Enhanced lipid utilization in infants receiving oral L-carnitine during long-term parenteral nutrition

Fourteen infants requiring long-term total parenteral nutrition but able to tolerate small quantities of enteral feedings were randomized into carnitine treatment and placebo control groups. All infants had received nutritional support devoid of carnitine. Plasma carnitine levels and observed plasma lipid indices were not different before supplementation. Under standardized, steady-state conditions, 0.5 g/kg fat emulsion (Intralipid) was administered intravenously over 2 hours both before and after infants received 7 days of continuous nasogastric or gastric tube L-carnitine (50 μmol/kg/day) or placebo. Plasma triglyceride, free fatty acid, acetoacetate, β-hydroxybutyrate, and carnitine concentrations were observed at 0 (start of lipid infusion), 2, and 4 hours for pre- and post-treatment periods, and in addition at 6 and 8 hours after carnitine supplementation. Infants receiving carnitine had significantly greater β-hydroxybutyrate plasma concentrations ($P < 0.05$) and carnitine ($P < 0.001$) at 0, 2, 4, 6, and 8 hours, and greater plasma acetoacetate concentrations ($P < 0.05$) at 2, 4, 6, and 8 hours, compared with controls. Twenty-four-hour urinary carnitine excretion was very low for both groups before supplementation; after supplementation, excretion was higher ($P < 0.05$) in the carnitine group. No significant differences were found between groups for plasma triglyceride or free fatty acid concentrations at any observation period. This study demonstrated enhanced fatty acid oxidation, as evidenced by increased ketogenesis, with L-carnitine supplementation in infants receiving long-term total parenteral nutrition. (J Pediatr 1986;109:984-8)

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Carnitine is a quarternary amine with the primary biochemical role of facilitating long chain fatty acid transport into the mitochondrial matrix, where beta oxidation occurs.[1] In the healthy adult or child, L-carnitine is synthesized in the liver and kidney from lysine and methionine.[2] Studies have suggested that fatty acid oxidative capacity is limited in small infants, which may relate to low acylcarnitine and free carnitine levels.[3-6] Carnitine is transported by the placenta in amounts adequate to support metabolism in the fetus.[7] However, rapid decreases

ANOVA	Analysis of variation
BOB	β-Hydroxybutyrate
FFA	Free fatty acids
TPN	Total parenteral nutrition

Supported in part by Grant 83487 from Abbott Laboratories.

Presented at the National Meeting of the Society for Pediatric Research, May 7, 1985.

Submitted for publication Dec. 30, 1985; accepted July 8, 1986.

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Table. Study population demographic data

Patient	Sex	Gestational age (wk)	Gestational development	Postnatal age (day)	Birth weight (kg)	Weight* (kg)	Plasma carnitine* (nmol/ml)	Diagnosis
Control								
1	F	35	AGA	78	2.05	2.87	7.6	Necrotizing enterocolitis
2	M	36	SGA	127	1.39	2.73	17.2	Tracheoesophageal fistula
3	M	40	AGA	78	3.29	4.32	6.2	Intractable diarrhea of infancy
4	M	34	AGA	57	2.00	2.82	3.9	Diaphragmatic plication
5	M	30	AGA	178	1.20	3.64	23.9	Midgut volvulus
7	M	33	SGA	58	1.15	1.77	3.9	Necrotizing enterocolitis
13	F	27	AGA	137	0.70	2.56	2.0	Necrotizing enterocolitis
Mean ± SD†		33.6 ± 4.2		102 ± 46	1.68 ± 0.86	2.96 ± 0.81	9.2 ± 8.2	
Treatment								
6	M	40	AGA	292	3.30	4.37	21.0	Failure to thrive
8	M	40	AGA	136	3.22	4.81	7.6	Ileitis
9	M	40	AGA	71	3.04	4.42	7.0	Gastroenteritis
10	F	30	AGA	39	1.46	2.11	12.0	Gastroschisis
11	F	40	SGA	42	2.42	3.66	9.4	Gastroschisis
12	F	34	AGA	48	1.85	2.47	3.8	Necrotizing enterocolitis
14	M	32	AGA	40	1.83	2.49	6.7	Necrotizing enterocolitis
Mean ± SD†		36.6 ± 4.4		95 ± 93	2.45 ± 0.75	3.48 ± 1.11	9.6 ± 5.6	
Population total		35.1 ± 4.4		99 ± 71	2.06 ± 0.88	3.22 ± 0.97	9.4 ± 6.7	

