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Carnitine deficiency in premature infants receiving total parenteral nutrition

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SUMMARY

Carnitine plays a significant role in fatty acid utilization and ketone body production. Its availability is especially important during the immediate postnatal period. To determine whether low birth weight infants who cannot be orally fed are at risk of developing carnitine deficiency, we compared the carnitine blood levels and urinary excretion of 12 premature infants (Group A) receiving total parenteral nutrition (TPN) with those of 8 infants of similar gestational age and birth weight (Group B) who received carnitine-containing milk formulas.

In Group A, serum levels of total and free carnitine fell after 5 days of carnitine-deficient parenteral nutrition, and urinary excretion was significantly reduced. Serum levels and urinary excretion increased after the onset of oral feedings. The control Group B exhibited no significant changes in carnitine blood levels between the first and fifth days of life, but did show a later increase. Children in Group A had lower carnitine blood levels compared to those in Group B on the fifth day of life.

These findings suggest that premature infants are not able to synthesize enough carnitine to maintain blood levels, and that carnitine deficiency can occur following TPN. Further investigation of metabolic consequences secondary to deficient carnitine intake in premature infants is necessary before carnitine supplementation should be considered.

carnitine; total parenteral nutrition; premature infants

INTRODUCTION

Carnitine (γ-trimethylamino-β-hydroxybutyric acid) is a naturally occurring

amino acid derivative, which plays an important role in the oxidation of fatty acids by facilitating their transport across the mitochondrial membrane via carnitine acyltransferase and -translocase systems [8, 23]. It is widely distributed in mammalian tissues, and is found in especially high concentrations in those organs which utilize fatty acids as a major source of energy, e.g. heart, skeletal muscle and brown adipose tissue [15]. Animal studies have suggested an important role for carnitine in non-shivering thermogenesis [11, 27], hepatic ketogenesis [17] and the preservation of myocardial function in the face of ischemia [22].

The immediate postnatal period is characterized by a sudden transition from a primarily carbohydrate-based metabolism to one which relies increasingly upon fat utilization [18, 28]. Postnatal accumulation of carnitine and increased carnitine acyltransferase activities have been demonstrated in several species [2, 10, 12], including man [24]. Administration of carnitine to newborn rabbits increases both core and skin temperature as well as lipolysis [27]. In vitro studies have demonstrated that carnitine enhances lipolysis and both the oxidation and esterification of fatty acids in human newborn subcutaneous adipocytes, but not in unstimulated adult fat cells [20, 24]. These observations indicate that carnitine may be especially important in postnatal fat metabolism, and suggest that its availability may be of clinical significance.

Biosynthesis of carnitine from lysine and methionine has been demonstrated in slices of liver and testes from adult rats [7]. However, data obtained in newborn mammals suggest that dietary intake is more important during the perinatal period. DL-[^{14}C]carnitine injected into lactating rats rapidly appears in milk, and can be demonstrated to accumulate in brown fat and other tissues of the suckling offspring [11].

Little is known about the de novo synthesis of carnitine in human newborn infants. Carnitine has been measured in amniotic fluid, in maternal and cord blood, and in the urine of newborn infants before the onset of feeding [13, 21]. It is not clear whether placental transfer or de novo synthesis is more important in the fetus. The accumulation of carnitine in human newborn adipose tissue after birth coincides with the onset of feeding, and suggests that oral intake of carnitine is important during the postnatal period [24]. Moreover, human infants fed exclusively carnitine-free soybean-based formulas have lower blood levels of carnitine than babies fed carnitine-containing cow's milk formulas or mother's milk [21].

We suspected that carnitine supply may be especially pertinent in low birth weight infants who cannot be adequately fed. Therefore, the present studies were undertaken to determine whether premature infants receiving total parenteral nutrition without carnitine supplementation were at risk of developing carnitine deficiency.

