Original Articles

Comparative acute effects of *l*-carnitine and *dl*-carnitine on hepatic catabolism of *l*-alanine and *l*-glutamine in rats[1]

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ABSTRACT

AIM: To compare the acute effects of *l*-carnitine (LCT) and *dl*-carnitine (DLC) on hepatic catabolism of *l*-alanine and *l*-glutamine in rats. **METHODS**: Livers from 24 h fasted and fed rats were perfused *in situ*. The substrates *l*-alanine (5 mmol/L) and *l*-glutamine (5 mmol/L) were employed. The gluconeogenic and ureogenic activity was measured as the difference between the rates of glucose and urea released during and before the infusion of *l*-glutamine or *l*-alanine. **RESULTS**: LCT (60 μmol/L) but not DLC (60 μmol/L and 120 μmol/L) increased the production of glucose and urea from *l*-glutamine. However, neither LCT (60 μmol/L and 120 μmol/L) nor DLC (60 μmol/L and 240 μmol/L) showed any significant effect on hepatic glucose and urea production from *l*-alanine. **CONCLUSION**: The results showed a different acute effect of LCT and DLC on the activation of hepatic gluconeogenesis and ureagenesis promoted by *l*-glutamine, reinforcing the idea that DLC could not replace LCT.

INTRODUCTION

It is quite clear that *l*-carnitine (LCT), orally administered has valuable effects in patients with carnitine deficiency caused by hemodialysis[1], genetic lesions[2,3], and certain types of hypoglycemia[4]. Furthermore, lipid-lowering properties has been described[5].

In Brazil, capsules containing a racemic mixture of *l*- and *d*-carnitine, ie, *dl*-carnitine (DLC), which is cheaper than LCT, is highly commercialized in pharmacies, stores and athletic academies, as a nutritional supplement. However, there is no scientific evidence supporting that DLC could show the same biological effects as LCT. On the contrary, the *D*-isomer competitively inhibits the carnitine acetyltransferase activity and consequently decreases the burning of fatty acids as well the mitochondrial transport of long chain fatty acids[3]. In agreement with these facts, LCT increases and DLC decreases exercise performance in patients with impaired exercise tolerance[6].

Since the liver receives high amount of carnitine after oral ingestion, we compared the effects on hepatic metabolism of the treatment during 1 week with LCT or DLC (1.2 mmol·kg^{-1}·d^{-1}). We observed[7,8] that the effects of LCT and DLC on hepatic metabolism of *l*-glutamine and *l*-alanine were different. However, these studies were performed using livers from rats chronically supplemented with LCT or DLC. Consequently, the possibility of a direct effect of LCT and DLC must be considered. Therefore, this study was performed to

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compare the acute effect of LCT and DLC on the liver metabolism of *l*-glutamine and *l*-alanine.

MATERIALS AND METHODS

Animals Male Wistar rats (*Rattus norvegicus*) weighing 180-220 g were employed. The manipulation of the animals followed the Brazilian Law on the protection of animals. Hepatic gluconeogenesis from fasting rats and hepatic ureagenesis from fed and fasting rats were investigated.

Liver perfusion technique The rats were anaesthetized by ip injection of pentobarbital sodium (35 mg/kg) and submitted to laparotomy. The liver was perfused *in situ*[9,10] 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 ºC) 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 experiment was adjusted according to the liver weight (4 mL/g of tissue fresh weight per min).

Research design To evaluate the acute effect of LCT and DLC on hepatic production of glucose and urea from *l*-alanine or l-glutamine, each amino acid was infused in the absence (control group) or presence of LCT (LCT group) or DLC (DLC group), followed by a post-infusion period to allow the return to basal levels. Samples of the effluent perfusion fluid were collected and analyzed for glucose and urea. Thus, gluconeogenesis and ureogenesis were measured as the difference between the production of glucose and urea during and before the amino acid infusion. The differences permitted to obtain and compare the areas under the curves (AUC), expressed as mmol/g.

Determination of the effect of LCT and DLC on the activation of hepatic glucose and urea production from *l*-glutamine (5 mmol/L) After a preperfusion period, (10 min), *l*-glutamine, alone (COG group) or combined with LCT (15 μmol/L, 30 μmol/L, 60 μmol/L) or DLC (60 μmol/L, 120 μmol/L) were dissolved in the perfusion fluid and infused between the 10^{th} and 60^{th} min of the perfusion period. Samples of the effluent perfusion fluid were collected at 2-min intervals and the levels of glucose[11] and urea[12] were analyzed. The differences in the glucose and urea production during (10-60 min) and before (0-10 min) the infusion of *l*-glutamine permitted to obtain the rate of gluconeogenesis and ureagenesis.

