Plasma carnitine concentration and lipid metabolism in infants receiving parenteral nutrition

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The relationships among plasma total carnitine concentration, postnatal age, and fatty acid metabolism were evaluated in 57 infants receiving parenteral nutrition. Concentrations of plasma carnitine, triglycerides, free fatty acids, acetoacetate, and β-hydroxybutyrate were determined before and at 2 and 4 hours from the beginning of a standardized 2-hour lipid infusion. Plasma carnitine concentrations declined with increasing postnatal age. There were no significant differences in gestational age or triglyceride concentrations between infants ≤4 weeks of age and those > 4 weeks of age, whereas free fatty acid concentrations were lower and acetoacetate and β-hydroxybutyrate concentrations were higher in the younger infants. Infants ≤4 weeks of age were further grouped according to plasma carnitine concentration >13 nmol/ml (group 1) and ≤13 nmol/ml (group 2) and were then compared with infants >4 weeks of age (group 3). There were no significant differences in triglyceride concentrations among the three groups; free fatty acids, acetoacetate, and β-hydroxybutyrate concentrations for group 2 patients were similar to those of group 1 patients or fell between values for group 1 and group 3 patients. These results demonstrate decreasing plasma carnitine concentrations and possibly impaired fatty acid metabolism in infants maintained on carnitine-free nutrients for more than 4 weeks. (J PEDIATR 1989;115:794-8)

Carnitine is essential for the optimal oxidative metabolism of fatty acids because it facilitates the transport of long-chain acyl–coenzyme A esters across the mitochondrial membrane.[1] Neonates appear unable to synthesize sufficient carnitine to maintain plasma and tissue concentrations similar to those found in the fetus or in the adult, and must rely on dietary intake to maintain adequate carnitine stores.[2] Low plasma carnitine concentrations may indicate depletion of body carnitine stores and have been associated with impairment of fatty acid oxidation.[3, 4] Of particular concern are the low plasma carnitine concentrations observed in neonates receiving parenteral nutrition lacking carnitine.[3-5] It remains unclear when carnitine supplemen-

BOB	β-Hydroxybutyrate
FFA	Free fatty acids
PNA	Postnatal age

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tation may be beneficial for infants receiving carnitine-free nutrients. Therefore we evaluated the relationships among postnatal age, plasma carnitine concentration, and fatty acid metabolism in infants receiving parenteral nutrition.

METHODS

Fifty-seven infants (30 male), with a median PNA of 2.5 weeks (range 0.5 to 19.5 weeks), a median gestational age of 32 weeks (range 27 to 41 weeks), and a median weight 1.62 kg (range 0.64 to 4.81 kg), were included in the study. Infants with limited enteral intake (<15% of caloric intake) consumed only carnitine-free formulas (Pregestimil and Nutramigen, Mead Johnson & Co., Evansville, Ind.).[6]

The clinical diagnoses included gastrointestinal tract surgery and necrotizing enterocolitis (32 infants), respiratory distress syndrome (23), and failure to thrive with malnutrition (2). Infants with suspected or documented major organ dysfunction, sepsis, or metabolic disturbances or who were receiving drugs known to affect fat or carnitine metabolism (e.g., cortiosteroids, insulin, heparin, valproic acid) were excluded from the study.

Lipid utilization test. Sixteen hours before the lipid utilization test, continuous lipid infusion or lipid-containing feedings were discontinued. The dextrose concentration in the parenteral nutrition solution remained at, or was reduced to, 10% dextrose to achieve a stable insulin output and promote optimal assessment of lipid-induced ketone body production. Infants then received a lipid infusion of 0.5 gm/kg for 2 hours.

Heparinized blood was collected at 0, 2, and 4 hours from the start of the lipid infusion and placed on ice immediately, and the plasma was separated by centrifugation. A sample of plasma was immediately frozen and a second sample was extracted with 6% perchloric acid to prevent degradation of acetoacetate and β-hydroxybutyrate before freezing at $-70°$ C until analysis.