MATERIALS AND METHODS

Twelve premature newborn infants who received total parenteral nutrition (TPN) for the first 5 days of life (Group A) were compared with 8 premature infants who were orally fed with cow's milk-based formulas or mother's milk after the first 24 hours (Group B). TPN consisted of glucose, electrolytes, a 5% or 6% amino acid mixture (containing lysine and methionine) and Intralipid. The indication for TPN was based on the clinical condition of the infants and their inability to tolerate oral feedings. Infants in Group A received their first oral feeding around the 5th day of life.

Determination of gestational age was based on the last menstrual period confirmed by clinical examination. All infants were appropriate for gestational age with respect to birth weight. As seen in Table I, there were no significant differences in gestational age or birth weight between the two groups. Diagnoses included hyaline membrane disease, transient tachypnea of the newborn, perinatal asphyxia, aspiration, pneumonia, apnea and hyperbilirubinemia. Infants in Group A generally were in poorer physical condition and required more ventilatory assistance than those in Group B.

The infants in Group A had a lower caloric intake than those in Group B on the 5th day of life. These differences had disappeared by the 10th—17th day. Carnitine intake was calculated on the basis of the total carnitine concentration of the milk given. There was no carnitine in the intravenous solution used, with the exception of very small amounts found in Intralipid. Infants of Group A had received minimal amounts of carnitine compared to those of Group B by the 5th day of life (Table I). The differences in total carnitine intake between the two groups persisted, although diminished, until the 10th-17th day of life.

Venous blood samples were obtained within the first 24 hours, on the 5th day and again between the 10th and 17th days of life. By the third blood sampling, all children had received a minimum of 7 days of carnitine-containing oral feedings. All samples were obtained shortly before the infusion of Intralipid or an oral feeding, to avoid possible changes in carnitine blood levels due to fat load [14]. Twenty-four hour urine collections were performed during the same time periods. All samples were centrifuged at 3000 r.p.m. and stored frozen at −28° C until analysis.

Free carnitine was measured by a modification of the method of McGarry and Foster [16]. This method is based on the measurement of labelled acetyl carnitine formed during the incubation of the sample with [^{14}C]-acetyl-CoA (New England Nuclear), carnitine acetyltransferase (Boehringer–Mannheim) and sodium tetrathionate (Fluka AG, Buchs). As suggested by Christiansen and Bremer [6], carnitine assays were performed in the presence of 0.1 M HEPES buffer (Sigma Chemical Co.) and 0.01 M EDTA instead of Tris · HCl. For the assay of total carnitine, samples were hydrolysed in the presence of 0.08 M KOH and 0.4 M Tris base, neutralized with HCl and assayed as described above. The difference between free and total carnitine was considered to be acyl carnitine. Urine creatinine was

TABLE I

Comparison of premature infants who received total parenteral nutrition for at least 5 days after birth (Group A) with those who were orally fed (Group B)

	n	Gestational age (weeks)	Birth weight (kg)	5th Day of life		n	10th—17th Days of life	
				Caloric intake $\left(\frac{\text{kcal}}{\text{kg} \cdot 24\ \text{h}}\right)$	Total carnitine intake from birth (μmol/kg)		Caloric intake $\left(\frac{\text{kcal}}{\text{kg} \cdot 24\ \text{h}}\right)$	Total carnitine intake from birth (μmol/kg)
Group A	12	34.3 ± 1.2	1.93 ± 0.34	76.8 ± 20.8	3.3 ± 5.1	8	134.5 ± 20.2	205.6 ± 82.4
Group B	8	34.7 ± 1.3	2.17 ± 0.25	101.1 ± 14.0	67.2 ± 14.3	7	142.4 ± 10.8	336.0 ± 66.4
Significance of difference		NS	NS	$P<0.01$	$P<0.01$		NS	$P<0.01$

The results are given as mean ± SD; NS = non-significant.

kindly measured in the laboratory of Prof. Dr. Roka, Institut für Klinische Chemie, Justus-Liebig-Universität, Giessen.

Statistical analysis was performed by the Wilcoxon signed ranks test for related samples when data from the same infant were compared, and by the Wilcoxon non-parametric test for independent samples when data from different children were compared.

