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Effects of Oral L-carnitine and DL-carnitine Supplementation on Alloxan-Diabetic Rats

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ABSTRACT

The effect of oral L-carnitine (LC) or DL-carnitine (DLC) supplementation during one or four weeks (200 or 400 $mg.kg^{-1}.day^{-1}$) *in diabetic rats was investigated. After the supplementation period, the blood was collected for the evaluation of total (TC) and free L-carnitine (FC), glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerol. Tissues were collected for the determination of TC and FC concentrations. The carnitine supplementation did not change levels of glucose, total cholesterol, HDL-C and LDL-C in the blood. Diabetic rats showed hypertriacylglycerolemia and decreased blood and tissue levels of FC and TC. Normalization of the blood triacylglycerol and increased blood and tissue levels of FC and TC were observed with the LC or DLC supplementation. However, the hyperglycemia remained unchanged. Thus, the reduction of blood triacylglycerol obtained with carnitine supplementation in the diabetic rats did not depend on an amelioration in the glycemia and was mediated partly at least by an increment of serum and tissue concentrations of FC and TC.*

Key words: carnitine, diabetes, dietary supplementation, lipids, rat

INTRODUCTION

L-Carnitine (LC) belongs to a group of food factors known as non-nutrient supplements. It is a normal component of the diet, being found in the animal products and vegetables. It is synthesized in the body from the lysine and methionine (Leibovitz and Mueller 1993). Several clinical conditions of carnitine deficiency have been described, including the genetic deficiency of L-carnitine, hemodialysis, muscular and liver disorders, kidney and cardiovascular diseases and diabetes mellitus (Proulx et al. 1997; Feng et al. 2001; Hong et al. 2002; Bellinghieri et al. 2003; Karlic and Lohninger 2004; Llias et al. 2004; Guarnieri et al. 2007; Rajasekar and Anuradha 2007; Cha 2008; Bernard et al. 2008).

When there is deficiency of LC, a common feature appears to be the increased serum concentration of triacylglycerol (Famularo et al. 1993; Malaguarnera et al. 1999; Bellinghieri et al. 2003; Tanaka et al. 2004). Therefore, a relevant aspect of the LC supplementation is a lipid-lowering effect observed in the experimental animals (Brady et al. 1986; Bell et al. 1987; Maccari et al. 1987; Feng et al. 2001; Hong et al. 2002; Rajasekar and Anuradha 2007) and human (Bellinghieri et al. 2003; Llias et al. 2004; Guarnieri et al. 2007; Cha 2008; Bernard et al. 2008). On the other hand, diabetes promotes carnitine deficiency (Mamoulakis et al. 2004) and increased blood

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triacylglycerol (Siqueira et al. 2006). However, studies investigating the effect of carnitine supplementation on the blood levels of triacylglycerol in type 1 diabetes are absent, not only in the human but also in the experimental animals.

Thus, considering that alloxan-diabetic rats is a suitable experimental animal model of type 1 diabetes and the fact that these rats show hypertriacylglycerolemia (Siqueira et al. 2006), this work investigated the effect of LC and DL-carnitine (DLC) supplementation on the blood levels of triacylglycerol in alloxan-diabetic rats. In addition, the free carnitine (FC) and total carnitine (TC) concentrations in the serum, liver, diaphragm and heart were evaluated.

MATERIALS AND METHODS

Materials

LC and DLC were purchased from the Ajinomoto Company (São Paulo, Brazil). Alloxan was obtained from the Sigma Chemical Company (Saint Louis, EUA). Reagents used to measure the FC and TC were obtained from the Sigma. Lab kits to measure glucose, cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from the Labtest (Lagoa Santa, MG, Brazil).

