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Low Blood and Plasma Carnitine Levels in Children Receiving Long-Term Parenteral Nutrition

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Summary: Total and free carnitine and acylcarnitine concentrations were analyzed in whole blood and plasma in 12 children with a mean age of 68.4 ± 42.9 months who had received carnitine-free total parenteral nutrition (TPN) for an average of 4 years. The purpose of the study was to see if the children had become carnitine deficient and, if so, whether this correlated with poor lipid clearance. Compared to controls, the TPN-dependent children had significantly decreased concentrations of total and free carnitine in blood (26.6 ± 9.4 (SD) μmol/L vs. 43.3 ± 9.1 μmol/L, $p < 0.001$, and 17.1 ± 7.7 μmol/L vs. 35.2 ± 8.1 μmol/L, $p < 0.001$, respectively). Similar results were found in plasma (total carnitine of 19.0 ± 8.0 μmol/L vs. 41.9 ± 5.2 μmol/L, $p < 0.001$, and free carnitine of 15.7 ± 7.3 μmol/L vs. 36.1 ± 5.2 μmol/L, $p < 0.001$, respectively). The acylcarnitine concentration in plasma was decreased in the TPN children (3.3 ± 1.5 μmol/L vs. 5.8 ± 3.0 μmol/L, $p < 0.01$) compared to controls. Despite the low carnitine concentrations, serum triglyceride levels and serum free fatty acid levels were within the normal range. There was no correlation between carnitine concentrations in plasma and serum triglyceride and free fatty acid levels. Our data show that children receiving carnitine-free TPN for many years developed markedly decreased concentrations of carnitine in blood and plasma. However, no adverse effects of the low carnitine levels were found on triglyceride and free fatty acid metabolism under stable conditions. **Key Words:** Plasma carnitine—Blood carnitine—Home parenteral nutrition—Serum triglycerides—Serum free fatty acids.

L-Carnitine is recognized as an essential cofactor for the transport of long-chain fatty acids across the inner mitochondrial membrane to the site of their β-oxidation. Another possible function for carnitine in metabolism is as a buffer for formed acetyl-CoA in the matrix of mitochondria, thereby modulating the CoASH/acetyl-CoA ratio (1). Evidence has been presented that fatty acid oxidation plays an important role in vivo in the regulation of gluconeogenesis (2). The hypothesis that carnitine plays a role in gluconeogenesis may be supported by the finding that many patients with genetic carnitine deficiency have severe bouts of hypoglycemia (3).

The ultimate precursors of carnitine are lysine and methionine. Enzymes for stepwise conversion from these precursors to γ-butyrobetaine are found in all human tissues. However, γ-butyrobetaine hydroxylase activity is present only in liver, kidney, and brain. Although hepatic γ-butyrobetaine activity is developmentally regulated in contrast to the renal enzyme activity in human infant, it has recently been shown that this enzyme activity is not rate limiting for carnitine biosynthesis (4).

It has been generally accepted that human adults are able to synthesize sufficient carnitine to supply the needs of the body. The picture in the human infant may be different in that they must provide carnitine for new tissue, particularly muscle, in addition to the maintenance need of carnitine. Preterm and newborn infants seem to be dependent on exogenous carnitine since plasma carnitine levels fall if no exogenous carnitine is given (5,6) but is maintained during breast milk feeding (7,8). The amount of carnitine supplied from human milk has been shown to be in the range of 4–7 μmol/kg of body weight (7,8). Depletion of tissue stores of carnitine

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has been demonstrated in newborn infants who received more than 15 days of total parenteral nutrition (TPN) (9). Despite that, the U.S. Food and Drug Administration (FDA) has not recommended routine supplementation of carnitine to TPN solutions.

We are not aware of any studies that have been performed in older children on long-term TPN. In the present study, we measured carnitine concentrations in plasma and whole blood in children on home parenteral nutrition for an average of more than 4 years. The purpose of this investigation was to investigate whether low exogenous carnitine supply influences the blood and plasma levels of carnitine and to correlate the carnitine levels with variables reflecting lipid metabolism under basal circumstances (10–12).

PATIENTS AND METHODS

Twelve patients, 7 boys and 5 girls, totally or partially dependent on parenteral nutrition were investigated over a period of 1 year. They were 68.4 ± 42.9 (SD) months old and had received TPN for 51.6 ± 41.1 months (range of 18–118 months). The children had normal height and weight, normal serum levels of the supplemented trace elements iron, copper, and zinc, and normal vitamin status as previously reported (10,13–15). At the time of evaluation, all patients were clinically stable without evidence of acute illnesses. No patients had renal or liver insufficiency. The clinical characteristics of the patients are shown in Table 1.