RESULTS

Figure 1 demonstrates the changes of serum total carnitine in individual infants of Group A during the study period. Carnitine levels fell ($P<0.01$) after 5 days of carnitine-deficient parenteral nutrition. Serum levels rose ($P<0.01$) after the onset of oral feedings with carnitine-containing formulas

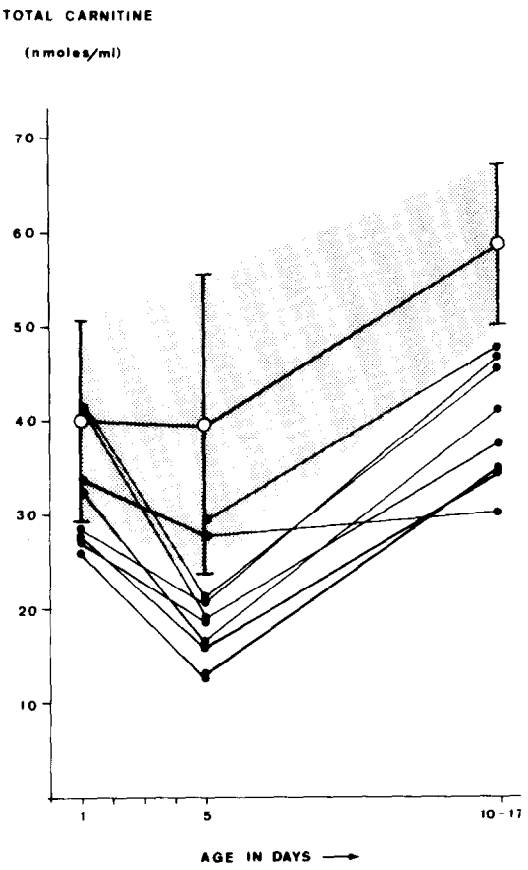

Fig. 1. Serum total carnitine levels of individual premature infants who received TPN for at least 5 days after birth (Group A; •——•) are compared to those of premature infants who were orally fed (Group B; the open circles represent the means; the shaded area represents ± SD).

TABLE II

Serum carnitine levels of premature infants who received total parenteral nutrition for at least 5 days after birth (Group A) compared to those of premature infants who were orally fed (Group B)

	Free carnitine (nmol/ml)			Acyl carnitine (nmol/ml)		
	1st Day of life	5th Day of life	10th—17th Day of life	1st Day of life	5th Day of life	10th—17th Day of life
Group A	21.7 ± 4.5	10.2 ± 2.7*	29.9 ± 7.7**	10.9 ± 4.7	9.2 ± 4.5	9.7 ± 2.8
(n)	(8)	(10)	(8)	(8)	(10)	(8)
Group B	19.2 ± 7.1	18.7 ± 6.0	31.3 ± 4.6**	20.8 ± 7.7	20.9 ± 11.1	27.1 ± 7.7
(n)	(7)	(7)	(7)	(7)	(7)	(7)
Significance of difference	NS	$P<0.01$	NS	$P<0.01$	$P<0.01$	$P<0.01$

*$P<0.01$ compared to the same group on the 1st day of life.
**$P<0.01$ compared to the same group on the 5th day of life.
The results are given as mean ± SD; NS = non-significant.

or mother's milk. In comparison, the control Group B (shaded area in Fig. 1) exhibited no significant changes between the first and fifth days of life, but did show a later increase ($P<0.05$). Children in Group A had significantly lower total carnitine serum levels ($P<0.01$) compared to their peers in Group B on the 5th and also on the 10th–17th day of life.

Table II shows the means and standard deviations of the free and acyl carnitine serum levels for both groups during the study period. Changes in free carnitine were similar to those of total carnitine. Although there were no consistent individual changes in acyl carnitine levels in either group, acyl carnitine levels were significantly lower in Group A compared to Group B at all times.