*On entrance to study.
†No significant differences between treatment and control groups for any parameter.

in plasma carnitine levels occur during the first 3 days after birth if no exogenous carnitine is given.[8] Decreased tissue levels of carnitine have been reported in infants after more than 15 days of TPN.[9] There are no data regarding the age when endogenous synthesis of carnitine will suffice to meet the needs of infants. We found plasma carnitine concentrations to be very low in older infants who had received TPN from birth. Therefore, we investigated carnitine deficiency and the effects of L-carnitine supplementation on fat clearance and utilization, plasma carnitine concentrations, and urinary excretion of carnitine in infants receiving parenteral nutrition for extended periods of up to 9 months.

METHODS

Patients. Fourteen infants, with a mean gestational age of 35.1 ± 4.4 (SD) weeks, postnatal age of 14.1 ± 10.1 weeks, and entrance weight of 3.22 ± 0.97 kg, were included in the study (Table). There were no significant differences for any demographic variable between treatment and control groups. All infants required a minimum of seven additional days of parenteral nutrition from time of study enrollment, were able to tolerate small quantities of enteral feedings, and had received nutritional support devoid of L-carnitine. All had received prolonged courses of TPN (10 of 14 subjects had received lifelong TPN, and all received TPN for longer than 1 month) before entrance into the study. Infants with limited enteral intake (<15% of caloric need) could have consumed only formulas containing no carnitine (Pregestimil, Nutramigen, Portagen, all Mead Johnson & Company, Evansville, Ind.)[10]

Carnitine supplementation. Patients were randomized by use of a set of randomly generated numbers into carnitine treatment and placebo control groups. The investigators and primary care physicians were blinded regarding the treatment regimen. Only the preparative pharmacist was aware of actual carnitine intake. After completion of a 2-hour lipid infusion ("bolus") and observations for plasma carnitine and lipid indices, study infants received either L-carnitine (Sigma Chemical Co., St. Louis) 50 μmol/kg/day (10 mg/kg/day) or placebo, via continuous nasogastric or gastric tube for 7 days. The placebo used was a pharmacy-prepared infant electrolyte solution, the same used as the diluent for the L-carnitine. L-Carnitine supplementation or placebo was continued until the termination of a second lipid bolus.

Parenteral nutrition and fat infusions. Parenteral nutrition fluids consisted of 2.5 to 3.0 g/kg/day protein (FreAmine III, Kendall McGaw, Irvine, Calif.), 2 to 3 g/kg/day lipid emulsion (Liposyn, Abbott Laboratories, North Chicago, Ill.), dextrose, electrolytes, vitamins, minerals, and trace elements. Most nutrients were received