Animals

Male Wistar rats obtained from the animal house, weighing 190-220 g (at 7-8 weeks of age) were used. All the studies involving the animals were approved by the Local Animal Ethics Committee (protocol number 008/2006 and approval number 042/2006). The rats had free access to water and commercial standard rodent chow. The animals were maintained under the controlled temperature (23ºC), humidity and 12 h light/12 h dark cycle during the period of carnitine supplementation. The rodent chow (Nutrilab CR1, Curitiba, PR, Brazil) was composed by the protein (22%), fibers (8%), mineral (10%), calcium (1.4%) and phosphorus (0.8%). Each kilogram of the product was enriched with the metionine (300 mg), lysine (100 mg), antioxidant (100 mg), vitamin A (12.000 IU), vitamin D3 (1.800 IU), vitamin E (30 mg), vitamin C (800 mg), vitamin K3 (3 mg), vitamin B1 (5 mg), vitamin B2 (6 mg), vitamin B6 (7 mg), vitamin B12 (20 µg), niacine (60 mg), pantotenic acid (20 mg), folic acid (1.0 mg), biotine (0.05 mg), coline (600 mg), iron (50 mg), zinc (60 mg), copper (10 mg), iodine (2 mg), manganese (60 mg), selenium (0.05 mg), cobalt (1.5 mg). Carnitine deficiency was obtained by the induction of diabetes. For this purpose, the rats received an intravenous injection (caudal vein) of alloxan (40 $mg.kg^{-1}$) as previously described (Siqueira et al. 2006). The control rats received an intravenous injection (caudal vein) of saline. After an additional week, a blood sample from the tail was collected for glycemia evaluation and all the rats with glycemia above 250 $mg.dL^{-1}$ were included in the diabetic group.

Carnitine supplementation

Diabetic and non-diabetic rats received LC or DLC dissolved in the water (200 or 400 $mg.kg^{-1}.day^{-1}$) during one or four weeks. During the supplementation period, body weight, water and food ingestion were measured. Moreover, after the supplementation period, all the rats were fasted (14 h) and killed by decapitation. The blood was collected for the evaluation of the FC and TC (Wieland et al. 1985), glucose (Bergmeyer and Bernt 1974), total cholesterol, HDL-C, LDL-C (Allain et al. 1974), triacylglycerol (Bucolo and David 1973), ALT and AST (Bergmeyer et al. 1978). After blood collection, the rats were submitted to laparotomy and the liver, heart and diaphragm muscle were collected and frozen in liquid nitrogen until the determination of the levels of FC and TC (Wieland et al. 1985).

Statistical Analysis

Data were analyzed by the ANOVA (one way and two way) with the software Graph Pad Prism 2. Values were reported as mean values (M) $\pm$ standard error (SE). $P < 0.05$ was accepted for all the comparisons.

RESULTS

Supplementation of non-diabetic rats

The LC or DLC (200 or 400 $mg.kg^{-1}.day^{-1}$) supplementation during one or four weeks did not change the water and food intake, body weight or the blood levels of enzymes AST and ALT (not shown). In addition, the blood levels of glucose,

total cholesterol, HDL-C, LDL-C and triacylglycerol also remained unchanged (Table 1). On the other hand, the serum concentrations of FC increased from the first week of LC or DLC supplementation (200 or 400 mg.kg^{-1}.day^{-1}). But, an increased serum concentration of TC was observed (Table 2) only after four weeks of LC or DLC supplementation (200 or 400 mg.kg^{-1}.day^{-1}).

Table 1 - Blood levels of glucose, triacylglycerol (TG), total cholesterol (COL), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in non-diabetic rats supplemented during 1 or 4 weeks with L-carnitine (LC), DL-carnitine (DLC), 200 or 400 mg.kg^{-1}.day^{-1}, or not supplemented (CONT). Blood were obtained from 14 h fasted rats. The results are expressed as M ±SE of 7 animals.

