Ketogenic effects of low and high levels of carnitine during total parenteral nutrition in the rat[1,2]

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ABSTRACT Male Wistar rats received total parenteral alimentation for 3 d. The animals were divided into three groups: group 1, without L-carnitine; group 2, 10 mg (62.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$; and group 3, 100 mg (621.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$. Fat oxidation was followed by indirect calorimetry. Maximal oxidative metabolism of fatty acids was achieved with supplementation of L-carnitine in small amounts (10 mg · $kg^{-1} \cdot d^{-1}$). This was demonstrated by a decrease of the RQ and of the serum concentrations of fatty acids and by an increase of β-OH-butyric acid. Decreased liver free and long-chain acylcarnitine and increased short-chain acylcarnitine concentrations in this group also demonstrate an increased ketogenicity. This ketogenic effect of carnitine decreases when higher concentrations of carnitine are used. This study demonstrates that the ketogenic effect of carnitine is dose dependendent. *Am J Clin Nutr* 1987;46:47–51.

KEY WORDS Carnitine, ketogenesis, total parenteral alimentation, carnitine dosage

Introduction

The major effect of L-carnitine in fatty acid metabolism is supposed to be the transport of long-chain fatty acids across the inner mitochondrial membrane (1). The use of L-carnitine during total parenteral nutrition with lipid emulsions is advocated in order to stimulate the oxidative degradation of long-chain fatty acids. In the literature however, contradictory results about a ketogenic as well as an antiketogenic effect of carnitine were published (2, 3). In those studies carnitine was administered in amounts ranging between 1.5 and > 200 mg · $kg^{-1} \cdot d^{-1}$. Our objective is to determine the effect of high and low levels of intravenous L-carnitine supplementation on fatty acid oxidation.

Methods and experimental design

Animals

Male Wistar rats (243 ± 28 g), cared for following the official guidelines for experiments in animals of the governmental district, were prepared for parenteral nutrition by cannulation of the jugular vein according to Steiger et al (4). The animals were anesthetized with ketamine-HCl (2 mg/kg or 7.4 μmol/kg intraperitoneally). The animals were individually housed in metabolic cages randomly assigned to three groups of parenteral alimentation. All animals received as nutrients per day 5 g amino acids/kg (Aminofusin 10%®, Pfrimmer & Co, Erlangen, FRG), 5 g glucose/kg, and 8 g long-chain triglycerides (Intralipid 20%®, KabiVitrum, Stockholm, Sweden) as well as minerals and vitamins. The animals in group 1 (n = 6) received no carnitine supplement. Those in group 2 (n = 6) received 10 mg (62.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$ (10C) and those in group 3 (n = 6) 100 mg (621.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$ (100C). L-carnitine was given as the free base (Biocarn®, Nefro-Pharma, Bad Aibling, FRG). The amount of glucose infused was calculated not to exceed by very much the endogenous glucose production rate of the animals in order to promote an effective oxidative lipid metabolism (5, 6). The total energy intake of the animals was considered hypocaloric (116 kcal · $kg^{-1} \cdot d^{-1}$). The fluid intake was 290 mL · $kg^{-1} \cdot d^{-1}$. The animals were intravenously alimented for 3 d. The urine was continuously collected and stored at −80°C until analysis.

After parenteral nutrition the animals were stunned, blood was withdrawn by aortic puncture, and the liver was freeze clamped and put immediately into liquid nitrogen. Serum and liver tissue were kept at −80°C until analysis.

Chemical analysis

The chemical analysis was performed according to the following methods: free fatty acids according to Novak (7); β-OH-butyric acid, enzymatically, according to Williamson and Mellanby (8); glycerol, enzymatically, according to Laurell and Tibbling (9); liver carnitine content as described by McGarry and

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TABLE 1
Results of indirect calorimetric studies during total parenteral nutrition*

	LCT	*LCT + 10C*	*LCT + 100C*
RQ (protein free)	0.78 ± 0.03	0.71 ± 0.04 †	0.75 ± 0.02 † ‡
Fat (g · kg^{-1} · d^{-1})	13.3 ± 4.49 §	20.7 ± 3.82 ‡	17.9 ± 2.37 †
Glucose (g · kg^{-1} · d^{-1})‖	10.3 ± 3.08 (57.2 ± 17.1) §	2.7 ± 4.54 (15 ± 25.2) †	6.8 ± 2.32 (37.7 ± 12.8) ‡
Protein (g · kg^{-1} · d^{-1})	1.6 ± 0.38 ‡	1.3 ± 0.88 ‡	1.9 ± 0.48 ‡
Total calories (kcal · kg^{-1} · d^{-1})	175 ± 34 †	215 ± 29 NS	203 ± 21 ‡

* 10C = 10 mg (62.1 μmol) L-carnitine · kg^{-1} · d^{-1}; 100C = 100 mg (621.1 μmol) L-carnitine · kg^{-1} · d^{-1}; amounts of oxidatively metabolized substrates as calculated by Hunker et al (12).
† $p < 0.01$.
‡ $p < 0.05$.
§ $p < 0.001$.
‖ SI values in mmol · g^{-1} · d^{-1} are given in parentheses.

