Effects of supplementary levels of L-carnitine on blood and urinary carnitines and on the portal-systemic blood-ethanol concentrations in the rat

Robert Berger* and Dileep S. Sachan

Department of Nutrition and Food Sciences, College of Human Ecology and Agricultural Experiment Station, The University of Tennessee, Knoxville, TN, USA

Male Sprague-Dawley rats of about 300 g body weight were fed ground Rodent Chow or the same mixed with 0.0025, 0.005, 0.0075, or 0.01 (wt/wt) L-carnitine for 10 days. Changes in blood and urinary carnitines were monitored on days 0, 1, 3, 5, 7, and 10 of feeding carnitine supplemented (CS) diets. Changes in the portal and systemic blood-ethanol were monitored on day 11 following an intraduodenal dose of ethanol. Blood and urinary carnitines peaked after days 3 and 5, respectively, following the 0.005 level of carnitine supplementation which was found adequate. The urinary carnitine fractions followed the pattern of blood carnitines and there were strong positive correlations (r = 0.83) between blood and urinary total and nonesterified carnitines. While rates of ethanol appearance in the portal blood remained essentially unaltered, the systemic blood ethanol concentrations were significantly elevated in the carnitine supplemented rats during the initial 30 minutes. It is concluded that feeding of 0.005 CS diet for three days was adequate to attain peak blood carnitine concentrations and that carnitine retarded ethanol metabolism without altering the rates of ethanol absorption from the small intestine.

Keywords: L-carnitine supplement; acylcarnitines; blood-ethanol; ethanol metabolism; ethanol absorption

The elevated blood-ethanol concentrations (BEC) were observed in male rats fed D,L-carnitine supplemented diet and given acute or chronic ethanol treatment.[1] It was further established that changes in BEC were related to the levels of supplementary carnitine and the pharmacokinetic data supported attenuation of ethanol metabolism by D,L-carnitine.[2] However, these studies did not rule out potential contribution of the D-isomer of carnitine present in the racemic mixture as well as possible effect of carnitine on the rates of ethanol absorption from the small intestine of these animals. Thus, the present study was designed to address these concerns.

Prior to examining the above concerns, it was deemed necessary to assess the levels and duration of L-carnitine supplementation necessary for maintaining steady state concentrations of carnitine in the blood. Unfortunately, such information is not available in the literature[3-5] and our earlier reports are confounded by the presence of D-carnitine.[2] Using D,L-carnitine, it was found that blood carnitine steady state concentrations were attained after three days of feeding diet supplemented at the level of 0.01 (wt/wt); however, longer than five days were needed at the 0.0025, 0.005, and 0.0075 levels.[2]

Therefore, the objectives of this study were to establish levels and duration of L-carnitine supplementation for blood carnitine steady state concentrations and to determine early changes in portal and systemic BEC following intraduodenal administration of ethanol in these rats.

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Address reprint requests to Dr. Sachan at the Department of Nutrition and Food Sciences, College of Human Ecology and Agricultural Experiment Station, The University of Tennessee, Knoxville, TN 37996-1900, USA.
*Dr. Berger's present address is Research Chemist, Assay Laboratories, Sigma Chemical Co., St. Louis, MO 63178.
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Table 1 Supplementary levels of L-carnitine in diet and the effect on weight gain and urine output

	Nonsupplemented (NS) group	L-carnitine supplemented (CS) groups			
		0.0025	0.005	0.0075	0.01
No. of animals	4	4	4	4	4
Duration of treatment (days)	10	10	10	10	10
Initial weight (g)	300.3 ± 18.7[a]	295.8 ± 13.8[a]	297.3 ± 14.9[a]	301.0 ± 15.0[a]	298.3 ± 13.0[a]
Weight gain (g)	20.3 ± 2.4[a]	20.0 ± 0.6[a]	18.0 ± 3.4[a]	14.0 ± 3.4[a]	16.5 ± 2.5[a]
Urine output (mL/24 hr)	11.9 ± 0.3[a]	12.0 ± 0.6[a]	12.9 ± 0.7[ab]	13.7 ± 0.5[b]	14.3 ± 0.4[b]

Note: Values are mean ± SEM. Values in a row bearing the common superscripts were not significantly different ($P < 0.05$).