Period (weeks)	Dose (mg.kg^{-1}.day^{-1})	Group	Blood levels (mg.dL^{-1})				
			Glucose	TG	COL	HDL-C	LDL-C
1	0	CONT	58.5±2.4	78.6±6.2	95.0±3.8	67.3±3.6	10.3±1.2
	200	LC	53.0±3.2	70.1±5.1	90.6±6.2	66.6±4.8	10.7±1.9
	200	DLC	54.4±3.2	74.8±7.8	97.4±5.3	68.6±3.1	10.9±2.6
1	0	CONT	59.1±2.1	78.0±9.9	91.3±6.2	64.4±4.2	10.4±1.9
	400	LC	55.8±3.0	69.7±3.1	93.1±4.5	63.0±4.1	10.4±1.8
	400	DLC	60.4±2.9	75.6±8.4	89.4±4.2	68.4±6.1	11.9±2.4
4	0	CONT	54.8±4.8	82.9±11.3	92.9±5.0	63.1±3.7	9.7±1.7
	200	LC	56.0±4.3	72.4±14.2	87.8±3.5	64.1±5.1	9.8±2.0
	200	DLC	59.2±1.5	82.9±8.9	90.7±3.4	64.9±4.8	8.3±1.5
4	0	CONT	58.9±2.9	81.7±5.1	93.6±8.8	60.9±2.2	8.1±2.8
	400	LC	53.4±6.2	70.9±14.1	90.5±6.7	64.5±2.1	10.3±1.2
	400	DLC	53.2±4.4	76.4±5.0	90.7±4.1	60.5±1.8	8.7±2.7

Table 2 - Serum and tissue levels of free L-carnitine (FC) and total L-carnitine (TC) in non-diabetic rats supplemented during 1 or 4 weeks with L-carnitine (LC) or DL-carnitine (DLC), 200 or 400 mg.kg^{-1}.day^{-1}, or not supplemented (CONT). Serum and tissues were obtained from 14 h fasted rats. The results are expressed as M±SE of 7 animals. [a]Dose of L-carnitine (LC) or DL-carnitine (DLC) expressed in mg.kg^{-1}.day^{-1}. * Different of CONT group. $p< 0.05$ (ANOVA). [b] Different of DLC group. $p< 0.05$ (ANOVA).

Sample	Period (weeks)	Parameter	CONT	LC 200 [a]	DLC 200 [a]	LC 400 [a]	DLC 400 [a]
Serum (µmol.L^{-1})	1	FC	17.5±0.5	23.4±1.3*	21.7±1.3*	24.6±1.4*	22.7±2.0*
		TC	42.8±0.9	46.3±2.4	44.2±2.7	47.9±3.3	46.9±3.4
	4	FC	18.2±0.5	26.3±1.4*	24.8±1.8*	27.6±1.5*	25.7±1.7*
		TC	41.6±1.0	49.7±2.4*	48.2±2.3*	54.3±2.4*	52.2±2.7*
Liver (nmol.g^{-1})	1	FC	86.3±2.0	109.3±2.3*b	93.6± 2.4*	121.1±3.4*b	94.1± 2.4*
		TC	225.4± 3.3	230.0± 3.6	223.1± 3.0	231.7± 3.4	228.9± 3.4
	4	FC	87.6± 1.9	123.1± 3.5*b	101.4± 1.8*	129.0± 4.2*b	105.4± 2.6*
		TC	224.0± 4.1	231.5± 3.6	226.2± 3.7	232.5± 3.9	229.3± 4.0
Diaphragm (nmol.g^{-1})	1	FC	412.2± 15.1	437.5± 10.8	426.1± 12.0	439.7± 14.2	434.7± 16.2
		TC	536.9±18.9	543.2± 15.1	541.3± 14.9	549.1± 16.3	546.4± 17.2
	4	FC	415.2±15.2	471.3±18.5*	441.4± 12.8	495.1±12.6*	445.8± 14.6
		TC	541.3±17.9	553.8± 17.4	550.7± 13.9	555.7±18.3	552.5±13.4
Heart (nmol.g^{-1})	1	FC	504.8± 6.1	515.7± 6.6	505.6± 7.3	521.8± 12.9	512.1±9.1
		TC	634.1±17.4	646.2±19.6	641.3± 19.4	650.8± 17.6	650.3±19.8
	4	FC	506.1± 7.1	555.8± 7.4*	521.6± 10.4	571.8± 9.5*	524.4± 12.1
		TC	635.3±18.1	651.1±16.4	635.7±19.6	650.0±19.8	647.9±17.7