Foster (10) and Fishlock et al (11); serum and liver triglycerides (after sonication), enzymatically, with an automated routine clinical laboratory method (Testomar-Triglyceride®, Behringwerke AG, Marburg, FRG); and urine total nitrogen with the classic Kjeldahl procedure (12).

Indirect calorimetry

Indirect calorimetry was performed for 15 min, several times a day in a system with controlled airflow through the metabolic cages. Infrared absorption was used for carbon dioxide detection. Oxygen was determined by its paramagnetic properties. Barometric pressure and room temperature were recorded at the moment of gas exchange. The necessary calculations were performed according to Hunker et al (13).

Flow cytometric analysis

The state of liver cell proliferation was defined by means of flow cytometric analysis of cellular DNA according to Beck et al (14). Fresh liver was treated with pepsin; DNA of individual cells was stained with 4,6-diamidino-2-phenylindole. The proportion of cells in S phase within the cellular proliferation cycle was measured and considered representative for cell proliferation.

Statistical analysis

Statistical analysis of the data was performed with the nonparametric U-test according to Mann and Whitney.

Results

The results of indirect calorimetry are shown in **Table 1.** The values of the protein free respiratory quotient in the three groups are representative for the overall results of this investigation. The mean RQ of 0.78 in unsupplemented group 1 reflects the fair contribution of fatty acids to total energy expenditure. The energy expenditure of ~200 kcal · kg^{-1} · d^{-1} shows that the animals were alimented hypocalorically by the parenteral regime and that an additional 6–12 g of fat stores had to be used. Supplementation with the small amount of L-carnitine (10 mg · kg^{-1} · d^{-1}) leads to a decrease of the RQ, reflecting an enhanced fatty acid oxidation. This effect is partially reversed when the carnitine supplementation is increased to 100 mg · kg^{-1} · d^{-1}. The pattern of a different ketogenicity with respect to the amount of L-carnitine supplemented could be demonstrated by different variables **(Table 2).**

The carnitine fractions in liver tissue at the low and high levels of carnitine **(Table 3)** show a decrease of free and long-chain acylcarnitine concomitant with an increase of short-chain acylcarnitine in group 2 when the β-OH-

TABLE 2
Serum concentrations of triglycerides, free fatty acids, glycerol, and β-OH-butyric acid and liver triglyceride concentrations after 3 d of total parenteral nutrition*

Serum	*LCT*	*LCT + 10C*	*LCT + 100C*
Triglycerides (mg/dL)†	238 ± 91 (2.72 ± 1.04)	nd	154 ± 94 (1.76 ± 1.07) ‡
Free fatty acids (μmol/L)	3.9 ± 1.6 ‡	2.4 ± 0.7 NS	2.2 ± 1.1 ‡
Glycerol (μmol/L)	1.5 ± 0.5 §	2.3 ± 0.6 ‡	3.1 ± 0.5 ‖
β-OH-Butyric acid (μmol/L)	1.1 ± 0.3 ‖	2.2 ± 0.4 ‖	1.1 ± 0.3 NS
Liver		§	NS
Triglycerides (mg/g wet weight)¶	43.8 ± 5.6 (500.5 ± 64)	37.6 ± 8.0 (429.7 ± 91.4)	41.5 ± 7.1 (474.2 ± 81.1) NS

* 10C = 10 mg (62.1 μmol) L-carnitine · kg^{-1} · d^{-1}; 100C = 100 mg (621.1 μmol) L-carnitine · kg^{-1} · d^{-1}; nd = not determined.
† Values in mmol/L are given in parentheses.
‡ $p < 0.01$.
§ $p < 0.05$.
‖ $p < 0.001$.
¶ Values in μmol/g are given in parentheses.

