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Comparative effects of diet supplementation with *l*-carnitine and *dl*-carnitine on ammonia toxicity and hepatic metabolism in rats[1]

Vilma A F G GAZOLA (*State University of Maringá, Maringá, PR, 87020-900, Brazil*); Gisele LOPES (*CNPq Fellowship*); Rose M M DIAS (*Undergraduate student*); Rui CURI (*University of São Paulo, São Paulo, SP, 05508-900, Brazil*); Roberto B BAZOTTE[2] (*Department of Pharmacy and Pharmacology, State University of Maringá, Maringá, PR, 87020-900, Brazil*)

KEY WORDS carnitine; liver; metabolism; gluconeogenesis; ammonia

ABSTRACT

AIM: To compare the effects of chronic supplementation with *l*-carnitine (LCT) and *dl*-carnitine (DLC) on ammonia toxicity and hepatic metabolism. **METHODS**: Three groups of male adult rats were studied: 1) supplemented with LCT (1.2 mmol·kg^{-1}·d^{-1}), 2) supplemented with DLC (1.2 mmol·kg^{-1}·d^{-1}), and 3) control group (COG) not supplemented. **RESULTS**: The treatment with LCT decreased the toxicity to ammonia. However, the supplementation with DLC did not show any significant effect. In contrast, the effects of the supplementation with LCT and DLC on hepatic metabolism were quite similar, ie, both groups showed: (a) intensified ammonia uptake and decreased urea production from ammonia; (b) increased glucose and urea production from *L*-glutamine (5 mmol/L). **CONCLUSION**: The results suggested that LCT supplementation might protect against ammonia toxicity by extra-hepatic mechanisms.

INTRODUCTION

There is a large and accumulating body of evidence indicating that orally administered *l*-carnitine (LCT) have beneficial effects in patients with *l*-carnitine deficiency, renal disorders requiring hemodialysis and certain types of hypoglycemia. Furthermore, experimental evidence in animal models indicates that *l*-carnitine prevents hyperlipidemia[1], ethanol-induced fatty liver[2], hepatotoxicity[3], hepatic steatosis[4], and ammonia intoxication[5].

However, there are only few studies comparing the effects of *l*- and *dl*-isomers. For example, *l*-carnitine increases and *dl*-carnitine decreases exercise tolerance in patients with impaired exercise tolerance[6]. In contrast, favorable results employing *dl*-carnitine (DLC) were found during the treatment of infant hypoglycaemia and diphtheria[7, 8].

The comparative effects of both carnitine isomers on the liver capacity and ammonia detoxification, however, still remains to be investigated. In fact, it is well known that *l*-carnitine reduces ammonia toxicity and increases urea production[9-12] but there is no report regarding the effect of *dl*-carnitine.

Thus, the present study was carried out to compare the effects of a chronic supplementation of *l*-carnitine and a racemic mixture of *l*- and *d*-carnitine on ammonia toxicity and liver metabolism.

MATERIALS AND METHODS

Animals and experimental procedure Wistar rats (*Rattus norvegicus*) weighing ♂ 180-220 g, were maintained in individual cages under controlled environmental conditions (temperature 23 ℃ ± 2 ℃, 12 h light-dark cycle). The animals had free access to water and standard laboratory diet (Nuvital®). LCT and DLC were dissolved in the drinking water. The rats were divided into 3 groups. The control (COG) group received water with no additions. The experimental groups received water containing LCT (1.2 mmol·kg^{-1}·d^{-1}) or DLC (1.2 mmol·kg^{-1}·d^{-1}) during 7 d. The ingestion of water and food was also evaluated during the treatment. The dose employed was equivalent to that given as diet supplementation of LCT and DLC in humans[8,13].

Induction of ammonia intoxication Acute in-

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[2] Correspondence to Roberto B BAZOTTE. Phn 55-44-261-4522. Fax 55-44-263-6231. E-mail rbbazotte@uem.br
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toxication to ammonia was induced by ip injection of ammonium acetate aqueous solution (14 mmol · kg^{-1}). The time elapsed to the occurrence of the first convulsion, and the percentage of rats with convulsions and the percentage of deaths were determined.

