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Effect of diet supplementation with *l*-carnitine on hepatic catabolism of *l*-alanine in rats[1]

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Vilma A F G GAZOLA, Gisele LOPES, Daniel M LIMEIRA, Ricardo GALLETTO, Sebastião GAZOLA, Rui CURI[2], Roberto B BAZOTTE[3] (*State University of Maringá, SUM, Department of Pharmacy and Pharmacology, Maringá, PR, 87020-900*; [2]*University of São Paulo, São Paulo, SP, 05508-900, Brazil*)

KEY WORDS carnitine; liver; metabolism; gluconeogenesis; alanine

ABSTRACT

AIM: To investigate the effects of chronic supplementation with *l*-carnitine (LCT) on hepatic catabolism of *l*-alanine. **METHODS**: Two groups of male adult Wistar rats were used: 1) supplemented with LCT (1.2 mmol·kg^{-1}·d^{-1}) dissolved in the drinking water (LCT group) and 2) control group (COG) without LCT supplementation. After one week of LCT supplementation livers from 24 h-fasted rats were perfused *in situ* and the production of glucose, urea, pyruvate, and *l*-lactate from *l*-alanine (5 mmol/L) were measured. **RESULTS**: LCT decreased the production of glucose and urea from *l*-alanine. In agreement, pyruvate and *l*-lactate production from *l*-alanine were decreased. However, the supplementation with LCT did not show any significant effect on hepatic glucose production from pyruvate (5 mmol/L) and *l*-lactate (2 mmol/L). **CONCLUSION**: LCT supplementation decreased the conversion of *l*-alanine to pyruvate. However the ability of the liver to convert pyruvate to glucose was not affected by LCT treatment.

INTRODUCTION

It is well known that *l*-carnitine (LCT) supplementation, orally administered, has valuable effects in patients with carnitine deficiency caused by genetic lesions[1,2], hemodialysis treatment[3], and certain types of hypoglycemia[4]. Additionally, increasing evidence suggests that the administration of LCT may reverse some of the metabolic changes following myocardial ischaemia[5].

On the other hand, carnitine treatment with no scientific validation to the claims has been recommended to people without carnitine deficiency. The recommendations are various such as increasing athletic performance and reduction of body weight. In Brazil, it is quite clear that the use of carnitine as nutritional supplement has increased very much in the last few years. However, scientific evidence supporting these effects are absent[6,7].

Since the liver is the organ, which received the highest concentration of LCT during oral administration, we investigated the effect of LCT treatment on hepatic catabolism of amino acids. In this context, our previous investigation[8] showed that LCT supplementation increased glucose and urea production from *l*-glutamine. Knowing that the hepatic metabolism of *l*-glutamine was affected by LCT treatment we expanded the investigation to *l*-alanine, the major gluconeogenic amino acid in mammalians.

MATERIALS AND METHODS

Animals and LCT supplementation ♂ Wistar rats (*Rattus norvegicus*), weighing 180 – 220 g, receiving food (Nuvital® commercial chow) and water *ad libitum* were employed. The animals were divided into 2 groups. The control group (COG) received water with no additions (n = 34). The experimental groups received LCT dissolved in the drinking water (1.2 mmol·kg^{-1}·d^{-1}) during 7 d (n = 34). As we previously demonstrated this amount of LCT and period of supplementation was enough to get the maximal blood levels of LCT without effect on food and water ingestion[8].

Liver perfusion experiments The rats were anaesthetised with pentobarbital (35 mg/kg) and

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[3] Correspondence to Roberto B BAZOTTE. Phn 55-44-261-4522. Fax 55-44-263-6231. E-mail rbbazotte@uem.br
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submitted to laparotomy. Afterwards, blood was collected from cava vein for determination of LCT[9]. The livers were perfused *in situ* using Krebs/Henseleit-bicarbonate buffer (KHB), pH 7.4, saturated with an O_2/CO_2 mixture (95 %:5 %). The perfusion fluid was pumped through a temperature regulated (37 ℃) membrane oxygenator prior to entering the liver via a cannula inserted into the portal vein. A constant flow rate in each individual experiment was adjusted according to the liver weight.

Experimental approach Saturating concentration of *l*-alanine (5 mmol/L) dissolved into the perfusion fluid between the 10th and the 30th min of liver perfusion was employed. In part of the experiments saturating concentration of *l*-lactate (2 mmol/L) and pyruvate (5 mmol/L) were employed. Employing saturating concentration of each substance above described it possible to measure the maximal capacity of the liver to produce glucose and/or urea.

During the liver perfusion period (40 min), samples of the perfusate were collected every 2 min and used for the determination of glucose[10] and urea[11]. In part of the experiments *l*-lactate[12] and pyruvate[13] were measured.