The patients received from TPN an average of 52.5 kcal/kg/day (range 36.2–75.0 kcal/kg/day) and the amount of fat delivered per night from Intralipid (KabiVitrum, Stockholm, Sweden) was 1.0 ± 0.5 g/kg/day (range 0.2–1.7 g/kg/day). The children were estimated to absorb less than 10% of their oral intake of food due to their underlying diseases. Furthermore, the majority of the children ingested only minimal amounts of food. Parenteral nutrition was administered at home each night for 10–14 h. Solutions were infused through a pediatric or standard Broviac catheter placed into the central vein using a volumetric infusion pump (IMED Corp., San Diego, CA, U.S.A.) as previously described (16).

TABLE 1. *Diagnosis and sex of children*

Diagnosis	Male	Female
Short bowel syndrome	6	3
Volvulus	2	
Intestinal malformation	3	3
Necrotizing enterocolitis	1	
Pseudo-obstruction syndrome		2
Radiation enteritis	1	

Venous blood samples were obtained from the patients at their monthly visit to the Home TPN Clinic. The children were fasting and had completed TPN infusions at least 6 h before the blood was drawn. Routine laboratory tests of blood drawn at the same occasion were analyzed at the UCLA Hospital Clinical Laboratory and normal values for children were used as reference values.

Twenty healthy children within the same age range were used for comparison of carnitine values. These control children were fasting for more than 4 h before blood was drawn.

This study was approved by the Human Subject Protection Committee and informed consent was obtained from the parents of all subjects.

Carnitine was determined in whole blood and plasma after chloroform–methanol extraction using an enzymatic radioisotopic method (17) modified as previously described (18). Acylcarnitine (long and short chain) was calculated as the difference between total carnitine, obtained after alkaline hydrolysis, and free carnitine.

Comparison of means was analyzed by Student's *t* test. Linear and multiple regression analysis were employed for correlations. Variance is expressed as mean ± SD.

RESULTS

Blood and plasma carnitine values are shown in Table 2. Mean values of total and free carnitine concentrations in the patients were about one-half the levels of controls in both whole blood and plasma. Those patients who were most dependent on TPN and ingested the least amount of food enterally had the lowest plasma carnitine concentrations (8.7, 12.0, and 12.3 μmol/L). The patients had lower acylcarnitine in plasma while no difference was seen in whole blood. On the other hand, the percentage of free carnitine fraction of total carnitine in plasma was lower in the patients, indicating a relative higher proportion of esterified carnitine.

Routine laboratory test values are shown in Table 3. Most of these values were statistically significantly different from the local reference values, as

TABLE 2. *Carnitine concentrations in blood and plasma*[a]

	Whole blood			Plasma		
	Patients	Controls	p	Patients	Controls	p
Carnitine (μmol/L)						
Total	26.6 ± 9.4	43.3 ± 9.1	<0.001	19.0 ± 8.0	41.9 ± 5.2	<0.001
Free	17.1 ± 7.7	35.2 ± 8.1	<0.001	15.7 ± 7.3	36.1 ± 4.5	<0.001
Acyl	8.9 ± 3.2	8.2 ± 5.3	NS	3.3 ± 1.5	5.8 ± 3.0	<0.01
Acyl/free ratio	0.57 ± 0.22	0.25 ± 0.19	<0.001	0.24 ± 0.13	0.16 ± 0.09	<0.05
% free of total	65.2 ± 9.0	81.7 ± 11.3	<0.001	81.6 ± 8.1	87.0 ± 6.2	<0.01

NS = nonsignificant.
[a] Mean ± SD.

we have previously shown in a similar group of patients (12). There was no correlation between blood and plasma levels of carnitine when compared to serum triglyceride and free fatty acid concentrations, respectively. This may indicate that the children adequately metabolized the relatively small amount of Intralipid infused (1.0 ± 0.05 g/kg/day). No correlation was found between the duration of TPN and plasma total carnitine concentrations. The percentage of free carnitine to total carnitine in plasma was positively correlated to the TPN duration and negatively to the amount of fat received from TPN. The regression lines for these two variables when compared to free carnitine of total were $y = 3.3x - 214$, $r = 0.638$, $p < 0.005$ and $y = 0.5x + 4.7$, $r = 0.785$, $p < 0.01$, respectively. Multiple regression analysis with the two variables [$y = 0.02x_1$ (TPN duration) $- 11.8x_2$ (TPN fat amount) $+ 91$, $p < 0.05$] showed that the TPN fat amount was the only important variable.