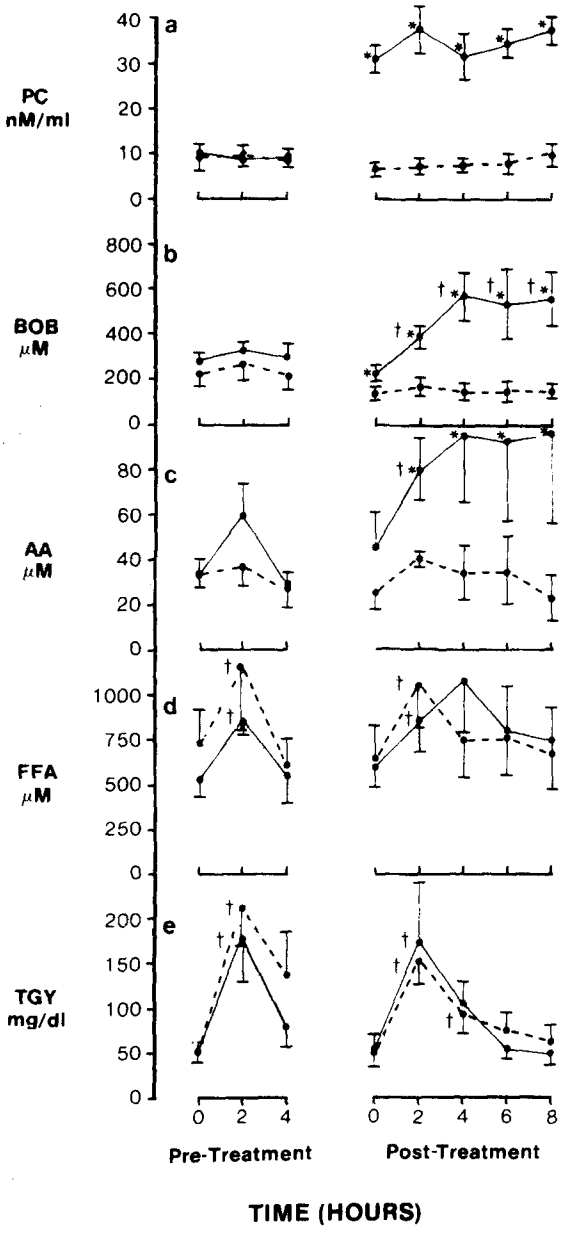

Figure. Mean ± SEM for treatment (●—●) and control (●- - -●) infants receiving lipid infusion (0.5 g/kg) at time 0 through 2 hours. Treated infants received 50 μmol/kg/d L-carnitine for 7 days.* Statistical significance between means of carnitine treatment versus placebo control groups for observed times. †Statistical significance within either group comparing time 0 (pre or post) and total plasma carnitine *(PC)* concentrations **(a)**, plasma β-hydroxybutyrate *(BOB)* concentrations **(b)**, plasma acetoacetate *(AA)* concentrations **(c)**, plasma free fatty acid *(FAA)* concentrations **(d)**, and plasma triglyceride *(TGY)* concentrations **(e)**.

parenterally, but limited (<15%) amounts were supplied enterally as Pregestimil, Nutramigen, Portagen, or infant electrolyte/dextrose solution (Pedialyte, Ross Laboratories, Columbus, Ohio).

Infants received a lipid bolus of 0.5 g/kg over 2 hours both before (day 1) and after (day 8) carnitine supplementation. For 16 hours before the lipid infusion and continuing throughout each observation period, continuous lipid infusion or lipid-containing feedings were discontinued, and the carbohydrate concentration in the parenteral nutrition solution remained or was reduced to 10% (8 to 10 g/kg/day glucose) to achieve a stable insulin output. These measures promoted optimal assessment of lipid-induced ketone body production.

Biochemical measurements. On day 1 of the study, blood was collected for plasma carnitine, β-hydroxybutyrate, acetoacetate, free fatty acid, and triglyceride concentrations at 0, 2, and 4 hours from the start of the lipid bolus. A 24-hour urine collection for carnitine excretion was completed before initiating supplementation with L-carnitine. On day 8, the blood measurements were obtained at 0, 2, 4, 6, and 8 hours from the start of the lipid bolus. A second 24-hour urine collection for carnitine excretion was completed just before the lipid bolus.