Materials and methods

Animals and diet

Twenty male Sprague Dawley rats (Harlan, Inc., Indianapolis, IN) of about 300 g body weight were divided into five groups, each consisting of four animals (*Table 1*). The animals were housed in wire mesh, stainless steel metabolic cages in the AAALAC accredited small animal facility. All animals were provided feed and water ad libitum. The diet consisted of Purina Rodent Chow #5001 (Ralston Purina Co., St. Louis, MO), which was fed as such to the nonsupplemented (NS) group and to the carnitine supplemented (CS) groups after mixing with 0.0025, 0.005, 0.0075, or 0.01 (wt/wt) L-carnitine (Sigma Tau, Rome, Italy) for a period of 10 days. The indigenous concentration of total L-carnitine in the nonsupplemented diet was 64 nmol L-carnitine/g of the chow. About 0.54 (54%) of the total carnitine was long-chain acylcarnitine and remaining was nonesterified carnitine.

Sample collection

Blood and urine samples were collected from the NS and CS animals on the day preceding carnitine supplementation and on days 1, 3, 5, 7, and 10 following supplementation. The 24-hour urine sample from each animal was collected in an Erlenmeyer flask containing five crystals of thymol as a preservative. Each collection was measured and frozen at −70° C for carnitine determination. At the end of each collection period, blood was drawn from the dorsal tail vein in a 20 μL capacity heparinized micro-hematocrit capillary tube (Clay Adams, Parsippany, NJ). The blood from the capillary tube was delivered into a 1.5 mL capacity microfuge tube (Walter Sarstedt, Inc., Princeton, NJ) and immediately frozen at −70° C for carnitine determination in the whole blood.

Cannulation of portal vein and posterior vena cava

After 10 days of treatment, each animal was anesthetized by an intramuscular injection of a mixture of Ketaset® (Ketamine hydrochloride, Bristol Laboratories, Syracuse, NY) and Acepromazine® (Ayerst Laboratories, New York, NY) in a 5:1 ratio. The doses of Ketaset® (182.3 μM/kg) and Acepromazine® (30.6 μM/kg) were calculated on the basis of body weight. The hair was shaved from the abdomen, extending from the upper torso to the pelvic region. The abdominal wall was opened exposing the liver, stomach, and duodenum, and the portal vein and posterior vena cava were catheterized. Catheters consisted of 0.5 mm i.d. × 0.92 mm o.d. Silastic® Medical-Grade Tubing (Dow Corning Corp., Midland, MI) with attached 27 gauge 12.5 mm needle (Beckman Dickinson Co., Rutherford, NJ). A dose of ethanol (65.2 mM/kg in a 0.13 aqueous solution) was infused into the duodenum at the rate of 1 mL/m using a peristaltic pump (Gilson Medical Electronics, Inc., Middletown, WI). One end of a 3.12 mm i.d. × 4.69 mm o.d. Tygon® R-3603 Silastic tubing (Fisher Scientific Co., Pittsburgh, PA) was attached to the peristaltic pump and the other end to a blunt 19 gauge needle which was placed at the gastroduodenal opening through stomach wall, and secured in place with a 00 silk suture.

Serial portal and systemic blood samples were collected in 20 μL capacity microhematocrit tubes (Clay Adams, Parsippany, NJ) through the indwelling catheters up to 30 min postduodenal ethanol infusion. Blood samples were treated as described earlier. At the end of portal and systemic blood sampling, a 50–100 mg of medial quadriceps muscle was quickly removed and frozen at −70° C. The animal was killed by decapitation.

Analysis

Blood, muscle, and urine samples were analyzed for nonesterified carnitine (NEC), acid soluble acylcarnitine (ASAC), and acid insoluble acylcarnitine (AIAC) according to the radioisotopic procedure of Cederblad and Linstedt[6] as modified by Sachan et al.[7] and adapted for a 20 μL sample of whole blood. The sum of the three carnitine fractions was called total carnitine (TC). Ethanol was determined according to the enzymatic procedure of Bernt and Gutmann[8] with modification to accommodate 20 μL blood sample.[2]

Statistics

All data were expressed as mean ± SEM and differences among the groups were determined by analysis

of variance and Duncan's new multiple range test. Correlations were calculated by linear regression and the statistical significance ($P < 0.05$) was established by the analysis of variance.[9]

Results

The weight gains were not significantly different among the groups (*Table 1*).The 24 hr urine output was significantly higher in the 0.0075 and 0.01 CS groups compared to the NS and the 0.0025 CS group, but not when compared to the 0.005 CS group.