Determination of the effect of LCT and DLC on the activation of hepatic glucose and urea production from *l*-alanine (5 mmol/L) After a preperfusion, (10 min), *l*-alanine, alone (COG group) or combined with LCT (60 μmol/L, 120 μmol/L) or DLC (60 μmol/L, 240 μmol/L) were dissolved in the perfusion fluid and infused between the 10^{th} and 30^{th} min of the perfusion period, followed by a period of post-infusion (20 min). The hepatic glucose and urea production from *l*-alanine were analyzed as described above to *l*-glutamine.

Statistical analysis The results, including AUC, were analyzed by ANOVA or Student unpaired *t*-test, using the Graph-Pad Prism–version 2.0 program. The data were presented as mean±SD. $P<0.05$ was accepted for all comparisons.

RESULTS

In the first set of experiments, the direct effect of LCT and DLC on the liver capacity to produce glucose and urea from *l*-glutamine were investigated. If we discount the basal rates of glucose production and determined the AUC during the infusion of *l*-glutamine, the rates of glucose production in the presence of LCT (15 μmol/L, 30 μmol/L, and 60 μmol/L) was higher ($P<0.05$) than in the absence of LCT (Fig 1A). In conformity with these results, urea production from *l*-glutamine was higher ($P<0.05$) in the presence of LCT (60 μmol/L) but no difference was found for LCT at the concentrations of 15 μmol/L and 30 μmol/L (Fig 1B). In contrast with LCT, DLC (60 μmol/L and 120 μmol/L) did not affect the activation of glucose (Fig 2A) and urea (Fig 2B) production promoted by *l*-glutamine. Similar results to urea production from *l*-glutamine during the infusion of LCT (30 μmol/L, 60 μmol/L) or DLC (60 μmol/L, 120 μmol/L) were obtained in livers from fed rats (Fig 3A and B).

In the second set of experiments, the direct effect of LCT and DLC on the liver capacity to produce glucose and urea from *l*-alanine were investigated. Differently of *l*-glutamine, the infusion of LCT (60 μmol/L, 120 μmol/L) or DLC (60 μmol/L, 240 μmol/L) did not affect the activation of hepatic urea and glucose production promoted by *l*-alanine in livers from fasted rats (Fig 4B, 4C). Similar results to urea production from *l*-alanine during the infusion of LCT (60 μmol/L, 120 μmol/L) or DLC (60 μmol/L, 240 μmol/L) were

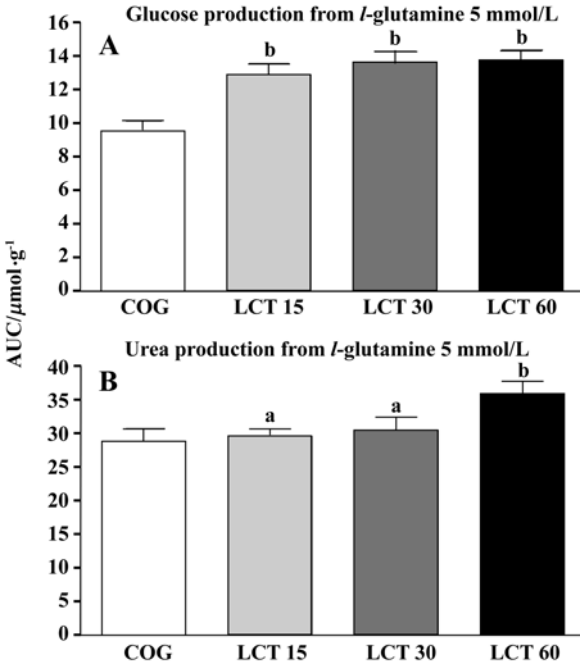

Fig 1. Glucose (A) and urea (B) production from *l*-glutamine alone (COG group) or combined with *l*-carnitine 15 μmol/L (LCT 15), *l*-carnitine 30 μmol/L (LCT 30) and *l*-carnitine 60 μmol/L (LCT 60) in perfused livers from 24 h-fasting rats. *n*=6. Mean±SD. [a]*P*>0.05 and [b]*P*<0.05 *vs* COG group. AUC=areas under the curves.