Liver perfusion The experiments were performed at 8:00 am. Livers from 24 h-fasted rats were perfused *in situ* as previously described[14]. On the day of the experiment, the rats were anaesthetized by ip injection of pentobarbital sodium (35 mg·kg^{-1}). After laparotomy, blood was collected from vena cava and the separated serum was used for determination of free *l*-carnitine[15] and glucose[16]. The livers were perfused using Krebs Henseleit bicarbonate buffer (KHB), pH 7.4, saturated with a mixture of O_2-CO_2 (95 % – 5 %). The perfusion fluid was pumped through a temperature controlled (37 ℃) membrane oxygenator prior to entering the liver via portal vein. The perfusion was performed in an open system with no recirculation of the perfusate. A constant flow rate in each individual experiment was adjusted according to the liver weight (4 mL · g^{-1} of tissue fresh weight per min).

Determination of ammonia uptake and urea production from NH_4Cl in isolated perfused liver The ammonia uptake was measured by the difference between the concentration of ammonia in the KHB and the corresponding concentration in the effluent perfusate during the period of NH_4Cl infusion. Urea[17] production was estimated in the KHB by the difference between the concentration of urea after and before NH_4Cl infusion.

Urea was determined with the aid of urease. The ammonia resulting from enzymatic hydrolysis of urea reacts with phenol and hypochlorite to give a blue dye indophenol, which is proportional to urea concentration and can be measured at 600 nm.

Determination of urea and glucose production from *L*-glutamine in isolated perfused liver After a pre-perfusion of the liver with KHB for 10 min, *L*-glutamine (5 mmol/L) was infused for 30 – 50 min followed by a period of post-infusion (10 – 20 min) to allow the return to the basal levels. Samples of the effluent perfusion fluid were collected at 2-min intervals and analyzed for glucose[16] and urea[17] content. All results were expressed as μmol · min^{-1} · g^{-1} tissue fresh weight. Thus, urea and glucose production was measured as the difference between rates of urea and glucose released after and before *L*-glutamine infusion.

Determination of urea and glucose production from *L*-glutamine in isolated hepatocytes Liver perfusion was employed to isolate hepatocytes as described above. The technique described by Berry & Friend[18] was used with some modifications[19]. Hepatocytes (2×10^9/L) with viability between 95 % – 100 % were incubated (KHB saturated with 95 % – 5 % : O_2-CO_2 mixture, at 37 ℃, under constant agitation) for one hour in the absence (control) or presence of *L*-glutamine 5 mmol/L. After this period, the samples were centrifuged and the soluble fraction collected for determination of glucose[16] and urea[17]. The difference between the concentration of urea and glucose in the control flasks and the flasks incubated with *L*-glutamine represented the amount of production.

Statistical analysis The results, including the areas under the curves (AUC), were analyzed by ANOVA or unpaired *t*-test, using the Graph-Pad Prism-version 2.0 program. The results are presented as $\bar{x} \pm s$. A 95 % level of confidence ($P < 0.05$) was accepted for all comparisons.

RESULTS

The treatment with *l*-carnitine or *dl*-carnitine did not affect glycemia, food ingestion, and water consumption (data not shown).

The efficacy of the supplementation imposed to the rats was verified by the increased ($P < 0.05$) blood free *l*-carnitine showed by LCT and DLC group when compared with COG group (Tab 1).

Tab 1. Blood concentration of free carnitine (μmol/L) after 1 week of supplementation with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT), *dl*-carnitine (DLC), and control group (COG). $n = 5$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* COG group.

	COG	LCT	DLC
Free carnitine	17.5 ± 1.1	23.4 ± 2.3^b	21.8 ± 3.0^b

As shown by Tab 2, the LCT group presented lower degree of convulsions and death than COG group after ip injection of ammonium acetate (14 mmol·kg^{-1}). It is noteworthy that the *dl*-carnitine supplementation did not show protection against ammonia toxicity.