As seen in Figure 2, urinary total carnitine excretion of individual infants in Group A fell ($P<0.01$) after 5 days of parenteral nutrition, and was lower than that of 4 infants in Group B. Seven of the 10 studied children in Group

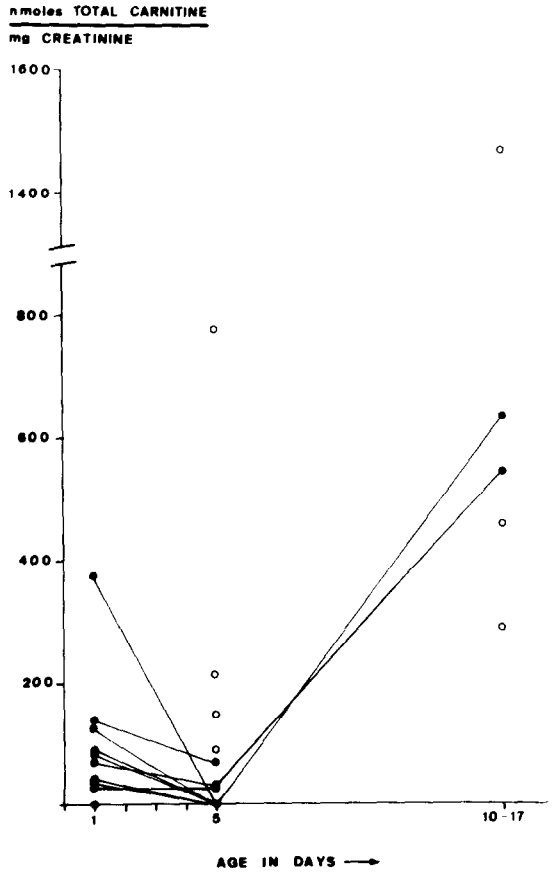

Fig. 2. Urinary total carnitine excretion of individual premature infants who received TPN for at least 5 days after birth (Group A; •—•) are compared to those of premature infants who were orally fed (Group B; ○—○).

TABLE III

Urinary carnitine excretion in premature infants who received total parenteral nutrition for at least 5 days after birth and were orally fed thereafter

	n	Total carnitine		Free carnitine		Acyl carnitine	
		μmol	nmol	μmol	nmol	μmol	nmol
		$m^2 \cdot 24$ h	mg creatinine	$m^2 \cdot 24$ h	mg creatinine	$m^2 \cdot 24$ h	mg creatinine
1st Day of life	10	5.7 ± 4.1	96.8 ± 105.6	2.5 ± 1.7	39.8 ± 34.3	3.2 ± 2.9	57.0 ± 76.5
5th Day of life	10	1.8 ± 3.2	12.6 ± 23.6	0.7 ± 1.1	11.4 ± 5.0	1.1 ± 2.1	8.1 ± 16.0
1st vs. 5th Day		P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01
10—17th Day of life*	5	37.2 ± 20.6	675.8 ± 459.4	28.6 ± 19.1	482.4 ± 307.5	9.0 ± 10.1	193.4 ± 231.2
5th vs. 10—17th Day		P<0.01	P<0.01	P<0.01	P<0.01	NS	P<0.05

*Including 3 children who received oral feedings from the 1st day of life onward.
The results are given as mean ± SD; NS = non-significant.

A had no measurable total carnitine excretion* on the 5th day of life. After the onset of oral feedings, carnitine excretion increased in the two cases measured, and reached levels comparable to those of children in Group B on the 10th—17th day of life.

Table III shows the means and standard deviations of total, free and acyl carnitine excretion of infants in Group A during the study period. In order to compensate for possible losses during urine collection as well as for the increasing glomerular filtration rate during the neonatal period, the data are also expressed as nmol carnitine per mg creatinine. Changes in free carnitine excretion were similar to those of total carnitine.

The per cent of carnitine made up by acyl carnitine esters in the urine was larger ($P<0.01$) than that in the blood of almost every child studied during the first 5 days of life. No carnitine was detectable in the urine of infants with total carnitine blood levels lower than 20 nmol per milliliter.

