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Parenteral nutrition in preterm neonates with and without carnitine supplementation

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The effects of carnitine supplementation on fat and glucose metabolism and carnitine balance were studied in 12 preterm neonates receiving full or partial parenteral nutrition (PN) for 5 to 21 days. The gestational age ranged from 27 to 32 weeks and the birth weight from 790 to 2090 g. The neonates were assigned at random to receive either L-carnitine 10 mg/kg (n=6) or saline (n=6). In the carnitine group, increased concentrations in plasma of total and free carnitine were observed. Less than 50% of the given dose was recovered in urine. In the placebo group no changes in the total plasma carnitine concentration were seen. In all neonates plasma triglycerides, free fatty acids, glycerol, alanine, 3-hydroxybutyrate (BOB), glucose and lactate were measured at predetermined intervals. The only significant difference between the groups was higher BOB-concentrations in the carnitine group 2 days after the start of parenteral nutrition. Elevated BOB concentrations are an indicator of improved fatty acid oxidation in the carnitine group. In this study, only a temporary effect of the carnitine supplementation was found.

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Carnitine is a cofactor for the transport of long-chain fatty acids into the mitochondrial matrix where the beta-oxidation takes place (1). In the neonate carnitine also has an influence on lipolysis, thermogenesis, ketogenesis and possibly on nitrogen metabolism (2). The fetus may obtain carnitine from either fetal synthesis or placental transport, or both. The tissue reserves of carnitine are lower in preterm than in term infants (3, 4), and preterm infants are therefore suggested to have an increased risk of developing carnitine deficiency. The neonate obtains extra carnitine from breast milk. The situation is different in infants receiving parenteral nutrition (PN) as available solutions do not contain carnitine (5). It has been shown that premature infants receiving carnitine-free parenteral nutrition have an increased risk of developing suboptimal carnitine levels, with consequences such as impaired fatty acid oxidation. Therefore carnitine has been regarded as an essential nutrient for at least the very low birthweight infant (6).

The aim of the present study was to evaluate the effects of carnitine supplementation on fat and glucose metabolism and carnitine balance in preterm neonates. In this group of infants a combination of enteral and parenteral nutrition is common, and during the study period no attempts were made to change the feeding routines.

PATIENTS AND METHODS

This study was approved by the Ethics Committee of the Medical Faculty, Gothenburg University. Informed consent was obtained from the parents of all infants.

Patients

The study was conducted in the units of Neonatology, Intensive Care and Pediatric Surgery in the Children's Hospital, East Hospital, Gothenburg University, Sweden. It was part of a more extensive study on carnitine, fat and glucose metabolism and carnitine balance in neonates. Newborn infants with a gestational age below 33 weeks and an expected need for PN with fat and amino acid solutions for at least 5 days were eligible for this study. Fourteen patients entered the study. One patient was excluded as full enteral nutrition was reached before 5 days and one patient died from intracranial bleeding on day 3. Data from these patients were not included in the results of the study. Of the remaining 12 patients, 6 received carnitine and 6 received placebo supplementation.

Nutrition

The day PN was started was defined as day 0 (the first day of the study). Complete PN included a 20% lipid emulsion, Intralipid®, (KabiVitrum, Stockholm, Sweden) 20 ml/kg (4 g/kg of fat), Vaminolac®, (KabiVitrum) 35 ml/kg (2.3 g/kg of amino acids) and a 10% dextrose solution 90–110 ml/kg (10 g/kg of glucose). The neonates were, by drawing cards, randomized in blocks of four into either a carnitine or a placebo group. Coded ampoules with either L-carnitine, the inner salt, 10 mg/kg (60 μmol/kg) (Sigma Tau, Rome, Italy) or placebo, saline 0.9 mg/ml, were added to the lipid solution. Except for the days when sampling was done, the PN solutions were administered continuously over 24 h using computerized microinfusion

Table 1
Clinical characteristics of patients receiving placebo or carnitine supplementation

Study group	Patient	Gestational age (weeks)	Birth weight (g)	Duration of PN (days)	Diagnosis
Placebo	1	28	1440	6	IRDS
	2	30	790	7	SGA
	3	31	1880	10	IRDS
	4	31	1940	7	IRDS
	5	32	2090	21	NEC
	6	32	2090	6	IRDS
Mean		30.7	1750		
Carnitine	1	27	930	7	IRDS
	2	27	870	7	IRDS
	3	28	1250	5	Immaturity
	4	29	1220	10	IRDS
	5	31	1105	10	IRDS
	6	31	1750	7	IRDS
Mean		28.8	1187		