Liver FC levels also increased from the first week of LC or DLC supplementation (200 or 400 mg.kg^{-1}.day^{-1}). However, the levels of FC were higher in the livers of the rats supplemented during one or four weeks with LC (200 or 400 mg.kg^{-1}.day^{-1}) than those supplemented with DLC. In contrast with FC, the liver concentration of TC remained unchanged for all doses or duration of LC or DLC supplementation (Table 2).

In the diaphragm and heart, increased levels of FC were observed after four weeks of LC supplementation (200 or 400 mg.kg^{-1}.day^{-1}). In contrast, the FC levels in the diaphragm and heart remained unchanged for all the doses or duration of DLC supplementation. Moreover, the TC levels remained unchanged for all the doses or duration of LC or DLC supplementation (Table 2).

Supplementation of alloxan-diabetic rats

The supplementation (one or four weeks) with LC or DLC (200 or 400 mg.kg^{-1}.day^{-1}) did not change the water and food intake, body weight or the blood levels of the enzymes AST and ALT (not shown). Moreover, the blood levels of glucose, total cholesterol, HDL-C and LDL-C also remained unchanged (Table 3). However, decreased triacylglycerol levels were observed (Table 3) after four weeks of LC or DLC supplementation (400 mg.kg^{-1}.day^{-1}). Before LC or DLC supplementation, diabetic rats showed decreased FC and TC levels in the serum, diaphragm and heart (Table 4). FC blood levels increased from the first week of LC or DLC supplementation (200 or 400 mg.kg^{-1}.day^{-1}).

Table 3 - Blood levels of glucose, triacylglycerol (TG), total cholesterol (COL), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in diabetic rats supplemented during 1 week (200 mg.kg^{-1}.day^{-1}) or 4 weeks (400 mg.kg^{-1}.day^{-1}) with L-carnitine (LC), DL-carnitine (DLC) or not supplemented (CONT). Blood were obtained from 14 h fasted rats. The results are expressed as M±SE of 7 animals. * Different of CONT group. $p< 0.05$ (ANOVA).

Weeks	Dose (mg.kg^{-1}.day^{-1})	Group	Blood levels (mg.dL^{-1})				
			Glucose	TG	COL	HDL-C	LDL-C
1	0	CONT	374.6±19.0	258.9±43.6	127.4±4.9	73.9±3.8	9.8±2.4
	200	LC	367.5±27.3	229.2±39.6	117.6±11.1	75.2±5.2	10.2±1.4
	200	DLC	395.2±25.5	233.4±38.3	124.3±4.2	67.9±3.7	8.4±3.2
4	0	CONT	368.1±27.7	223.8±33.4	119.7±5.0	73.7±5.1	10.4±2.3
	400	LC	363.7±38.1	79.2±9.5*	118.6±4.8	75.3±4.3	9.0±2.0
	400	DLC	402.3±13.0	96.3±16.9*	121.5±3.5	71.2±4.2	10.2±2.8

Table 4 - Serum and tissue levels of free L-carnitine (FC) and total L-carnitine (TC) in diabetic and non-diabetic rats. The animals were fasted (14 h) before the blood and tissue collection. The results are expressed as M ±SE of 7 animals. * Different of non-diabetic group. $p< 0.05$ (ANOVA).