Blood was collected in heparinized tubes, placed on ice, and plasma immediately separated by centrifugation. The plasma was either immediately frozen or processed to a point at which storage would not result in degradation. Triglyceride concentrations were measured by an enzymatic assay with correction for free glycerol.[11] FFA levels were determined by a microfluorometic enzymatic assay sensitive to 10 μmol/L.[12] Acetoacetate and BOB concentrations were determined by microfluorometric enzymatic assays sensitive to 1 μmol/L.[13] Plasma carnitine concentrations were determined by a modification of the method of Cederblad and Lindstedt.[14]

Statistics. Statistical analyses were performed using the Student t test for determining differences in means between groups, Wilcoxon signed rank test for differences within groups, chi-square analysis for differences in proportions, and ANOVA for repeated variables for assessing the contribution of selected factors to variation of data. Significance was established at $P < 0.05$.

The research study was approved by the Institutional Review Board of the University of Tennessee and Le Bonheur Children's Medical Center; an Investigational New Drug application was approved for L-carnitine liquid prior to review. Parental informed consent was obtained before enrollment.

RESULTS

Plasma and urinary carnitine values. Plasma carnitine levels were very low at time 0, did not change over 4 hours, and were not different between groups in the prestudy period (Figure). On study day 8, after supplementation, values from the treatment group were three and one half to five times higher than those from the placebo control group

(P <0.001) and three to four times higher than pretreatment values (P <0.001). Plasma carnitine concentrations for the control group did not change from day 1 to day 8. Likewise, prestudy values of urinary carnitine excretion did not differ significantly between the control (2.87 ± 3.56 μmol/24 hr) and treatment groups (2.17 ± 1.09 μmol/24 hr), and both were very low. After 1 week of carnitine supplementation, urinary excretion was significantly higher for the treatment group than for controls (P <0.05) (26.38 ± 19.1 μmol/24 hr vs 2.55 ± 1.32 μmol/24 hr). Paired analysis revealed significant increases (P <0.05) in urinary excretion of carnitine from pre- to post-supplementation sampling, whereas no changes were demonstrated for the placebo group.

Fat utilization. On day 1, before carnitine supplementation, the 2-hour fat infusion did not result in significant increases in plasma BOB or acetoacetate concentrations. No differences were found between treatment and control groups in this regard (Figure). The two groups had similar increases in plasma triglyceride and FFA levels after the fat infusion (Figure).

On day 8, after carnitine supplementation, treated infants had significantly (P <0.05) greater plasma concentrations of BOB at all observed times, and greater concentrations of acetoacetate at 2, 4, 6, and 8 hours after the fat bolus than did control infants. In the control infants, plasma BOB and acetoacetate concentrations remained low, near baseline values after the 2-hour fat infusion, whereas both ketone bodies increased significantly (BOB at 2, 4, 6, and 8 hours; acetoacetate at 2 hours; P <0.05) after the fat infusion in the treatment group (Figure). Plasma triglyceride and FFA concentrations did not differ between groups. Peak plasma triglyceride levels occurred for both groups at the end of the 2-hour lipid infusion. FFA levels peaked at 2 hours in the placebo group and at 4 hours in the treatment group.

DISCUSSION

Most studies evaluating L-carnitine supplementation have focused on the neonate.[3-6, 15-18] We chose to investigate the supplementation of L-carnitine in older infants, because random observations in this population receiving long-term TPN showed extremely low plasma carnitine concentrations. We have demonstrated increased BOB and acetoacetate production in infants receiving carnitine supplementation during TPN. Previous studies have shown a positive correlation between acylcarnitine and total plasma carnitine and BOB in preterm infants,[15] and a positive correlation between acylcarnitine and BOB with preinfusion total plasma carnitine levels.[3,15] These same investigators could not show significant increases in ketone body production, except as expressed as a decrease in FFA/BOB ratio in preterm infants receiving L-carnitine.