PN = parenteral nutrition, IRDS = idiopathic respiratory distress syndrome, SGA = small for gestational age, NEC = necrotizing enterocolitis. No significant differences were found when comparing placebo and carnitine groups.

pumps, allowing a flow of 0.1 ml/h (IMED 965, Milton Trading Estate, Abingdon, Oxon, Great Britain). PN was gradually increased during the first 5 days. In the neonates that were also orally fed (breast milk), the volumes of all PN solutions were decreased, to maintain unchanged total fluid volumes. When enteral feedings constituted more than 75% of recommended intake, PN was stopped.

Sampling

On day 0, blood samples for determination of carnitine, glucose, triglycerides (TG), free fatty acids (FFA), 3-hydroxybutyrate (BOB), alanine, glycerol and lactate were obtained before any intravenous fat and amino acids were given. Samples for carnitine determination were taken from both blood and urine. Plasma for TG, FFA, BOB, alanine and glycerol was separated by centrifugation, and plasma fractions were portioned and frozen separately. Blood for lactate analysis was frozen after precipitation in 0.6 mol/l perchloric acid. The sampling was repeated on day 2, 5, 10, 15 and 20. Before this sampling the Intralipid-Vaminolac-infusion was stopped for 4 h. On day 5 samples were also taken immediately after stopping the Intralipid-Vaminolac infusion. The blood samples were taken via indwelling catheters not used for Intralipid-infusions. If additional breast milk was given, at least 3 h elapsed between a portion being given and sampling taking place. Urine volumes were measured via urine catheters and collection bags. Aliquots of 24-h urine, the breast milk given, infused plasma and blood were taken for carnitine determination. The carnitine intake from sources other than the PN was calculated from the carnitine concentrations and the volumes given of breast milk, plasma and blood.

Chemical methods

Blood glucose was measured using a glucose-6-phosphate dehydrogenase method. TG and FFA were determined by gas-liquid chromatography (7). Alanine, glycerol, BOB and lactate were analysed using enzymatic tests (8–11).

Carnitine was assayed by a radioenzymatic method (12) modified as described previously (13). The breast milk samples were usually diluted with distilled water to twice the volume (14) before the chloroform-methanol extraction. Total carnitine refers to values obtained after alkaline hydrolysis, and free carnitine without hydrolysis. Acylcarnitine was calculated as the difference between these values.

Statistical methods

Student's *t*-test was used for the comparison of means. A *P*-value <0.05 was considered significant.

Table 2
Enteral and parenteral intake in infants receiving placebo or carnitine supplementation (mean ± s.e.mean)

	Placebo			Carnitine		
	Day 0	Day 2	Day 5	Day 0	Day 2	Day 5
Intralipid 20% (ml/kg)	8 ± 2	12 ± 2	10 ± 2	9 ± 2	12 ± 3	11 ± 2
Vaminolac (ml/kg)	12 ± 3	21 ± 3	17 ± 3	14 ± 3	18 ± 4	15 ± 4
Dextrose 10% (ml/kg)	55 ± 7	62 ± 11	53 ± 9	50 ± 9	59 ± 11	59 ± 11
Breast milk (ml/kg)	15 ± 7	33 ± 9	65 ± 15	21 ± 10	41 ± 13	82 ± 20
Total volume (ml/kg)	89 ± 6	128 ± 10	144 ± 6	94 ± 7	129 ± 7	167 ± 8

No significant differences were found when comparing placebo and carnitine groups.

Table 3

Carnitine intake, carnitine urine excretion and plasma carnitine concentrations in infants receiving placebo or carnitine supplementation (mean ± s.e.mean).