Our research design, summarized in Fig 1, demonstrated that livers from fasted rats showed low rate of glucose, urea, *l*-lactate, and pyruvate production before the infusion of the substrate. All biochemical parameters were measured as the difference between metabolic rates during (10 – 30 min) and before (0 – 10 min) the substrate infusion. The difference was obtained by the calculation of the area under curve (AUC). Thus, all data showed in the results (Fig 2 – 4) were obtained from experiments similar to that described in Fig 1.

Statistical analysis The AUC was calculated with the help of Prism software. $P < 0.05$ was the accepted level of significance. The results in the text are presented as $\bar{x} \pm s$. A 95 % level of confidence ($P < 0.05$) was accepted for all comparisons.

RESULTS

LCT supplementation (1.2 mmol · kg^{-1} · d^{-1}) during 1 week was confirmed by the increased ($P < 0.05$) blood levels of free carnitine showed by LCT group (23.4 ± 2.3) μmol/L *vs* COG group (17.5 ± 1.1) μmol/L.

In the first set of experiments, the effect of supplementation with LCT on the liver capacity to produce glucose and urea from *l*-alanine were investigated (Fig 2). If we discount the basal rates of glucose production and determined the AUC during the infusion of *l*-alanine, the rates of glucose production showed by LCT group (Fig 2A) was slight lower (4.1 ± 0.6) μmol/g than COG group (4.5 ± 1.0) μmol/g. In conformity with these results, urea production from *l*-alanine (Fig 2B) was decreased ($P < 0.05$). The values of AUC for LCT and COG rats were respectively (8.8 ± 1.0) and (12.2 ± 1.0) μmol/g.

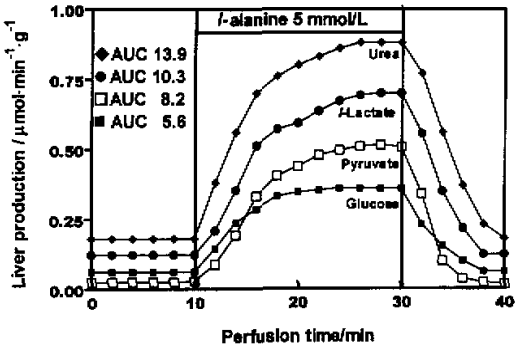

Fig 1. Demonstrative experiment of urea, *l*-lactate, pyruvate, and glucose production from *l*-alanine in perfused livers from 24 h-fasting rats. The effluent perfusate was sampled in 2 min intervals and analyzed for urea, *l*-lactate, pyruvate, and glucose. AUC = areas under the curves (μmol/g).

In the second set of experiments the effect of LCT supplementation on hepatic production of pyruvate and *l*-lactate from *l*-alanine were measured (Fig 3). As showed by Fig 3A, LCT group showed lower ($P < 0.05$) pyruvate production during *l*-alanine infusion. The values of AUC for LCT and COG rats were respectively (6.2 ± 0.3) and (7.9 ± 0.5) μmol/g. Similar results were obtained to *l*-lactate (Fig 3B), ie, LCT group showed lower ($P < 0.05$) *l*-lactate production during *l*-alanine infusion. The values of AUC for LCT and COG rats were respectively (7.2 ± 1.3) and (10.5 ± 0.4) μmol/g.

In the third set of experiments the effects of LCT supplementation on glucose production from pyruvate and *l*-lactate were measured (Fig 4). Livers from LCT and COG rats showed basically the same hepatic glucose production during the infusion of pyruvate (Fig 4A). The values of AUC for LCT and COG rats were respectively (10.5 ± 1.1) and (10.9 ± 0.9) μmol/g. On the other hand, the rates of glucose production from

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E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

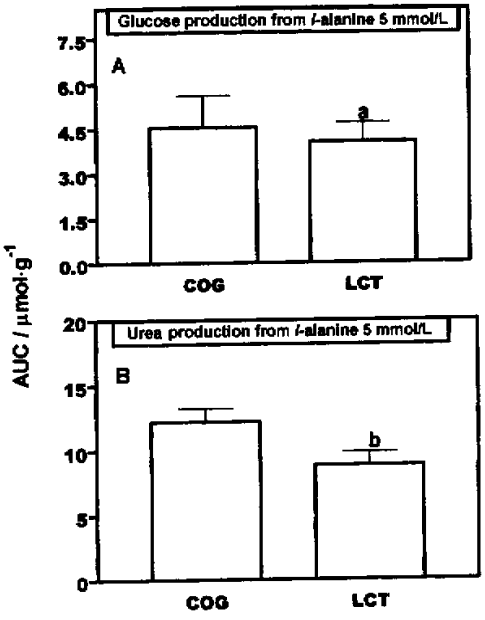

Fig 2. Glucose (A) and urea (B) production from *l*-alanine in perfused livers from 24 h-fasting rats supplemented during 1 week with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT) and not supplemented (COG). $n=7$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* COG group.

l-lactate showed by LCT group (Fig 4B) were slight higher (9.6±1.2) μmol/g than COG group (7.4±1.3) μmol/g.