The effects of supplementation with *l*- and *dl*-carnitine on the liver capacity to uptake ammonia and produce urea were investigated. The ammonia uptake at NH_4Cl 0.25 mmol/L (Fig 1A) was similar in all groups. Differently, the LCT and DLC groups showed higher

Tab 2. Toxicity to ammonium acetate in 24 h-fasted rats. The rats were supplemented during 7 d with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT), *dl*-carnitine (DLC) or not supplemented (COG). The observations were performed during 90 min after ip injection of ammonium acetate (14 mmol·kg^{-1}). $^bP < 0.05$ *vs* the COG group. Observation: the time elapsed for the first convulsion is not the mean for all group, but represents the time when the first rat of each group showed the first convulsion.

	n	Time (s) elapsed for the first convulsion	Percentage of rats with convulsions	Percentage of deaths
COG	8	420	100 %	63 %
LCT	9	450	67 %^{b}	45 %^{b}
DLC	8	450	100 %	75 %

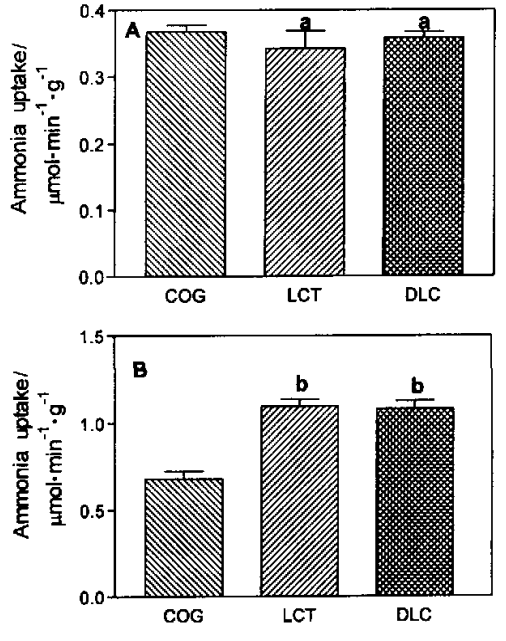

Fig 1. Ammonia uptake at NH_4Cl 0.25 mmol/L (A) and 1.0 mmol/L (B) in isolated perfused livers from 24 h-fasted rats supplemented during 1 week with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT) or *dl*-carnitine (DLC) and control group (COG). $n = 7$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* COG group.

($P < 0.05$) ammonia uptake at NH_4Cl 1 mmol/L than the COG group (Fig 1B). On the other hand, the LCT and DLC groups showed lower ($P < 0.05$) urea production in both ammonia concentrations (Fig 2A and 2B).

Urea and glucose production from *L*-glutamine (30 min of infusion) was slightly raised in both groups

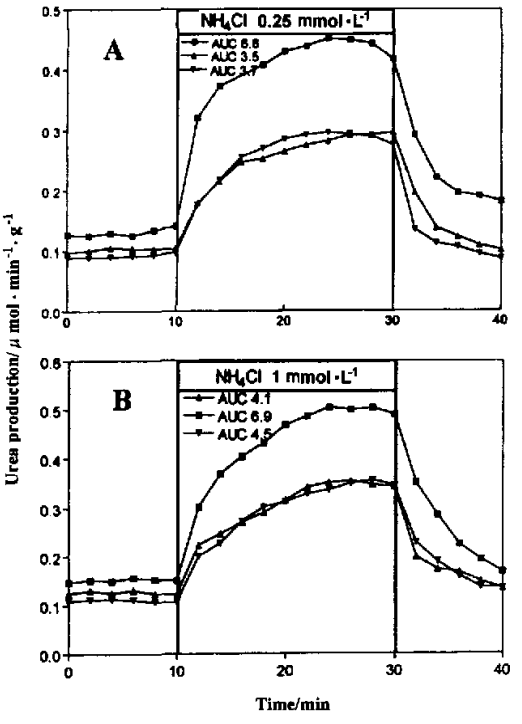

Fig 2. Urea production from NH_4Cl at 0.25 mmol/L (A) and 1.0 mmol/L (B) in isolated perfused livers from 24 h-fasted rats supplemented during 1 week with 1.2 mmol/L kg^{-1}·d^{-1} of *l*-carnitine (LCT) or *dl*-carnitine (DLC) and control group (COG). $n = 7$. AUC = areas under the curves.

