 0.001$) and AC ($p < 0.05$) excretion after 30 days of abstinence in comparison to the urinary excretion at admission (Fig. 1), is an argument against nephrotoxic action of applied drugs, and may be an indicator of improvement of renal function during controlled abstinence. Also, lack of significant changes in urinary excretion of FC and TC at T30 versus T49 may support opinion assuming restoration of the renal function during controlled abstinence.

There were no significant changes of the urinary AC/FC ratios between T0 vs T30 and T49 vs control group (Fig. 2). Urinary AC/FC ratio significantly increased at T0 ($p < 0.05$) and T30 ($p < 0.01$) as compared to controls, and significantly decreased at T49 ($p < 0.05$) in comparison to T30. Urinary acylcarnitine/free carnitine ratio reflects balance of the AC and FC and it is a very sensitive indicator of mitochondrial carnitine metabolism. Increase of the AC/FC ratio indicates on accumulation of AC and/or on decreased amount of FC, and reflects an increase of acetylcarnitine production. Decrease of the AC/FC ratio in our patients during abstinence suggests restoration of carnitine metabolism to normal values after 49 days of hospitalization. Our results show that excretion of carnitine at

T30 and T49 was similar to that in the control group (Fig. 1), where creatinine excretion was significantly lowered (Fig. 3). Faster normalization of carnitine than creatinine excretion during abstinence after drinking may be good marker of restoration of health in persons addicted to alcohol.

Alcoholism itself and treatment of nephrotoxic alterations caused by alcohol during abstinence may be connected with a use of medications that increase urinary excretion of carnitine and its esters. Therefore administration of carnitine during alcohol abuse and abstinency should be considered as preventive mean, in order to avoid complications concerning multiple organ such as kidney, liver and brain as carnitine supplementation is safe [14] and not expensive. In alcoholics we propose research on the administration of carnitine containing nutraceutical formulation e.g. (NF: folate, α-tocopherol, vitamin B_{12}, S-adenosylmethionine, N-acetylcysteine, acetylcarnitine), since a recent randomized trail demonstrated that nutraceutical formulations with carnitine significantly improved cognition and mood in Alzheimer's Disease, neurodegenerative disorders [34] and community dwelling adults without dementia [35]. It was reported that acetylcarnitine protects disturbed redox state in the alcohol treated rats [35].

5. Conclusions

In conclusion, carnitine concentration in urine may be useful for evaluating of the renal tubular function in patients with chronic alcohol abuse. In addition to detection of alcohol abuse, urinary carnitine and acylcarnitine can also be useful for monitoring restoration of carnitine metabolism and alcohol abstinence. Our data indicate that the increased urinary excretion of carnitine and its esters induced by chronic alcohol consumption is reversible within 49 days of abstinance. Despite numerous limitations of our study, it seems that our results provide encouragement for further systematic studies, including, nutraceuticals containing carnitine in treatment of damages caused by chronic alcohol consumption.

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Conflict of interests

The authors declare no conflict of interests.

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