Changes in concentrations of the blood carnitine fractions in relation to the levels and durations of carnitine supplementation are shown in *Figure 1*. The data for the 0.0075 CS group have been omitted from *Figure 1* because they were not significantly different from those of the 0.005 CS group. The concentrations of all carnitine fractions in the NS animals remained unchanged during the 10-day period. However, the blood concentrations of TC and NEC were significantly elevated after one day of feeding 0.0025, 0.005, 0.0075, or 0.01 CS diet. The peak concentrations of TC and NEC were reached after three days of feeding 0.005 CS diet and neither higher supplementary levels nor longer durations of feeding CS diets significantly elevated blood concentrations of these carnitine fractions. The blood ASAC were significantly increased (10–20 μM/L) after one day of feeding 0.0025 and 0.005

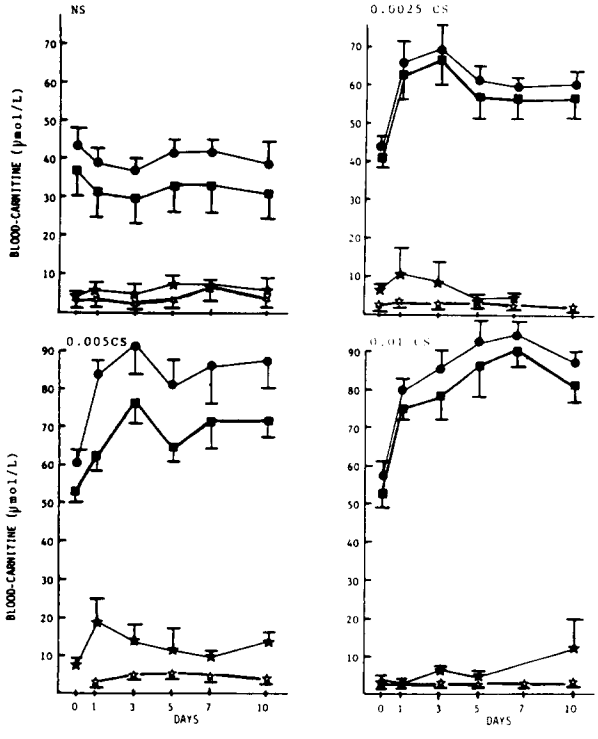

Figure 1 Effects of duration of L-carnitine supplementation on blood total carnitine (●), nonesterified carnitine (■), acid soluble acylcarnitine (★), and acid insoluble acylcarnitine (☆) during 10 days of feeding nonsupplemented (NS), and 0.0025, 0.005, or 0.01 carnitine supplemented (CS) diets to the rat. Each point represents group mean ± SEM.

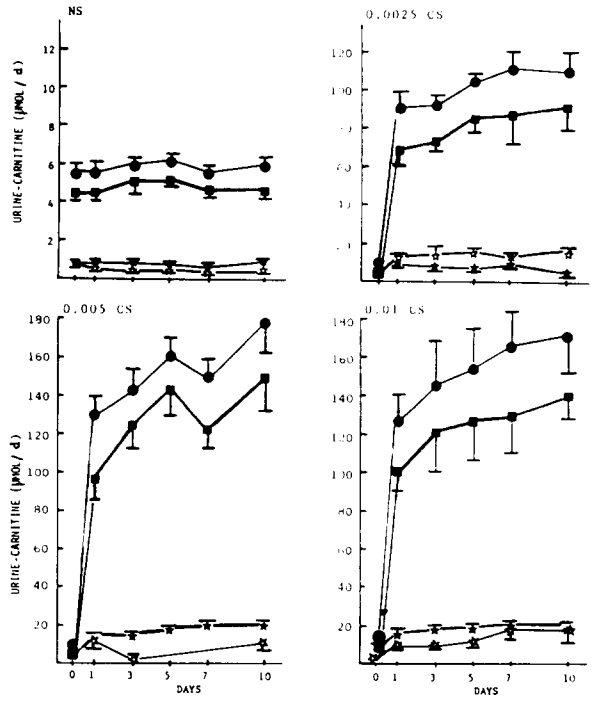

Figure 2 Effects of duration of L-carnitine supplementation on urinary total carnitine (●), nonesterified carnitine (■), acid soluble acylcarnitine (★), and acid insoluble acylcarnitine (☆) during 10 days of feeding nonsupplemented (NS), and 0.0025, 0.005, or 0.01 carnitine supplemented (CS) diets to the rat. Each point represents group mean ± SEM.

L-carnitine and declined thereafter but not significantly. The blood AIAC concentrations were not significantly altered by L-carnitine supplementation at any level or duration of supplementation.