Parameter	Groups	Serum (µmol.L^{-1})	Liver (nmol.g^{-1})	Diaphragm (nmol.g^{-1})	Heart (nmol.g^{-1})
LC	Non-diabetic	17.5±0.5	86.3±1.9	412.6±15.1	504.8±6.1
	Diabetic	10.3±1.5*	110.7±4.0*	349.2±19.3*	445.8±18.0*
TC	Non-diabetic	42.8±0.9	225.4±3.3	536.9±18.9	634.1±17.4
	Diabetic	35.2±1.1*	263.4±11.0*	470.1±17.2*	572.7±18.3*

However, serum concentrations of TC increased after four weeks of supplementation with 400 mg.kg^{-1}.day^{-1} of LC or DLC (Table 5). Furthermore, liver, diaphragm and heart, showed increased concentrations of FC and TC after four weeks of LC or DLC supplementation (400 mg.kg^{-1}.day^{-1}) (Table 5).

Table 5 -Serum and tissue levels of free carnitine (FC) and total L-carnitine (TC) in diabetic rats supplemented during 1 or 4 weeks with L-carnitine (LC) or DL-carnitine (DLC), 200 or 400 $mg.kg^{-1}.day^{-1}$, or not supplemented (CONT). Blood and tissues were obtained from 14 h fasted rats. The results are expressed as M±SE of 7 animals. [a] Dose of L-carnitine (LC) or DL-carnitine (DLC) expressed in $mg.kg^{-1}.day^{-1}$. * Different of CONT group. $p< 0.05$ (ANOVA).

Sample	Parameter	CONT	LC 200[a] 1 week	DLC 200 [a] 1 week	LC 400 [a] 4 weeks	DLC 400 [a] 4 weeks
Serum	FC	10.3±1.5	18.9±1.6*	17.0±1.6*	21.6±1.7*	18.6±1.9*
($\mu mol.L^{-1}$)	TC	35.2±1.1	38.5±2.0	36.7±1.9	44.2±1.9*	41.8±1.9*
Liver	FC	110.7±4.0	118.6±4.4	115.5±4.6	137.8±4.3*	130.9±4.6*
($nmol.g^{-1}$)	TC	263.4±11.0	281.6±12.6	274.6±13.5	339.2±16.9*	320.3±15.7*
Diaphragm	FC	349.2±19.3	381.6±17.2	375.0±16.1	425.7±20.1*	419.0±19.0*
($nmol.g^{-1}$)	TC	470.1±17.2	512.3±16.4	496.1±15.2	541.0±18.0*	535.0±16.6*
Heart	FC	445.8±18.0	462.6±20.6	459.2±22.2	525.7±25.2*	519.9±24.3*
($nmol.g^{-1}$)	TC	572.7±18.3	610.2±20.7	599.5±21.4	660.6±23.6*	648.2±21.6*

DISCUSSION

In agreement with the previous studies (Bell et al. 1987; Bobyleva-Guarriero et al. 1988, Bell et al. 1992), the supplementation (one or four weeks) with LC or DLC (200 or 400 $mg.kg^{-1}.day^{-1}$) did not modify the blood lipids in the non-diabetic rats (Table 1). The absence of effect of LC or DLC could not be attributed to the lack of intestinal absorption, since an increased serum levels of FC were found in the non-diabetic rats supplemented with LC or DLC (Table 2). Thus, in spite the fact that β-oxidation depended on the activity of the carnitine dependent transport of fatty acids (Kuroda et al. 1996; Longo et al. 1996), the increased availability of LC or DLC did not change the blood lipid levels in the non-diabetic rats. However, in contrast with these results, LC administration decreased the blood lipids in the animals and humans. However, this effect occurred if the blood levels of cholesterol and/or triacylglycerol were elevated due to the metabolic disorders or dietary manipulations (Hoppel and Brass 1978; Paulson and Shug 1981; Pola et al. 1983; Vacha et al. 1983; Brady et al. 1986; Bell et al. 1987; Maccari et al. 1987; Shimura and Hasegawa 1993). In the non-diabetic rats, the blood levels of FC increased from the first week of LC or DLC supplementation (200 or 400 $mg.kg^{-1}.day^{-1}$). However, an increased blood level of TC was observed (Table 2) after four weeks of LC or DLC supplementation (200 or 400 $mg.kg^{-1}.day^{-1}$). Thus, the duration of supplementation, but not the daily dose was crucial to increase the blood levels of TC. On the other hand, there was no change in the concentration of the TC in the liver, diaphragm and heart (Table 2) one or four weeks of the supplementation (200 or 400 $mg.kg^{-1}.day^{-1}$).