Plasma carnitine concentration was determined by a modification of the Cederbald and Lindstedt method.[7] Triglyceride concentration was measured by an enzymatic assay with correction for free glycerol.[8] Free fatty acids, acetoacetate, and BOB concentrations were determined by microfluoremetric enzymatic assays.[9, 10]

Statistics. Statistical analysis was performed with the Student t test for determining differences in means between groups. Analysis of variance with the Tukey multiple comparison test was used when more than two groups were compared. Simple linear regression analysis was used to determine relationships between patient characteristics and measures of lipid metabolism. Repeated measures analysis of varianace was used to determine whether differences occurred over time and whether a difference existed between groups. Significance was established at $p < 0.05$.

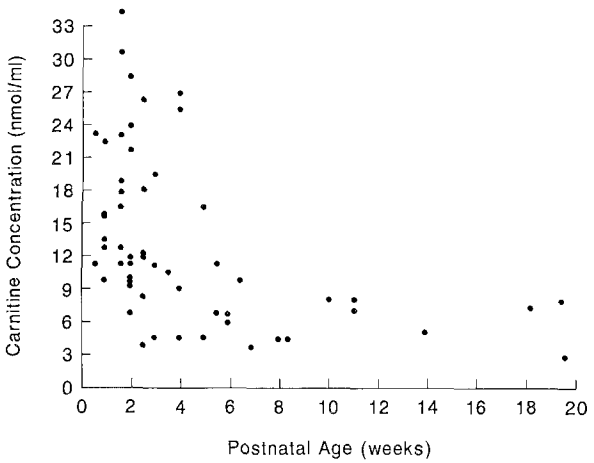

Fig. 1. Relationship between postnatal age and plasma carnitine concentration.

The research study was approved by the institutional review board, and parental informed consent was obtained before enrollment.

RESULTS

The 57 infants were receiving a mean protein intake of 2.22 ± 0.53 gm/kg/day and a mean caloric intake of 77.0 ± 17.1 kcal/kg/day at the time of study. Plasma carnitine concentrations (determined from the mean of the three observations) declined with increasing PNA (Fig. 1). There was no significant relationship between gestational age and plasma carnitine concentration for all infants ($r = 0.11$, $p = 0.41$) nor for infants <4 weeks of age ($r = 0.15$, $p = 0.36$). When weight, gestational age, and PNA were compared by linear regression analysis with measures of lipid metabolism (FFA, acetoacetate, and BOB concentrations), PNA had the best correlation (data not shown).

To assess the influence of PNA on lipid metabolism, we grouped infants on the basis of a PNA ≤4 weeks (n = 40) or >4 weeks (n = 17). There was no significant difference in gestational age between these two groups (31.6 ± 4.7 vs 33.2 ± 5.2 weeks, $p = 0.26$). All infants were receiving parenteral nutrition as clinically indicated, including glucose, amino acids, fats, electrolytes, minerals, and vitamins, and there were no significant differences between groups. Infants in the younger group weighed less (1.47 ± 0.70 vs 2.58 ± 1.16 kg) and had a higher plasma carnitine concentration (16.0 ± 7.8 vs 7.0 ± 3.3 nmol/ml). There was no significant difference in baseline triglyceride concentrations between the two groups (Fig. 2). After the 2-hour lipid infusion, there was a significant rise in the 2-hour triglyceride concentration for both groups, which by 4 hours had