The 24-hour excretion of carnitine fractions in the urine of NS and CS animals in relation to the levels and durations of carnitine supplementation is shown in *Figure 2*. The amounts of TC, NEC, ASAC, and AIAC were not significantly altered in the NS animals during the 10-day experimental period. However, in all CS groups, the 24 hr excretion of all carnitine fractions was significantly elevated after one day of feeding CS diet and remained elevated for the rest of the period. The apparent peak of TC and NEC excretion was after five days of feeding 0.005 CS diet. The 5-day value was accepted because it was not significantly different from either the 3-day or 10-day value which were significantly different between themselves. Furthermore, higher levels or longer durations of carnitine supplementation did not significantly increase 24-hour carnitine excretion. The concentrations of ASAC were relatively higher than those of AIAC in urine of all groups. The CS animals excreted significantly higher amounts of ASAC and AIAC than the NS animals. The peak excretion of the acylcarnitines was reached after one day of feeding CS diets and was not changed significantly during the rest of the 10-day period. Unlike blood and urinary compartments, the carnitine concentration in the quadriceps muscle was not significantly affected by the supplementary doses of L-

Table 2 Blood-ethanol concentration (mmol/L) in portal and systemic blood following a duodenal infusion of ethanol in rats fed diets with varying levels of supplementary L-carnitine

Minutes post-ethanol infusion	Nonsupplemented (NS) group	L-carnitine supplemented (CS) groups			
		0.0025	0.005	0.0075	0.01
			Portal		
5	128.5 ± 7.3[a]	133.3 ± 9.1[a]	70.6 ± 8.0[b]	145.2 ± 40.2[ab]	70.9 ± 13.9[b]
10	190.2 ± 16.1[a]	207.2 ± 19.8[a]	132.3 ± 11.9[b]	142.2 ± 40.0[ab]	98.9 ± 12.6[b]
15	125.6 ± 16.8[a]	144.3 ± 12.3[a]	100.4 ± 8.0[a]	150.2 ± 44.1[a]	121.9 ± 19.6[a]
20	107.6 ± 21.5[a]	106.0 ± 12.1[a]	74.4 ± 8.0[a]	150.4 ± 41.0[a]	124.2 ± 29.3[a]
30	74.4 ± 11.2[a]	115.8 ± 11.4[a]	82.5 ± 12.2[a]	113.5 ± 28.0[a]	116.2 ± 25.1[a]
			Systemic		
5	25.0 ± 5.5[a]	55.6 ± 9.5[a]	45.7 ± 3.1[b]	43.8 ± 6.5[b]	46.5 ± 8.8[b]
10	40.8 ± 2.7[a]	67.0 ± 8.8[b]	56.2 ± 2.3[b]	67.8 ± 5.9[b]	57.1 ± 5.0[b]
15	47.5 ± 2.7[a]	72.2 ± 5.5[b]	59.9 ± 4.2[b]	68.0 ± 4.3[b]	62.7 ± 5.6[b]
20	51.9 ± 5.9[a]	73.3 ± 5.7[b]	64.0 ± 7.8[b]	67.2 ± 8.7[b]	67.8 ± 4.2[b]
30	36.6 ± 6.4[a]	57.2 ± 0.7[b]	56.0 ± 4.9[b]	61.8 ± 5.0[bc]	69.9 ± 1.9[c]

Note: There were four rats in each group. All animals were fed ground Purina Rodent Chow #5001 with or without various levels of supplementary carnitine for 10 days prior to ethanol infusion. The values are group means ± SEM. Values in a line bearing the same superscript letter are not statistically different ($P \geq 0.05$).

carnitine at the end of the 10-day period (data not shown).

Portal and systemic blood ethanol concentrations following a duodenal infusion of ethanol are shown in *Table 2*. Although portal blood-ethanol concentrations did not differ statistically between the NS and CS groups over the 30-minute period, systemic blood-ethanol concentrations were significantly elevated in all CS groups compared to the NS group. However, the systemic blood-ethanol concentrations of the CS groups did not differ significantly among themselves (except for the 0.0075 CS and 0.01 CS animals at 30 min) and remained significantly higher than in the NS group at all times. In both NS and CS groups, systemic blood-ethanol concentrations peaked at 20 min and declined at 30 min at the rates inversely related to the levels of carnitine supplementation. This decrease in systemic blood-ethanol concentration between 20 and 30 min post-ethanol infusion diminished with increasing L-carnitine supplementation; NS (-0.42) < 0.0025 CS (-0.28) < 0.005 CS (-0.14) < 0.0075 CS (-0.09) < 0.01 CS ($+0.03$).