The results of this investigation are consistent with those of others demonstrating improved lipid utilization in neonates receiving carnitine supplementation. Age and the duration of carnitine abstinence appear to play a role in determining poor lipid utilization,[15, 16] and studies that have failed to demonstrate an improvement probably reflect inadequate repletion with the short-term use of carnitine.[17] The results of some may have been affected by the simultaneous infusion of glucose as a major source of energy.[18]

In contrast to the popualtion of Schmidt-Sommerfeld et al.,[15] our study subjects were older (more than 3 months of age on average, and all more than 4 weeks of age) and had been receiving carnitine-free nutrition since birth. Schmidt-Sommerfeld's study population consisted of appropriate for gestational age premature newborn infants (2 days of age) requiring TPN because of intolerance to feedings, but who had a mean duration of TPN of less than 1 week. Because of the short duration of carnitine abstinence, total plasma carnitine concentrations were higher than in our population (all >8 nmol/ml). Our study included both small for gestational age (n = 3) and appropriate for gestational age (n = 11) infants requring TPN for at least 1 month. Their carnitine status was consistent with profound deficiency, as evidenced by extremely low plasma carnitine concentrations and urinary carnitine excretion. These differences in our study population likely improved our recognition of carnitine-related alterations in fat utilization.

Our results show that orally administered carnitine supplementation (50 μmol/kg/d) increased total plasma carnitine concentrations, but values remained at the lower end of the normal age for similarly aged infants being breast-fed or receiving formula containing L-carnitine.[4, 19, 20] Higher orally administered doses of L-carnitine may be useful in future studies. The total plasma carnitine values of the placebo and pretreatment groups were very low, consistent with symptomatic systemic carnitine deficiency.[21, 22] The average plasma concentrations were lower than any cited in the literature for preterm or term infants (nine of 14 infants on enrollment with total plasma carnitine values of <8 nmol/ml), again reflecting longer deprivation. Urinary excretion of carnitine was also very low, supporting the conclusion that these infants were depleted not only in plasma but in tissue. The increase in carnitine excretion to values more typically observed in infants receiving carnitine in the diet indicates repletion of tissue stores, which may not have been the case in studies of short-term carnitine supplementation.[17]

One interesting observation in our study was the increased mean plasma BOB concentrations seen in the treatment groups after L-carnitine supplementation but before the 2-hour fat infusion. The treatment and control

groups had similar ketone body responses in the pretreatment period. Therefore the significant difference in mean BOB concentrations at time 0 in the posttreatment period likely represents modestly improved endogenous fat utilization in the carnitine-supplemented group in response to caloric reduction, rather than some underlying population difference. No difference in acetoacetate concentrations was found prior to fat loading.

Solutions currently used for intravenous alimentation contain no carnitine,[2] although they contain all precursor materials required for endogenous production. Infants maintained with parenteral nutrition solutions have decreased total plasma carnitine concentrations.[3-6, 8-9] Decreased tissue levels of carnitine have also been found in neonates receiving TPN for more than 15 days.[9] Our data suggest that not only preterm infants but also term infants receiving long-term TPN are incapable of producing adequate quantities of carnitine. It remains to be determined whether this inability to generate carnitine relates to continued immaturity in endogenous biosynthesis, selective inhibition of synthesis by a component or components of the nutritional solution, or alterations in liver function (common in infants receiving long-term TPN).[23]

In this study, infants as old as 9 months of age receiving long-term TPN, with or without small quantities of oral feedings devoid of L-carnitine, had plasma carnitine levels and total urinary carnitine values consistent with those observed in carnitine deficiency. The age of transition from dependence on to independence of exogenous carnitine remains unclear; however, in the sick infant receiving TPN, it may not occur until after 9 months. Orally administered supplementation with L-carnitine resulted in significant increases in concentrations of plasma carnitine, repletion of tissue stores (significant rises in urinary excretion), and enhanced ketogenesis. Thus this patient population should be considered for L-carnitine supplementation.