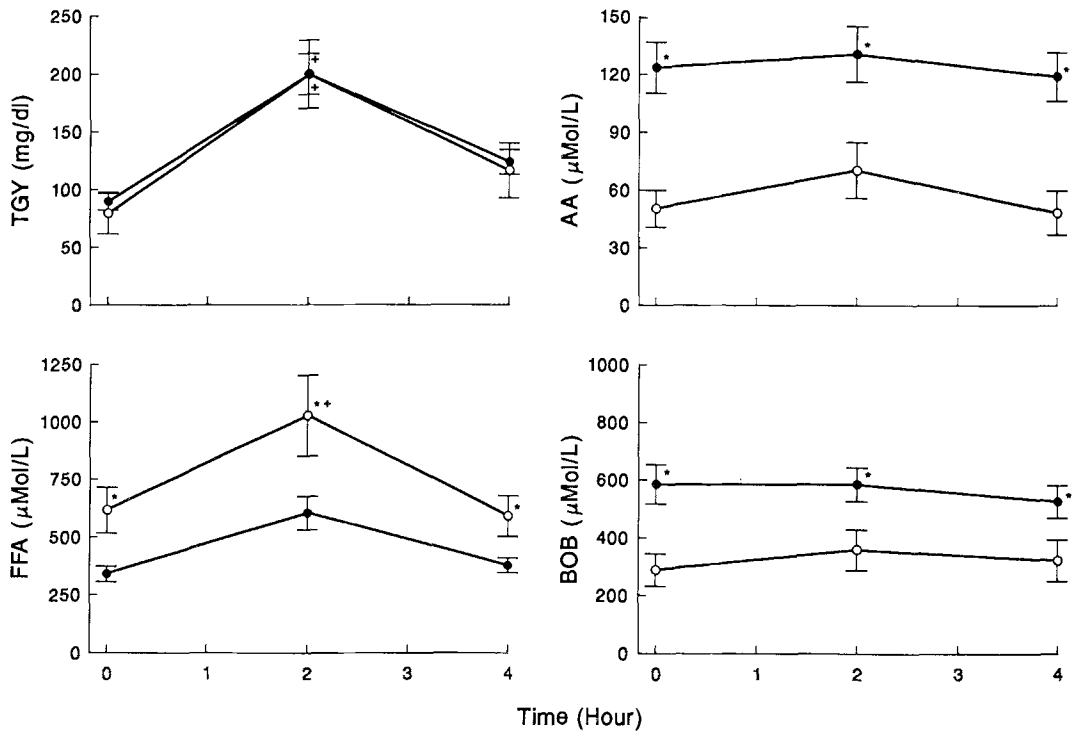

Fig. 2. Concentrations (mean ± SEM) for infants ≤4 weeks of age (●—●) and >4 weeks of age (○—○) who were receiving a lipid infusion (0.5 gm/kg) at time zero through 2 hours. *Asterisks* indicate significant difference between groups; *plus signs* indicate significant difference within either group compared with time zero. *AA*, Acetoacetate; *TGY*, triglycerides.

Table. Clinical characteristics and lipid metabolism in the study groups

	Group 1* (n = 19)	Group 2† (n = 21)	Group 3‡ (n = 17)
Gestational age (wk)	33.0 ± 4.9	30.5 ± 4.3	33.2 ± 5.2
PNA (wk)	1.9 ± 0.9	2.2 ± 1.0	9.8 ± 5.1§
Weight (kg)	1.61 ± 0.69	1.36 ± 0.69	2.58 ± 1.16§
Plasma carnitine concentration (nmol/ml	22.9 ± 5.2§	9.8 ± 2.9	7.0 ± 3.3
Male/female ratio	11/8	10/11	9/8
Acetoacetate (μmol/L)	122 ± 99	125 ± 72	59 ± 49§
BOB (μmol/L	532 ± 407	562 ± 247	339 ± 285

Values (except male/female ratio) are expressed as mean ± SD.
*Group 1 PNA ≤ 4 weeks; plasma carnitine concentration >13 nmol/ml.
†Group 2 PNA ≤ 4 weeks; plasma carnitine concentration ≤13 nmol/ml.
‡Group 3 PNA > 4 weeks.
§Significantly different from two other groups.

declined to near baseline concentrations. Infants in the younger age group had a significantly lower baseline FFA concentration and higher baseline acetoacetate and BOB concentrations (Fig. 2). The older infants had a significant rise in FFA concentrations at 2 hours, whereas the younger infants had a significantly smaller rise. Both groups had a decline in FFA concentrations at 4 hours, with values returning essentially to baseline. Acetoacetate and BOB concentrations remained relatively constant after the lipid infusion in both groups, but significantly higher values were observed in the younger infant group at all time points.

To assess the influence of both PNA and plasma carnitine concentration on lipid metabolism, we further grouped infants ≤4 weeks of age by plasma carnitine concentration >13 nmol/ml (group 1, n = 19) and plasma carnitine concentration ≤13 nmol/ml (group 2, n = 21). Additionally, infants >4 weeks of age were included in group 3 (n= 17). The clinical characteristics for the three groups are summarized in the Table. There were no significant differences in gestational age and gender distribution among the three groups. Patients in groups 1 and 2 were similar in age and weight but significantly younger and smaller than group 3 patients. Patients in groups 2 and 3 had similar plasma carnitine concentrations, which were significantly lower than those in group 1 patients. There were no significant differences in triglyceride concentration among the three groups at the three observation times. There were no significant changes with time for acetoacetate and BOB concentrations in any group; therefore the mean data for all observations are presented in the Table. The mean FFA concentrations