	Placebo			Carnitine		
	Day 0	Day 2	Day 5	Day 0	Day 2	Day 5
Carnitine intake (μmol/kg)	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	60.4 ± 0.1***	61.0 ± 0.2***	60.8 ± 0.1***
Carnitine excretion (μmol/kg)	5.7 ± 1.0	2.5 ± 0.6	1.3 ± 0.2	4.3 ± 2.5	21.0 ± 10.2	25.1 ± 4.5 **
Total carnitine (μmol/l)	18 ± 4	13 ± 1	15 ± 2	25 ± 2	54 ± 6***	76 ± 11***
Free carnitine (μmol/l)	15 ± 4	10 ± 1	11 ± 2	22 ± 2	47 ± 5***	71 ± 10***
Acylcarnitine (μmol/l)	3 ± 1	3 ± 1	4 ± 1	4 ± 1	6 ± 2	6 ± 2

*** = $P < 0.001$. ** = $P < 0.01$. (Comparison with corresponding values in placebo group).

RESULTS

Table 1 describes the clinical data of the patients. Both the mean gestational age and the birth weight were lower in the carnitine group. The difference was, however, not significant. In 2 of 6 neonates in each group the duration of PN was more than 1 week.

The volumes of PN and breast milk given per kg body weight were similar in the two groups (Table 2). PN constituted more than half of the caloric intake on day 5.

The carnitine intake was substantially higher in the carnitine group than the placebo group (Table 3). Since the carnitine added to the PN was 60 μmol/kg, the daily carnitine intake from other sources was less than 1 μmol/kg body weight in both groups during the first 5 days of the study. This intake came mainly from breast milk but also from blood and plasma. (The total carnitine concentration in breast milk ranged from 0.4 to 36.9 μmol/l, mean 11.0 μmol/l). In the carnitine group a gradual increase in the excretion was observed. On day 5 less than 50% of the carnitine was recovered in the urine. In the placebo group, the mean carnitine intake was less than the urinary excretion on all days studied. A gradual decrease in urinary excretion was found and on day 5 the excretion in the two groups differed significantly ($P < 0.01$). On day 0, no significant differences in total carnitine, free carnitine or acylcarnitine in plasma were found. In the carnitine supplemented group, total and free carnitine concentrations in plasma were increased more than 100% on day 2 and 5. In the placebo group carnitine concentrations were unchanged during the first 5 days.

Blood glucose concentration and plasma concentrations of TG, FFA, BOB, lactate, glycerol and alanine on day 0 and 4 h after the cessation of Intralipid-Vaminolac-infusions on day 2 and 5 are shown in Table 4. The only significant difference between the two groups was a higher mean BOB-value in the carnitine group on day 2 ($P < 0.05$).

On day 5, samples were obtained at the end of the Intralipid-Vaminolac-infusion and at the end of the 4-h infusion break (Fig. 1). In both groups plasma concentrations of TG, FFA, glycerol and BOB differed significantly before and after the fat-free period ($P < 0.05$). When comparing plasma clearance of the different biochemical variables, no significant differences were found between the two groups.

DISCUSSION

This study was designed to compare routine PN in preterm neonates with and without carnitine supple-

Table 4

Blood and plasma biochemical variables in infants receiving placebo or carnitine supplementation (mean ± s.e.mean).

	Placebo			Carnitine		
	Day 0	Day 2	Day 5	Day 0	Day 2	Day 5
Glucose (mmol/l)	4.4 ± 0.6	4.6 ± 0.3	4.6 ± 0.8	4.9 ± 0.8	3.9 ± 0.5	4.7 ± 0.3
TG (mmol/l)	0.78 ± 0.13	1.00 ± 0.25	0.84 ± 0.12	1.28 ± 0.34	1.54 ± 0.37	1.13 ± 0.19
FFA (mmol/l)	0.42 ± 0.05	0.41 ± 0.05	0.52 ± 0.17	0.60 ± 0.11	0.75 ± 0.21	0.37 ± 0.05
BOB (mmol/l)	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.02	0.18 ± 0.04*	0.08 ± 0.02
Lactate (mmol/l)	2.25 ± 0.20	1.97 ± 0.34	1.44 ± 0.12	2.56 ± 0.27	1.58 ± 0.21	2.09 ± 0.58
Glycerol (mmol/l)	0.16 ± 0.06	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.04	0.06 ± 0.01
Alanine (mmol/l)	0.24 ± 0.03	0.25 ± 0.04	0.24 ± 0.04	0.24 ± 0.03	0.18 ± 0.03	0.21 ± 0.03

TG = triglycerides, FFA = free fatty acids, BOB = 3-hydroxybutyrate.
*$P < 0.05$. (Comparison with corresponding value in placebo group).