In the non-diabetic rats supplemented with LC or DLC (200 or 400 $mg.kg^{-1}.day^{-1}$) for one or four weeks, higher liver concentration of FC were observed (Table 2). However, the levels of FC in the liver was higher in the rats supplemented with LC (200 $mg.kg^{-1}.day^{-1}$) than the rats supplemented with DLC (200 or 400 $mg.kg^{-1}.day^{-1}$).

In the non-diabetic rats, the FC concentration in the diaphragm and heart tissue did not differ between the groups after one week of supplementation (200 or 400 $mg.kg^{-1}.day^{-1}$). However, after four weeks of supplementation, the concentrations of FC in the groups supplemented with LC (200 or 400 $mg.kg^{-1}.day^{-1}$) were higher than the DLC group (Table 2). Thus, the duration of the supplementation, but not the daily dose, was crucial to increase the levels of FC in the diaphragm and heart. This result also suggested that the D-isomer influenced the liver carnitine uptake, but in lesser extent than in the diaphragm. Therefore, as described in other studies (Paulson and Shug 1981; Bremer 1983), the liver pool of LC appeared to be less affected by the D-carnitine (DC). Therefore, the results from non-diabetic rats suggested the existence of selectivity to the LC isomer (Huth and Shug 1980; Vary and Neely 1982; Lopes et al. 2003). In agreement with this proposition, several studies showed the existence of a competitive inhibition of the LC uptake by the DC (Yokogawa et al. 1999; Georges et al. 2000.

Since diabetic rats showed reduced levels of the FC and TC in the plasma, kidney, heart and muscles (Brooks et al. 1985; Rodrigues et al. 1988; Rodrigues et al. 1990; Reddi et al. 1991) and the supplementation with LC restored the normal concentration of carnitine in these tissues (Marzo et al. 1993; Malone et al. 1999), they could be used as an animal model of carnitine deficiency and carnitine supplementation. In agreement with this proposition, lower levels of FC and TC (serum, diaphragm, heart and muscles) were found, before the supplementation with the LC or DLC (diabetic *vs.* non-diabetic rats).

Alternatively, diabetic rats supplemented with the LC or DLC during one week (200 $mg.kg^{-1}.day^{-1}$) or four weeks (400 $mg.kg^{-1}.day^{-1}$) showed higher concentration of FC than non-supplemented diabetic rats. But, the TC levels in the blood, liver, diaphragm and heart only increased after four weeks of LC or DLC (400 $mg.kg^{-1}.day^{-1}$) supplementation (Table 5), suggesting that the duration of supplementation influenced the serum and tissue levels of the TC in the diabetic rats.

Carnitine deficiency promotes changes in lipid metabolism (Famularo et al. 1993). But LC supplementation restores the lipid metabolism, including reduction of blood levels of triacylglycerol, phospholipids and free fatty acids (Rebouche 1992; Dayanandan et al. 1994; Ji et al. 1996; Llias et al. 2004; Guarnieri et al. 2007). In agreement with these studies, supplementation with LC or DLC (400 $mg.kg^{-1}.day^{-1}$) during four weeks normalized the increased serum triacylglycerol concentrations of the diabetic rats but did not change the hyperglycemia (Table 3). Thus, it was concluded that the reduction of blood triacylglycerol obtained with the LC or DLC supplementation in the diabetic rats did not depend on an amelioration in the glycemia and was mediated partly at least by an increment of serum and tissue concentrations of the FC and TC, particularly in the liver. Furthermore, these findings suggested that the LC or DLC supplementation could be recommended in the patients with type 2 diabetes with high blood levels of triacylglycerol. However, additional studies will be necessary to confirm this suggestion.

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