(measured in micromoles per liter) at baseline and 4 hours were significantly higher in group 3 infants (baseline, 618 ± 407; 4 hours, 594 ± 361) than in groups 1 and 2 infants (baseline, 368 ± 219 and 316 ± 179; 4 hours, 377 ± 141 and 382 ± 236, respectively), whereas at 2 hours, group 3 infants (1030 ± 720) had significantly higher values than those in group 1 (535 ± 285). The mean acetoacetate concentration was significantly lower in group 3 infants than in groups 1 and 2 infants (Table). There were no significant differences in the mean BOB concentrations among the three groups.

DISCUSSION

Dietary intake appears to be the critically important factor affecting total body carnitine status during the neonatal period. Preterm infants are born with limited tissue reserves and are at increased risk for the development of carnitine deficiency.[11] Infants also appear to have a reduced capacity for biosynthesis secondary to low activities of several enzymes involved in carnitine synthesis.[12] Infants maintained on carnitine-free nutrients from birth are at increased risk for the development of abnormalities in FFA metabolism. Our data demonstrate a direct relationship between PNA (or duration of carnitine-free nutrition) and plasma carnitine concentration. These results also indicate that the resulting low plasma carnitine concentrations are associated with apparently impaired fatty acid metabolism.

Low plasma carnitine concentrations and decreased tissue carnitine concentrations have been observed in infants receiving parenteral nutrition.[3-5, 13, 14] Impaired fatty acid metabolism has been observed in preterm infants receiving carnitine-free parenteral nutrition during the first 6 to 10 days of life,[4, 5] but alterations in fatty acid metabolism were not observed in a group of mostly term infants who received a single dose (100 mg/kg) of carnitine.[15] Although these latter infants had low plasma carnitine concentrations and had been receiving parenteral nutrition for the first 7 days of life, tissue stores of carnitine may not have been depleted. A study of older infants who were maintained for prolonged periods (4 to 40 weeks) on parenteral nutrition noted low plasma carnitine concentrations associated with impaired fatty acid metabolism.[13]

In the current study, declining plasma carnitine concentrations were noted with increasing PNA. There were significant relationships for both plasma carnitine concentration and PNA with measures of fatty acid metabolism, but the correlation with PNA was stronger.

We grouped infants by PNA more or less than 4 weeks because of our earlier results[13] indicating impaired fatty metabolism in older infants and because the study by Orzali et al.[15] showed no impaired fatty acid metabolism at 1 week of age. Infants who were ≤4 weeks of age had better utilization of infused lipids than those >4 weeks of age. About half of the infants ≤4 weeks of age had plasma carnitine concentrations similar to those in the group >4 weeks. Our results suggest that the very low plasma carnitine concentrations in the older infants probably reflect depletion of tissue carnitine stores, whereas low plasma concentrations in the infants on a shorter regimen of parenteral nutrition in this and other studies do not reflect tissue depletion of carnitine.

The current study included a heterogenous group of infants, all of whom had received carnitine-free parenteral nutrition since shortly after birth. A standardized lipid utilization test was used to produce a controlled environment to study lipid metabolism.[13] All lipids were withheld for 16 hours before study to allow for the clearance and metabolism of exogenously administered lipids. The carbohydrate load was decreased to reduce insulin levels and minimize the effects of infused glucose on lipid metabolism.

The effect of exogenous carnitine supplementation on lipid metabolism has been studied by several investigators. Schmidt-Sommerfeld et al.[16] demonstrated improved lipid utilization in a group of preterm infants receiving carnitine supplementation approximately 1 week. Orzali et al.[15] did not observe improvements in lipid utilization after the administration of a single dose of carnitine. Oral carnitine supplementation for 7 days was shown to improve lipid utilization in older infants maintained for prolonged periods of parenteral nutrition.[13] From these studies and our current study, it appears that the duration of carnitine abstinence is important in determining poor lipid utilization in infants. Those who are premature may develop depletion of total body carnitine more rapidly than term infants because of decreased tissue stores. Carnitine supplementation should be considered for infants who will receive carnitine-free nutrients for longer than 4 weeks. It remains unclear when endogenous biosynthesis of carnitine is adequate to meet the metabolic demands in infants, so that exogenous carnitine supplementation is no longer necessary.

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