supplemented with carnitine (Fig 3A and 3C). Similar results were obtained when the dose of *l*- and *dl*-carnitine supplemented was increased from 1.2 to 2.4 mmol·kg^{-1}·d^{-1} (data not shown). However, when the period of *L*-glutamine infusion was prolonged to 50 min, the LCT and DLC groups showed higher ($P < 0.05$) urea and glucose production (Fig 3B and 3D). Similar findings were obtained in incubated hepatocytes isolated from 24 h-fasted rats, ie, the LCT and DLC groups presented higher ($P < 0.05$) urea production from *L*-glutamine than COG group. However, there was only a slight increase in glucose production due to carnitine supplementation (Tab 3).

DISCUSSION

As we expected, LCT and DLC supplementation increased ($P < 0.05$) blood free *l*-carnitine levels (Tab 1). The treatment with LCT or DLC (1.2 mmol·kg^{-1}·d^{-1})

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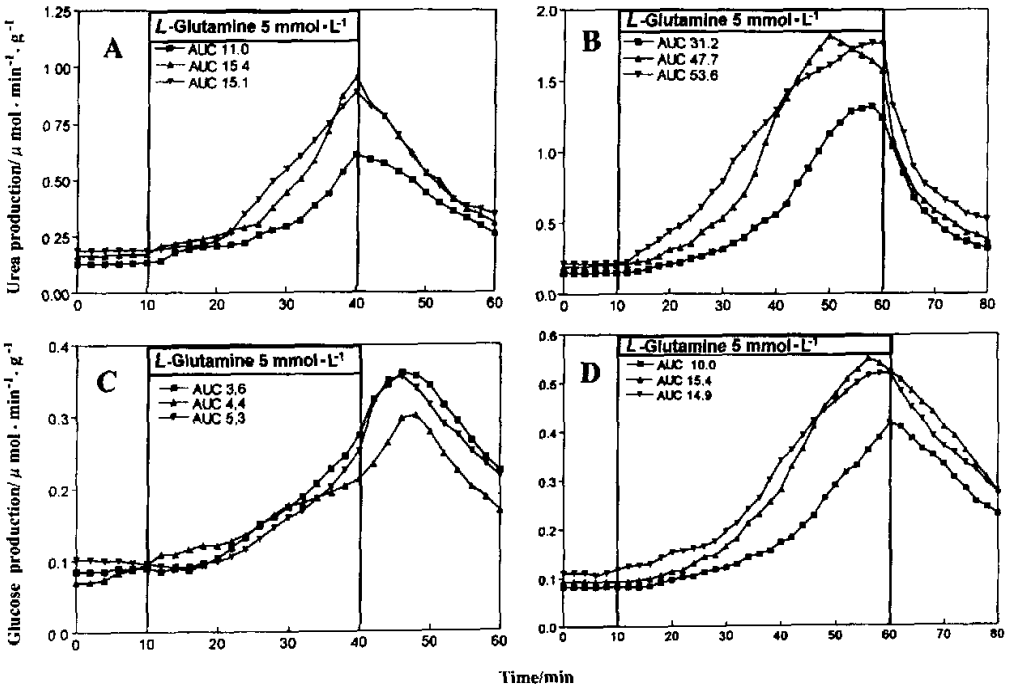

Fig 3. Urea and glucose production from *L*-glutamine in isolated perfused livers from 24 h-fasted rats supplemented during 1 week with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT) or *dl*-carnitine (DLC) and control group (COG). $n = 7$. AUC = areas under the curves. Left side: 30 minof *L*-glutamine infusion. Right side: 50 min of *L*-glutamine infusion.