REFERENCES

1. Bremer J. Carnitine metabolism and functions. Physiol Rev 1983;63:1420-80.
2. Borum PR. Carnitine. Ann Rev Nutr 1983;3:233-59.
3. Schmidt-Sommerfeld E, Penn D, Wolf H. Carnitine blood concentrations and fat utilization in parenterally alimented premature newborn infants. J PEDIATR 1982;100:260-4.
4. Schiff D, Chen F, Seccombe D, et al. Plasma carnitine levels during intravenous feeding of the neonate. J PEDIATR 1979; 95:1043-6.
5. Yeh Y, Cooke RJ, Zee P. Impairment of lipid emulsion metabolism associated with carnitine insufficiency in premature infants. J Pediatr Gastroenterol Nutr 1985;4:795-8.
6. Takahashi M, Sawaguchi S. Lipid metabolism in parenterally alimented neonates: carnitine blood concentrations and fat utilization. Indian J Pediatr 1983;50:161-8.
7. Schmidt-Sommerfeld E, Penn D, Sodha RJ, Progler M, Novak M, Schneider H. Transfer and metabolism of carnitine and carnitine esters in the in vitro perfused human placenta. Pediatr Res 1985;19:700-6.
8. Novak M, Monkus EF, Chung D, et al. Carnitine in the perinatal metabolism of lipids. I. Relationship between maternal and fetal plasma levels of carnitine and acylcarnitines. Pediatrics 1981;67:95-100.
9. Penn D. Decreased tissue carnitine concentrations in newborn infants receiving total parenteral nutrition. J PEDIATR 1981; 98:976-8.
10. Borum PR, York CM, Broquist HP. Carnitine content of liquid formulas and special diets. Am J Clin Nutr 1979; 32:2272-6.
11. Kreutz FH. Enzymatische Glyzerinbestimmung. Klin Wochenschr 40:362, 1962.
12. Miles J. A microfluorometric method for determination of free fatty acids in plasma. J Lipid Res 1983;24:96.
13. Persson B. Determination of plasma acetoacetate and β-hydroxybutarate in newborn infants by an enzymatic fluorometric micro-method. Scand J Clin Lab Invest 1969; 25:918.
14. Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. Clin Chim Acta 1972;37:235-43.
15. Schmidt-Sommerfield E, Penn D, Wolf H. Carnitine deficiency in premature infants receiving total parenteral nutrition: effect of L-carnitine supplementation. J PEDIATR 1983; 102:931-5.
16. Orzali A, Donzelli F, Enzi E, et al. Effect of carnitine on lipid metabolism in the newborn. I. Carnitine supplementation during total parenteral nutrition in the first 48 hours of life. Biol Neonate 1983;43:186-90.
17. Orzali A, Maetzke E, Donzelli F, et al. Effect of carnitine on lipid metabolism in the neonate. II. Carnitine addition to lipid infusion during prolonged total parenteral nutrition. J PEDIATR 1984;104:436-40.
18. Curran JS, Williams PR, Kanarek KS, et al. An evaluation of orally supplemented L-carnitine in premature infants receiving Intralipid 20%. Acta Chir Schand [Suppl] 1983;517:157-64.
19. Warshaw JB, Curry E. Comparison of serum carnitine and ketone body concentrations in breast- and in formula-fed newborn infants. J PEDIATR 1980;97:122-5.
20. Novak M, Monkus EF, Burch M, et al. The effects of a L-carnitine supplemented soybean formula on the plasma lipids of infants. Acta Chir Scand [Suppl] 1983;517:149-55.
21. Duran M, deKlerk JBC, Wadman SK, et al. Systemic carnitine deficiency: benefit of oral carnitine supplements vs. persisting biochemical abnormalities. Eur J Pediatr 1984; 142:224-8.
22. Chapoy PR, Angelini C, Brown WJ, et al. Systemic carnitine deficiency: a treatable inherited lipid-storage disease presenting as Reye's syndrome. N Engl J Med 1980;303:1389-94.
23. Whitington PF. Cholestasis associated with total parenteral nutrition in infants. Hepatology 1985;5:693-6.