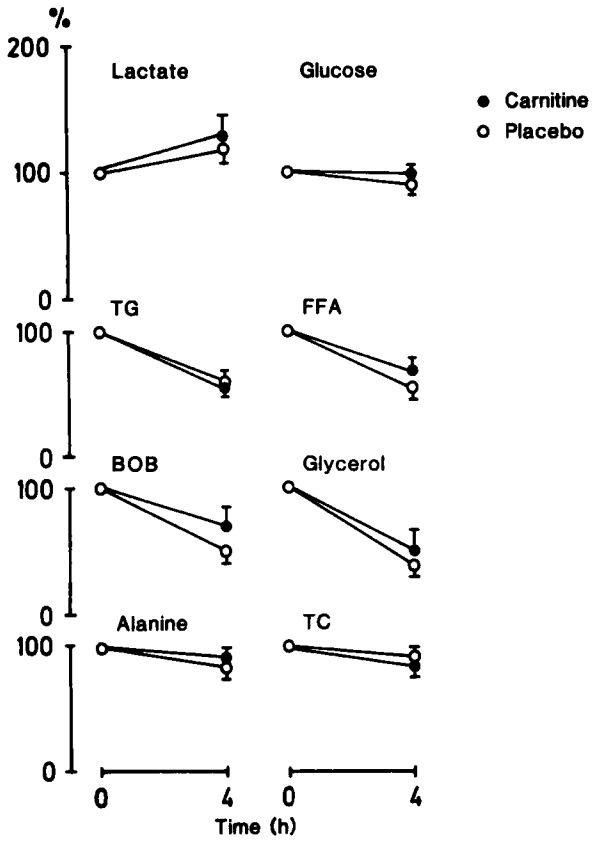

Fig. 1. Percentage changes in lactate, glucose, triglycerides (TG), free fatty acids (FFA), 3-hydroxybutyrate (BOB), glycerol, alanine and total carnitine (TC) after a 4-h break in the Intralipid-Vaminolac-infusion on day 5.

mentation. Due to our routine with early breast-milk feeding, total PN was seldom needed. Carnitine supplementation resulted in higher plasma and urine concentrations of both total and free carnitine from day 2 of the study. No significant differences were found in plasma acylcarnitine concentrations. By measurement of carnitine intake and excretion, the carnitine balance could be calculated. In the carnitine group, less than 50% of the supplemented carnitine was recovered in the urine, thus indicating retention of carnitine. In adults 75–80% of a bolus dose is found in the urine within 24 h (15). The reasons for a high uptake in neonates may be low tissue reserves (3, 4) and/or the continuous infusion, thereby avoiding very high plasma levels.

The carnitine intake from sources other than carnitine supplementation was very small. The carnitine concentrations in breast milk were lower than found in other studies (14, 16). We have no explanation for this finding as the general handling of breast milk (freezing, pasteurisation) does not seem to influence the carnitine concentration (14).

In the neonates on PN without carnitine supplementation, the carnitine excretion was, despite a small carnitine intake, significantly higher than the intake. During the first 5 days of the study no decrease in plasma carnitine concentrations was observed. First, after more prolonged PN, plasma carnitine concentrations in the low range seen in patients with systemic carnitine deficiency have been observed (17, 18). This situation creates a risk of a further reduction of the tissue carnitine reserves (3, 4).

Conflicting results have been reported regarding improved fatty utilization with carnitine supplementation during PN in neonates. Increased BOB concentrations are considered to be an indicator of increased fatty acid oxidation. Under basal conditions and during carnitine supplementation, no increase in ketogenesis was seen in one study (19), whereas BOB was increased in another study (18). In the present study, BOB in the carnitine-supplemented group was significantly higher only on day 2.

In some studies the effects of a 2- and 4-h fat infusion, 0.25 g/h, have been investigated (18, 20, 21). In these studies, the carnitine-supplemented groups showed higher BOB-values (18, 21), at least in the most premature infants (20). In the present study, PN was given as a continuous infusion, as it has been shown that this form of administration has favorable effects on clinical and metabolic variables (22, 23). A standard fat load was not infused in this study since we wanted to evaluate the effects on PN as it is used in our clinical setting. Instead, a 4-h break was made in the fat (and amino acid) infusion. The maximal fat infusion rate was 0.2 g/h for 20 h. After the 4-h infusion break, there was no significant difference in the percentage changes of FFA and BOB concentrations between the two groups.

In conclusion, the carnitine supplementation in this study had only a minor influence on the fat metabolites studied. It has yet to be shown whether carnitine supplementation is of major importance in the immediate postnatal period.

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