TABLE 3
Liver carnitine concentrations (nmol/g wet wt) after 3 d of total parenteral nutrition*

	LCT	LCT + 10C	LCT + 100C
Total carnitine	3760 ± 1407	4458 ± 974	3927 ± 551
	NS	NS	
	NS		
Free carnitine	820 ± 72	765 ± 62	949 ± 207
	†	†	
	NS		
Short-chain acyl-carnitine	255 ± 77	469 ± 67	376 ± 80
	‡	NS	
	‡		
Long-chain acyl-carnitine	2685 ± 1273	2229 ± 856	2602 ± 755
	†	†	
	NS		

* 10C = 10 mg (62.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$; 100C = 100 mg (621.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$.
† $p < 0.05$.
‡ $p < 0.001$.

butyric acid levels indicate an enhanced ketogenicity. The lower serum and liver triglycerides in the carnitine-supplemented groups agree with these results. Serum free fatty acids are equally decreased in both the low- and the high-carnitine groups compared with the animals without carnitine supplementation. Serum glycerol concentrations increase almost in a dose-effect relationship when carnitine is supplemented. As shown in **Figure 1**, the supplementation of total parenteral nutrition with 10 mg L-carnitine · $kg^{-1} \cdot d^{-1}$ leads to a higher percentage of liver cells in S phase. **Figure 2** shows a better total-nitrogen retention with 10 mg of L-carnitine supplementation.

Discussion

The effect of carnitine supplementation on oxidative fatty acid metabolism is controversial. A stimulation of ketogenesis by carnitine (2) as well as an inhibition of ketone synthesis (3, 15) were described. Our results show that carnitine at low concentrations stimulates ketogenesis and that ketogenicity cannot be further increased at higher concentrations. This is in accordance with Yeh (16), whose in vitro studies essentially reported the same dependence of ketogenesis on carnitine concentrations. The stimulated β-oxidation at low-carnitine concentrations is supported by the results of indirect calorimetry, β-OH-butyric acid formation, as well as the pattern of liver carnitine metabolites. The decrease of free and long-chain acylcarnitine and the increase of short-chain acylcarnitine are in agreement with a stimulated oxidation of fatty acids. There is no influence of L-carnitine administration on the content of total liver carnitine. Yeh (16) showed that the liver carnitine content did not increase when carnitine was administered at a rate < 500 mg/kg (3.1 mmol/kg). The effect of carnitine in small amounts on nitrogen balance as well as on the degree of liver cell proliferation (as determined by flow cytometric analysis) could well be in accordance with a more anabolic state of metabolism. This result is of importance for the use of carnitine during total parenteral nutrition.

These results tell us little about the mechanism by which carnitine exerts its peculiar dose-dependent influence on fatty acid oxidation. Decreased fasting blood ketone levels were observed by Wolff et al (17) in patients with disorders of organic acid metabolism who were treated with L-carnitine. Blood ketone formation decreased with increasing doses of carnitine supplementation. Wolff et al speculate that decreased ketogenesis may be caused by reducing transport of fatty acids into mitochondria, by increasing fatty acid synthesis from acetyl-CoA, by reducing conversion of acetyl-CoA to ketones, by inhibition of 3-OH-3-methyl-glutaryl synthase, and by increasing oxidation of acetyl-CoA through the citric acid cycle (17).

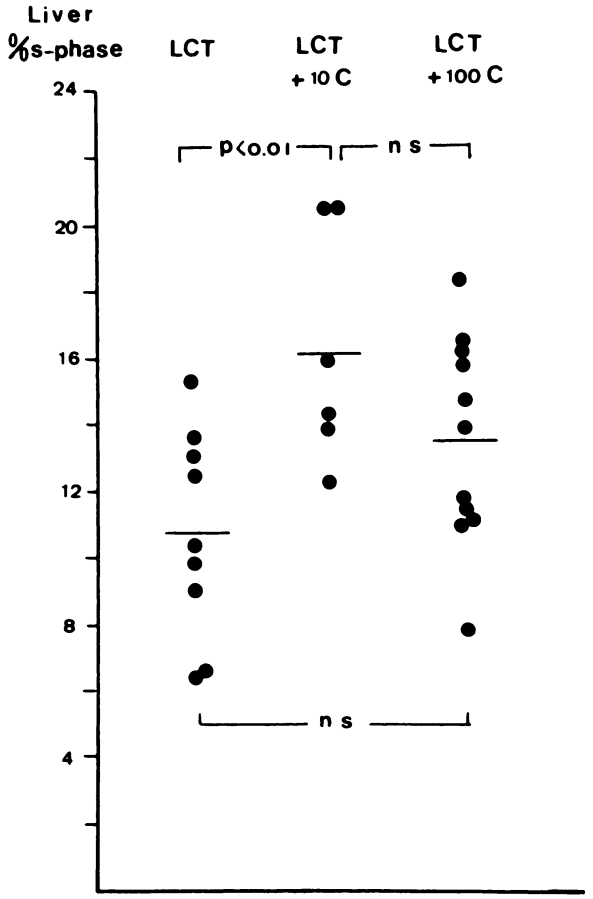

FIG 1. Percentage of S-phase cells in the cellular proliferation cycle. The supplementation of total parenteral nutrition with 10 mg (62.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$ (10C) leads to a significant increase of liver cells in S-phase. 100C = 100 mg (621.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$.