DISCUSSION

Hepatic catabolism of *l*-alanine can be affected by several factors depending of the experimental condition. As we previously demonstrated the hepatic catabolism of *l*-alanine was influenced by diabetes[14] and hypoglycemia[15], but no effect was found for meal feeding schedule[16] and exercise[17].

Since LCT treatment without scientific corroboration has been used to make thin or increase execise performance and considering that the liver receives high amount of LCT after oral ingestion, we studied the effect of LCT treatment on liver catabolism of *l*-alanine.

In contrast to *l*-glutamine[8], hepatic urea (Fig 2B) production from *l*-alanine was decreased in livers from LCT supplemented rats. These effects were consequence of chronic supplementation of LCT since the infusion of LCT at physiological (30 μmol/L) and pharmacological (60 μmol/L) levels did not affected urea production from

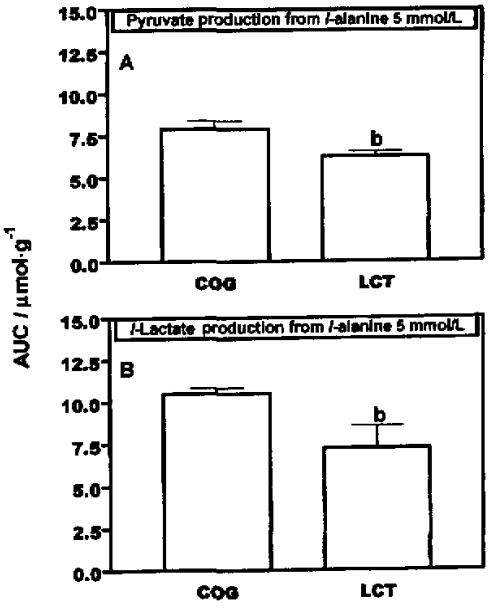

Fig 3. Pyruvate (A) and *l*-lactate (B) production from *l*-alanine in perfused livers from 24 h-fasting rats supplemented during 1 week with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT) and not supplemented (COG). $n=6$. $\bar{x}\pm s$. $^bP<0.05$ *vs* COG group.

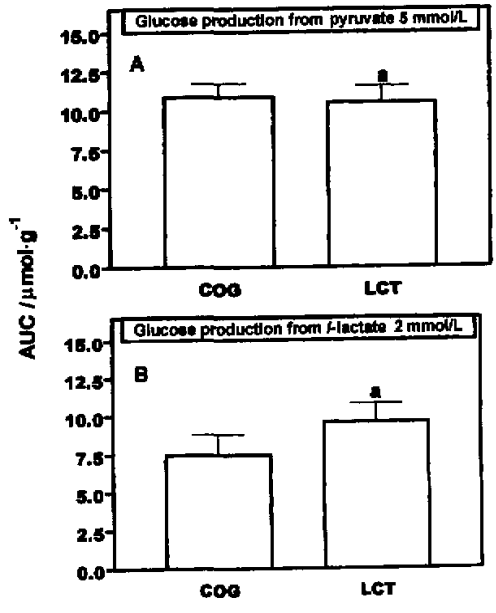

Fig 4. Glucose production from pyruvate (A) and *l*-lactate (B) in perfused livers from 24 h-fasting rats supplemented during 1 week with 1.2 mmol·kg^{-1}·d^{-1} of *l*-carnitine (LCT) and not supplemented (COG). $n=4$. $\bar{x}\pm s$. $^aP>0.05$ *vs* COG group.

l-alanine 5 mmol/L (results not shown).

In view of the fact that livers from LCT rats exhibit decreased gluconeogenesis and ureagenesis from *l*-alanine (Fig 2), we considered the possibility of a decreased catabolism of *l*-alanine induced by LCT supplementation. Thus, hepatic production of pyruvate and *l*-lactate from *l*-alanine was measured. As shown by Fig 3, LCT group showed lower ($P < 0.05$) pyruvate (Fig 3A) and *l*-lactate (Fig 3B) production during the infusion of *l*-alanine.

Since glucose production from pyruvate (Fig 4A) and *l*-lactate (Fig 4B) were similar for both groups, we can propose that the gluconeogenic capacity of the liver was not affected by LCT treatment. Thus, the decreased hepatic glucose production from *l*-alanine can be attributed to the lower catabolism of this amino acid, inferred by urea production. Taken together, our results from *l*-alanine and *l*-glutamine[8] suggest that the hepatic catabolism after LCT supplementation can be different to each amino acid. Moreover, in view of the significant effects on liver metabolism, LCT supplementation in the absence of LCT deficiency cannot be recommended.

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食物中添加 *l*-卡尼汀对大鼠肝代谢 *l*-丙氨酸的作用[1]

关键词 卡尼汀; 肝; 代谢; 糖原异生; 丙氨酸

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