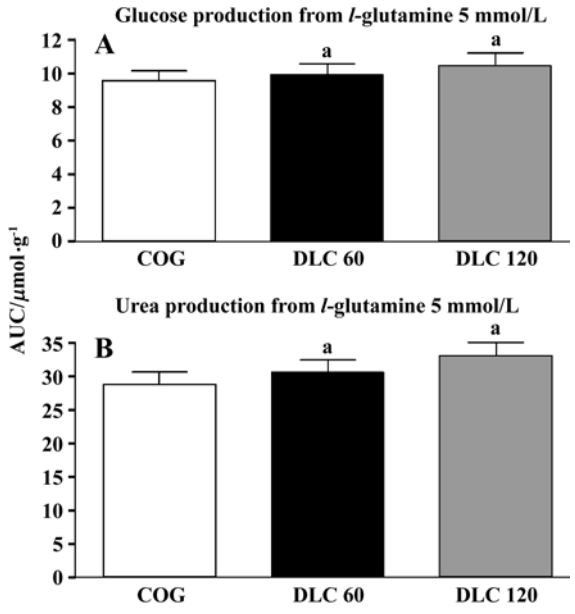

Fig 2. Glucose (A) and urea (B) production from *l*-glutamine alone (COG group) or combined with *dl*-carnitine 60 μmol/L (DLC 60) and *dl*-carnitine 120 μmol/L (DLC 120) in perfused livers from 24 h-fasting rats. *n*=6. Mean±SD. [a]*P*>0.05 *vs* COG group. AUC=areas under the curves.

obtained in livers from fed rats (Fig 4A).

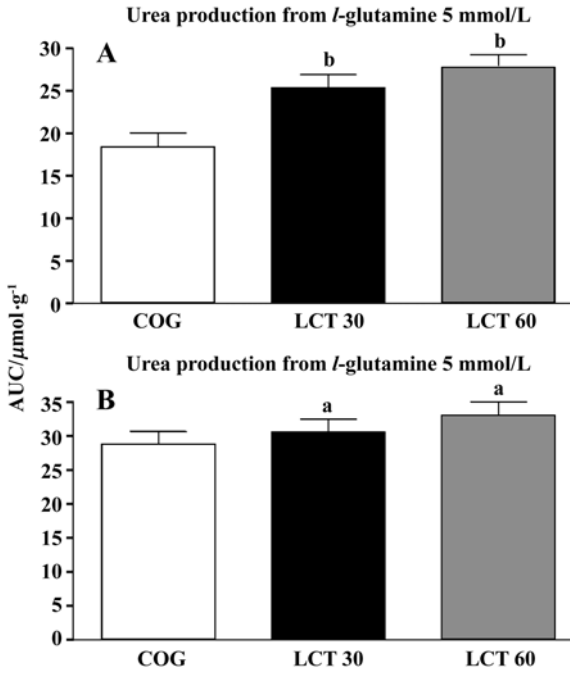

Fig 3. Urea (A and B) production from *l*-glutamine in perfused livers from fed rats. *l*-glutamine was infused alone (COG group), combined with *l*-carnitine 30 μmol/L (LCT 30), and *l*-carnitine 60 μmol/L (LCT 60) or *dl*-carnitine 60 μmol/L (DLC 60) and *dl*-carnitine 120 μmol/L (DLC 120). *n*=6. Mean±SD. [a]*P*>0.05 and [b]*P*<0.05 *vs* COG group. AUC=areas under the curves.

DISCUSSION

Our previous studies[7,8] measured the portal blood levels of free carnitine in rats supplemented with LCT and DLC (1.2 mmol·kg^{-1}·d^{-1}) during 1 week. The values obtained were respectively 23.4±2.3 μmol/L and 21.8±3.0 μmol/L. These higher values (P<0.05), if compared with the physiological blood portal level of free carnitine obtained from no supplemented rats, ie, 17.5±1.1 μmol/L, could help to explain the changes in the catabolism of *l*-glutamine and *l*-alanine promoted by LCT and DLC treatment[7,8]. In agreement with our results Molica *et al*[13] also found influence of acetyl-*l*-carnitine supplementation on hepatic gluconeogenesis and ureagenesis.

Therefore, the possibility of an acute effect of LCT and DLC must be considered.