Discussion

The feeding of diets supplemented with L-carnitine at 0.0025, 0.005, 0.0075, or 0.01 (wt/wt) significantly elevated blood carnitine concentrations after one day of feeding; however, the peak concentrations were reached after feeding the CS diets for three days (*Figure 1*). The peak concentrations of TC attained by 0.005 supplementary level (90 μM/L) and NEC (80 μM/L) were not significantly altered by higher levels or longer durations of feeding CS diets. Therefore, it is concluded that feeding of 0.005 CS diet for three days is adequate to attain peak and steady state concentrations of carnitine in blood of the rat. These carnitine concentrations are within the range reported by other investigators[10,11] as well as our earlier report using D,L-carnitine,[2] where blood carnitine steady state concentration was reached after five days of feeding 0.01 D,L-carnitine supplemented diet. Thus L-carnitine is twice as efficacious as the D,L-carnitine in achieving peak blood carnitine concentrations.

The mean urinary excretion of TC and NEC (6 and 4 μmol/day · 0.320 g bw) in the NS animals is well within the expected range of 1–2 μM carnitine excretion per day per 100 g body weight quoted in literature.[5] In the CS animals, levels and duration of carnitine supplementation affected urinary carnitines akin to the blood carnitines (*Figures 1* and *2*). The daily excretion of TC and NEC (about 160 and 140 μM/24 hr, respectively) peaked after five days of feeding 0.005 CS diet because it was neither significantly different from the 3- and 10-day excretion in the 0.005 group nor from those supplemented with 0.0075 or 0.01 L-carnitine (*Figure 2*). This increase amounted to 26–28-fold greater than that found in the NS group. Even at these levels, only about 0.30–0.35 of the ingested carnitine was excreted in urine which is consistent with reported values.[11] The excess carnitine at the higher levels of supplementation was either not fully absorbed or compartmentalized in tissues other than skeletal muscle. The fate of high doses of carnitine is not really known. There is some evidence that over 40% of orally administered carnitine may be lost in urine and feces as carnitine or its metabolites due to action of gut microorganisms.[12] Because there was a significant positive correlation between the urinary and blood TC ($r = 0.83$) and NEC ($r = 0.79$), it is proposed that the urinary carnitine profiles are good indicators of blood carnitine profiles under these conditions.

None of the supplementary levels of carnitine feeding for 10 days altered skeletal muscle-carnitine concentrations. A longer duration of feeding would be expected to increase muscle carnitine as indicated by our earlier observations, where 0.01 D,L-carnitine supple-

mentation for 56 days resulted in significantly higher carnitine concentrations in skeletal muscles of CS than NS chronic alcoholic rats (unpublished). Thus, 10-day feeding of 0.005 or higher levels of L-carnitine supplemented diet was sufficient to cause significant increases in the carnitine concentrations in the blood and urine, but it was not administered long enough to alter carnitine concentrations of the skeletal muscle.

Pharmacokinetics of ethanol in the CS animals reported earlier[2] showed that the rates of ethanol appearance in the first 60 min post-ethanol administration were not significantly different between NS and CS rats. However, the peak concentrations were not reached at the same time in the two groups which left room for some concern over the conclusion that carnitine affected only the metabolism and not at all the absorption of ethanol.[2] Therefore, ethanol concentrations were monitored simultaneously in the portal and systemic blood of NS and CS rats (*Table 2*). Initial rates of ethanol appearance in the portal blood were not significantly different between NS and CS groups (26 vs. 27 mM/L.m). However, the appearance of ethanol in the systemic blood of the CS animals was more than doubled compared to that found in the NS rats (11 vs. 4 mM/L.m) during the same period of time. The rates of ethanol disappearance from the systemic blood of the various CS groups (0.0025–0.01) ranged from 1.8–2.8 mM/L.m compared to 3.8 mM/L.m found in the NS group. Because these disappearance rates were significantly slower in all CS groups than the NS group, the data presented here lead to the conclusion that carnitine attenuates ethanol metabolism without altering the rates of ethanol absorption from the small intestine. It is hypothesized that carnitine may modulate ethanol transport and/or ethanol metabolizing enzyme systems.

In summary, feeding of 0.005 L-carnitine supplemented diet for three days produced peak blood carnitine concentrations and the urinary carnitine profiles closely resembled blood carnitine profiles. Supplementary L-carnitine did not alter ethanol absorption from the small intestine; however, it did retard ethanol metabolism.

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