DISCUSSION

We have documented markedly decreased levels of carnitine and its derivatives in blood and plasma in children who have received TPN for an average of 4 years as their main nutritional source. Our hypothesis was that low blood and plasma carnitine levels would adversely affect free fatty acid oxidation and triglyceride metabolism after long-term TPN. However, no correlation was found between the plasma total carnitine concentrations and duration of TPN. Those children who were most dependent on TPN and ingested the least amount of food enterally were the ones who had the lowest plasma carnitine concentrations. The relative contribution from exogenous carnitine and endogenously synthesized carnitine with respect to the carnitine need of the body is not known. It has been shown that prolonged breast feeding of up to 12 months had no reducing effect on serum carnitine in infants (8). Infants receiving TPN for extended periods up to 9 months have very low plasma carnitine values [9.4 ± 6.7 (SD) μmol/L] and L-carnitine supplementation normalized carnitine levels and improved fatty acid oxidation and ketogenesis in these children (19). The children in our study were older [mean age of 68.4 ± 42.9 (SD) months] and their plasma carnitine values were higher than these infants but only one-half the values found in our age-matched controls. Ten of our patients had total plasma carnitine values below the reference range for adults (23–70 μmol/L) in this laboratory. Plasma free carnitine values were also markedly lowered and the acylcarnitine/free carnitine ratio was increased compared to controls. This may indicate an increased conversion of free carnitine to acylcarnitine from the carnitine pool in the patients. Moreover, the percentage of free carnitine to total carnitine was the only parameter that showed a significant correlation to any of the other variables measured, i.e., it was negatively correlated to the amount of fat given in the TPN. This might be of clinical relevance, especially in times of crises, since increasing evidence suggests that carnitine may also help regenerate in-

TABLE 3. *Biochemical variables in serum*[a]

	Patients	Reference[b]	
Hemoglobin (g/dl)	10.8 ± 1.3	12.5 ± 0.9	<0.005
Hematocrit (%)	32.6 ± 3.3	37.0 ± 6.0	<0.001
Total lymphcyte count ($\times 10^3/\mu l$)	2.3 ± 0.9	4.5 ± 3.0	<0.001
GOT (U/L)	73.4 ± 48.5	21.0 ± 15.0	<0.005
GPT (U/L)	56.6 ± 37.9	16.0 ± 15.0	<0.005
Cholesterol (mg/dl)	121 ± 34.1	160 ± 90	<0.005
Triglyceride (mg/dl)	93 ± 58	92 ± 63	NS
Free fatty acids (mmol/L)	0.76 ± 0.58	0.70 ± 0.40	NS

NS = nonsignificant.
[a] Mean ± SD.
[b] Reference values at UCLA clinical laboratory, mean ± SD.

tramitochondrial free coenzyme A by accepting short-chain acyl moieties from acyl-CoA (1). There is no difference in carnitine concentration in normal children from the second year of age and adults (20).

The reasons for the low plasma and blood carnitine values are not clear but probably multifactorial. It has been shown that γ-butyrobetaine hydroxylase activity is not rate limiting in carnitine biosynthesis (4). Other factors that can affect the plasma carnitine level are a decreased glomerular filtration rate and the release of carnitine from muscle in catabolic states (21,22). However, the investigated children had normal serum creatinine levels and were metabolically stable. Dietary intake of carnitine plays a definite role for carnitine balance. In adults, lower values are seen in subjects on a vegetarian diet, which is low in carnitine (23), chronically tube-fed patients (24), and during long-term TPN (25). Thus, possible mechanisms for the low carnitine concentrations include decreased intake, impaired production, or lack of substrate since both plasma methionine and especially lysine concentrations are low in these patients (11). On the other hand, lipid-supplemented TPN is shown to cause increased free and acylcarnitine levels (26,27). Moreover, children probably need to receive carnitine supplementation not only for maintenance but also for growth.

It must be stressed that about 1% of the body carnitine pool is estimated to be in the extracellular pool and over 90% in skeletal muscle tissue (28). In normal subjects, there is no correlation between carnitine levels in plasma and skeletal muscle (29). However, it is not known whether the low plasma carnitine levels found indicate suboptimal levels of tissue carnitine in this group of children receiving carnitine-free TPN over several years. There were no clinical or biochemical abnormalities that indicated muscle or liver biopsy in these stable children.

The functional significance of the low carnitine concentrations is not clear. Serum concentrations of triglycerides and free fatty acids were normal in the investigated children under stable conditions. This is in contrast to a recent study in infants up to 4 months old fed soy-protein-based formulas with or without added carnitine (6). Serum free fatty acids concentrations were higher in the infants not receiving dietary carnitine. Regarding the abnormalities in liver function tests, carnitine supplementation to adult home TPN patients normalized plasma and hepatic carnitine levels but failed to improve the abnormal liver function tests and hepatic steatosis (25). However, there is one case report in which an adult patient on long-term TPN had beneficial effects from carnitine supplementation (30). Similarly, in infants, intravenous carnitine supplementation has resulted in divergent findings (19,31). The low blood and plasma carnitine levels found in these older children on prolonged home TPN had no adverse effect on lipid metabolism. These results need to be followed with functional studies to clarify the significance of the low carnitine concentrations.

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