DISCUSSION

On the first day of life, blood levels of carnitine in both groups were within the range previously reported for premature and full-term newborn infants [21, 24] and generally lower than those described for adults [3]. The fall of carnitine blood levels following 5 days of carnitine-deficient total parenteral nutrition suggests that, in spite of an adequate supply of the amino acids lysine and methionine, premature infants are not able to synthesize enough carnitine to maintain blood levels. The relatively higher blood levels of carnitine in infants of the control Group B who received carnitine-containing formulas or mother's milk, as well as the rise of carnitine following the institution of oral feeding in the study Group A, indicate that adequate carnitine intake can improve a carnitine-deficient state.

At the present time, we do not know whether decreased carnitine blood levels reflect tissue carnitine deficiency. In studies with chronic renal patients, Böhmer et al. [4] and König et al. [14] found a 50% reduction in serum free carnitine following dialysis. Levels generally returned to pre-dialysis values within 20 hours of dialysis [14]. Muscle biopsies demonstrated that tissue carnitine concentrations in patients undergoing chronic hemodialysis were only 10% of those found in controls [4], indicating that blood levels are maintained at the expense of tissue stores. Whether tissue carnitine depletion occurs in our patients after only 5 days of carnitine-deficient parenteral nutrition remains to be investigated.

The fall in urinary carnitine excretion following 5 days of carnitine-deficient parenteral nutrition suggests that the kidney is capable of conserving carnitine. Similar findings have been reported in adult volunteers following carnitine-deficient diets [19] or fasting [9]. The absence of

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*In our laboratory, the lower limit of reliable total carnitine measurement is 2.0 nmol/ml.

measurable carnitine excretion at blood levels below 20 nmol per ml suggests that this level may represent the renal threshold of the children studied.

The functional significance of diminished carnitine blood levels in premature infants receiving TPN has not yet been evaluated. Under physiological conditions, most infants receive fat along with carnitine in their natural diet. Our patients in Group A, however, received up to 2 grams of Intralipid per kg body weight per day without significant amounts of carnitine. This raises the question whether these infants are capable of adequately utilizing this fat load. Serum acyl carnitine (presumably mostly acetyl carnitine) is especially relevant in evaluating fat utilization. It is elevated in conditions of increased fatty acid turnover and ketogenesis [25]. In our study, the children receiving TPN had significantly lower acyl carnitine levels in comparison to the orally fed control group, indicating impairment of fatty acid oxidation. This was still evident after they had received at least 7 days of regular oral feedings and after free carnitine levels had reached normal values.

Low birth weight infants have been shown to have an impaired ability to utilize lipid emulsions [26, 29]. The prolonged rise of free fatty acid blood levels following Intralipid infusion has been thought to reflect impaired fatty acid utilization in these infants, but has not yet been associated with possible disturbances of carnitine-dependent fatty acid oxidation. However, patients with inborn errors of the carnitine/carnitine acyltransferase system have been reported to have impaired fatty acid oxidation and diminished ketone body production [5]. In some cases, administration of carnitine to patients with carnitine deficiency syndrome has resulted in clinical improvement and decreased lipid accumulation in muscle [1].

Reporting the case of a chronic renal patient who died of cardiac arrhythmia during hemodialysis while receiving a parenteral triglyceride infusion, Böhmer suggests that intracellular carnitine deficiency may contribute to functional disturbances in cardiac function and nerve conduction [4]. Heart block and respiratory insufficiency are two of the major causes of death in fatal cases of carnitine deficiency syndrome [1]. Whether clinical symptoms due to carnitine deficiency occur in parenterally alimented premature infants and are currently unrecognized due to their generally poor condition is not yet known.

The present study emphasizes the precariousness of carnitine availability to premature infants who may have deficient carnitine synthesis and who are dependent on long-term parenteral nutrition. Further investigation of possible metabolic consequences secondary to deficient carnitine intake in premature infants is necessary before carnitine supplementation should be considered.

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