Tab 3. Urea and glucose production (μmol·10^{-6} cells·h^{-1}) from hepatocytes incubated in the absence (A) or presence (P) of *L*-glutamine (5 mmol/L). The cells were isolated from livers of 24 h-fasted rats supplemented during 7 d with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT), *dl*-carnitine (DLC) or not supplemented (Control). $n = 5$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* the control group (COG).

	COG	LCT	DLC
Urea			
(A)	0.051 ± 0.005	0.043 ± 0.006	0.041 ± 0.006
(P)	0.126 ± 0.009	0.227 ± 0.037^b	0.210 ± 0.005^b
Glucose			
(A)	0.061 ± 0.005	0.044 ± 0.005	0.051 ± 0.006
(P)	0.229 ± 0.023	0.282 ± 0.030	0.262 ± 0.024

for 1 week was enough to get the maximal levels of *l*-carnitine, since the supplementation with LCT or DLC (2.4 mmol·kg^{-1}·d^{-1}) for 1 or 4 weeks did not increase the values showed by Tab 1 (data not shown).

The higher ammonia uptake (Fig 1B) and lower urea production (Fig 2B) showed by livers from LCT and DLC supplemented rats probably is consequence of the increased amount of ketoacids disposed for ammination to amino acids[5].

In contrast to ammonia (Fig 1B), livers from LC and DLC groups showed increased urea production from *L*-glutamine (Fig 3B). These results were obtained not only in isolated livers (Fig 3B), but also in isolated hepatocytes (Tab 3). Thus, it is possible that carnitine treatment may enhance hepatic glutaminase activity. Moreover, the stimulated catabolism of *L*-glutamine could provide the liver with sufficient skeleton of carbons to assure increased glucose production (Fig 3D). However, the results for glucose production were not totally confirmed in isolated hepatocytes, since there was no statistical significant difference between carnitine supplemented (LCT, DLC groups) and COG group (Tab 3). These findings are apparently contradictory, but it is important to point out that the hepatocytes were submitted to lower degree of oxygenation during the incubation period if compared with perfused livers. Thus, it could be argued that the differences in the results obtained from liv-

ers (Fig 3) and isolated hepatocytes (Tab 3) can be explained by the fact that incubated hepatocytes show less capatibility to respond to glucose precursors.

As previously demonstrated[9,11,12,20] the treatment with LCT reduced the toxicity to ammonia (Tab 2). But, in contrast, DLC supplementation did not give protection against ammonia toxicity (Tab 2). Since DLC and LCT groups showed similar alterations on hepatic metabolism of ammonia but only LCT group was able to reduce ammonia toxicity, we can suggest that extra-hepatic mechanisms might be involved. This proposition is supported by the demonstrated protective effect of LCT on precipitated encephalopathy in rats with portocaval shunt[22]. In addition, it has been shown that the protecting effect of LCT against ammonia involves increased ammination of glutamate to glutamine in the muscle[5].

Thus, our findings showed that LCT supplementation protects against ammonia toxicity by mechanisms not involving increased ammonia uptake and hepatic ureogenesis. Moreover, in view of a significant difference in the biological effects of both isomers, we recommended that LCT supplementation could not be replaced by DLC.

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比较饮食摄取 *l*-卡尼汀和 *dl*-卡尼汀对氨中毒和肝代谢的作用[1]

Vilma A F G GAZOLA (*State University of Maringá, Maringá, PR, 87020-900, Brazil*); Gisele LOPES (*CNPq Fellowship*); Rose M M DIAS (*Undergraduate student*); Rui CURI (*University of São Paulo, São Paulo, SP, 05508-900, Brazil*); Roberto B BAZOTTE[2] (*State University of Maringá, Maringá, PR, 87020-900, Brazil*)

关键词 卡尼汀; 肝; 代谢; 糖原异生; 氨

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