The main point concerning the results of this study, which should be emphasized is the observation that LCT at physiological levels, 15 μmol/L, was capable to promote acute activation (P<0.05) of hepatic glucose production from *l*-glutamine (Fig 1A). It should be noted that this effect was independent of the activation of

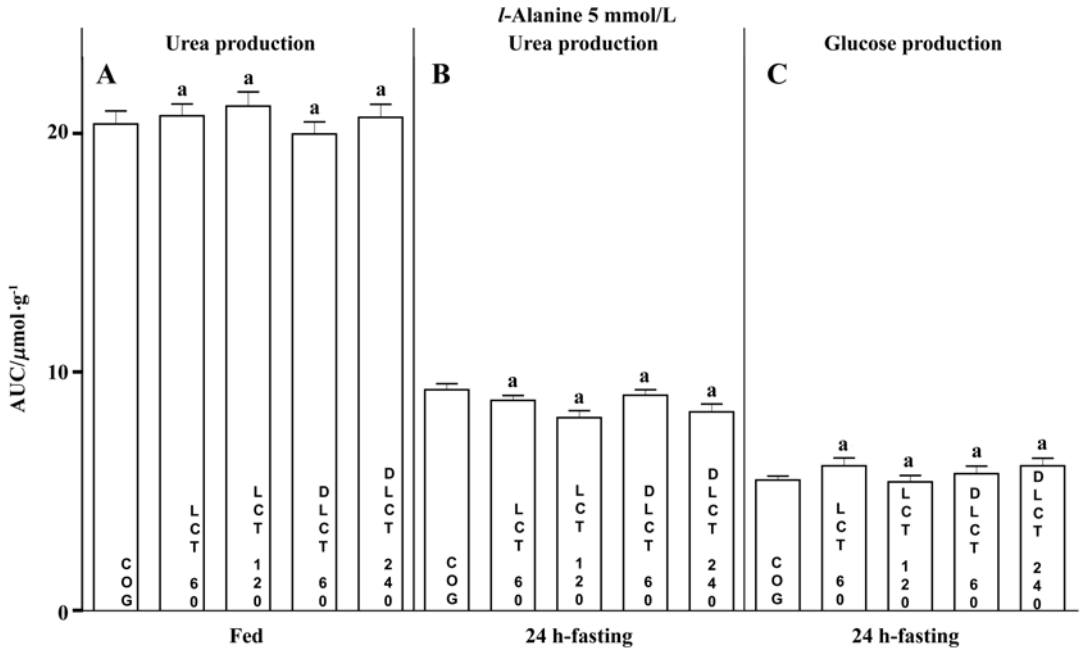

Fig 4. Urea production from *l*-alanine in perfused livers from fed rats (A); urea (B) and glucose production (C) from *l*-alanine in perfused livers from fasted rats. *l*-Alanine was infused alone (COG group), combined with *l*-carnitine 60 μmol/L (LCT 60), *l*-carnitine 120 μmol/L (LCT 120) or *dl*-carnitine 60 μmol/L (DLC 60) and *dl*-carnitine 240 μmol/L (DLC 240). *n*=6. Mean±SD. [a]*P*>0.05 *vs* COG group. AUC=areas under the curves.

ureagenesis, which was observed only to LCT 60 μmol/L (Fig 1B). The immediate consequence of this observation is the fact that the liver, the organ which received the highest concentration of carnitine after oral ingestion, is very sensitive to LCT. Differently of LCT we observed absence of effect to DLC on hepatic metabolism of l-glutamine not only in livers from fasted rats (Fig 2A, 2B) but also in livers from fed rats (Fig 3B).

Considering that the transport of *d*-carnitine and LCT in the liver is similar[2] the absence of effect of DLC could be attributed to the fact that *d*-carnitine inhibits the effects of LCT[14].

In contrast with *l*-glutamine, our results suggest that the glucose and urea production from *l*-alanine, which was influenced by diabetes[15], hypoglycemia[16], and LCT[8] supplementation was not affected by the direct infusion of LCT and DLC in the liver (Fig 4).

The absence of acute effects of DLC in the liver metabolism can not be considered a general rule for all acute effects of this isomer. For example, we demonstrated recently that DLC and LCT promote similar degree of acute tetanic fade in rat neuromuscular preparation[17].

Finally, our previous results and the data showed here reinforce the recommendation that DLC could not replace LCT as a nutritional supplement.

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