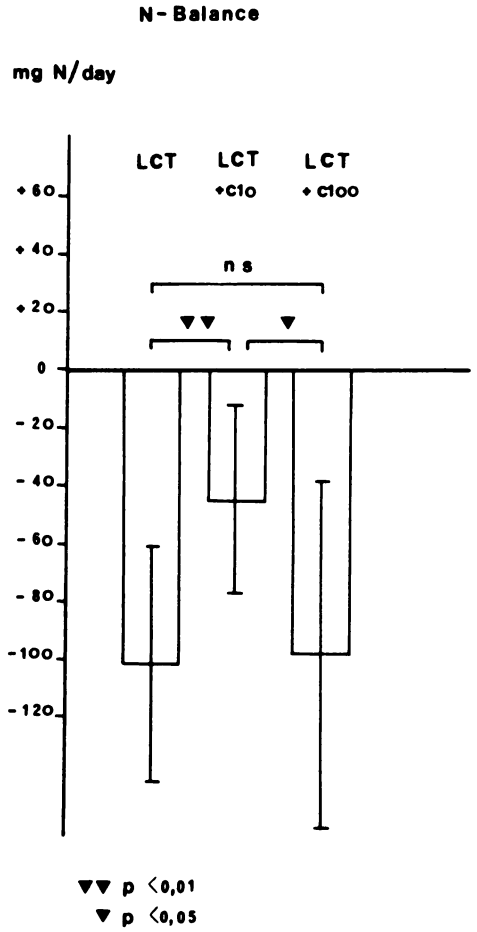

FIG 2. Nitrogen balance during total parenteral nutrition of rats. The supplementation of total parenteral nutrition with 10 mg (62.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$ (C10) leads to a significant amelioration of nitrogen retention. Because of the hypocaloric alimentation regime all nitrogen balances are negative. C100 = 100 mg (621.1 μmol) L-carnitine · $kg^{-1} \cdot d^{-1}$.

We would like to add an additional speculative idea on this peculiar dose-dependent effect of carnitine on ketogenesis: it was shown that as well as being a substrate for carnitine acyltransferase palmitoyl-CoA is a competitive inhibitor for the second substrate, carnitine (18), ie, increased concentrations of long-chain acyl-CoA increase the K_m of carnitine acyl transferase I for carnitine. Increases in the carnitine concentration may compensate for increases in intracellular long-chain acyl-CoA concentration so that maximal rates of oxidation of incoming fatty acids are achieved. Small amounts may be sufficient for this ketogenic effect of carnitine. However, this explanation requires an increase of the tissue carnitine concentration that was not observed in the liver. Explanations for this discrepancy could be *1*) changes of the tissue carnitine concentration are hidden behind the large standard deviation; *2*) compartmentalization of the effect (ie, muscle), and *3*) dynamic effect on the flow through the cellular compartment. On the other hand we learned from the investigation of organic acidemias that a pathologically accumulating acid is excreted as carnitine conjugate and may lead to secondary carnitine deficiency.

Roe et al (19, 20) showed that carnitine therapy is able to cause an intramitochondrial transformation of acyl-CoA into acylcarnitine conjugates. This leads to the intramitochondrial liberation of free CoA, which is beneficial for the flow of intermediary metabolism. If we transfer this knowledge to our experimental situation of carnitine-supplemented total parenteral nutrition, high levels of carnitine may not be able to further stimulate the carnitine acyl transferase I activity. High levels of carnitine may increase the formation of intramitochondrial fatty acid-carnitine conjugates and their transport out of the matrix space, ie, via the inversion of the carnitine acyl translocase direction or via the carnitine acetyl transferase activity. This would signify a withdrawal of activated substrate for β-oxidation when higher concentrations of carnitine are administered (antiketogenic activity of carnitine).

From our results we conclude that carnitine dosage should stay within the low range when used during total parenteral nutrition (possibly not > 10 mg · $kg^{-1} \cdot d^{-1}$, or 62.1 μmol · $kg^{-1} \cdot d^{-1}$) and should be in the high range when used for the purpose of intramitochondrial formation of free CoA.

After this experiment we are left with continuously higher serum glycerol concentrations with increasing carnitine supplementation. This may indicate an additional lipolytic activity of carnitine. We also have the equally low free fatty acid concentrations in groups 2 and 3 and a lower fatty acid oxidation in group 3. This means that free fatty acids must have disappeared in pathways other than β-oxidation. One possibility could be that fatty acids either are liberated by lipolysis or leave the matrix space as carnitine conjugates and are reesterified.

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