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FEEDING PRETERM INFANTS WITH L-CARNITINE SUPPLEMENTED FORMULA

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A total of 29 preterm infants maintained on mixed enteral nutrition (50 % pooled human milk, 50 % formula daily) were studied over a 15 days period. 16 of them received L-carnitine supplemented formula during the first seven consecutive days (600 nmol/ml, as added supplement), 13 infants served as controls. In response to enhanced dietary intake, the plasma levels and urinary excretion rates of carnitines were increased by the 7th day of study. The plasma carnitines then returned to the initial values, whilst the urinary excretion remained elevated at the 14th day of study. The elevated daily urinary excretion of carnitines was accompanied by increased clearance and decreased relative reabsorption rates in the supplemented group. In the control group the plasma carnitine levels remained unchanged throughout the observations, while the daily excretion of free carnitine decreased by the end of the study. In the supplemented group statistically significant decrease was found in the daily excreted ammonia and urea with a decrease of plasma alanine and glutamine levels by the 7th day of study. The plasma levels of beta-hydroxybutyrate, glucose and creatinine remained unchanged in both groups.

INTRODUCTION

Hitherto a wide spectrum of the carnitine deficiency syndromes has been documented /8,32/. Since the carnitine plays an essential role in the oxidation of long chain fatty acids and in ketogenesis /5,14/ in these syndromes the mitochondrial oxidation of fatty acids and the ketogenic process may be impaire Moreover, due to secondary metabolic cascade a

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multiplex deterioration of intracellular processes may develop /28/ leading to altered metabolism of various metabolites, even if their metabolism is not directly linked to fatty acid oxidation /19/.

The carnitine supply of the body derives in part from dietary intake and in part from endogenous synthesis. In the absence of exogenous intake the plasma and tissue carnitine levels of term and preterm infants decline, suggesting an insufficient endogenous synthesis in the early neonatal period /2,23,27/. The quantities of optimal daily needs are not known. The carnitine content of mature human milk declines as a function of time after delivery /24,26/, the available formulas contain it in variable amounts /3/. Moreover, there are indications that the bioavailability of carnitine from some formulas may be lower than from breast milk /30/. Thus, the exogenous intake may vary normally on a wide scale depending on the feeding regimen of an individual infant.

The aim of the present work was to study some possible aspects of carnitine supplementation in preterm infants.

MATERIALS AND METHODS

29 appropriate for gestational age infants were selected for the study. The relevant clinical data of infants are summarized in Table I. All of them were free from major medical problems and they did not require any parenteral liquid intake for at least one week before the start of study. The infants were fed with a mixed enteral nutrition during the study period, they received alternatively pooled breast milk and a formula (Robébi A). Thus, the half of daily intake was human milk and the other half was formula. The study period covered 15 days. In male subjects on the 0., 7., and 14. days urine collection was performed over 24 hr periods with the help of urine collector bags. The urine samples were sucked from the bags with plastic canula after voidings and were stored under a few drops of toulene. After termination of collection the urine was frozen under -20^{o}C until analysis. On the days of urine collection blood samples were taken from a peripheral vein into heparinized tubes. The blood was immediately centrifuged and the plasma was stored under -20^{o}C.

Started by various postnatal ages the children were randomly divided into two groups. 16 of them (supplemented group) received L-carnitine supplemented formula during the first week

TABLE 1

Clinical and nutritional data of infants (means with ranges in parentheses)

	Supplemented group (n=16)	Controls (n=13)
Gestational age(wk)	30.2 (27-33)	29.5 (28-32)
Postnatal age (days)	26.5 (14-42)	24.9 (14-38)
Birthweight (g)	1368 (980-1750)	1466 (1100-1710)
Weight (g)		
day 0	1521 (1170-1930)	1664 (1100-2095)
day 7	1684 (1300-2180)	1830 (1200-2250)
day 14	1903 (1560-2470)	2008 (1300-2490)
Growth rate (g/wk)		
day 0-7	163 (60-250)	167 (40-240)
day 7-14	193 (70-290)	177 (100-280)
Food intake (ml/kg)		
day 0	188 (148-215)	181 (153-204)
day 7	179 (157-206)	179 (151-203)
day 14	173 (140-198)	168 (145-203)
Carnitine supplement (μmol/kg/day)		
day 1	56.4 (44.0-64.4)	---
day 7	53.6 (47.2-61.9)	---

of study (day 1 to 7). The dose of administered carnitine was 600 nmol/ml formula (96.7 μg/ml) over its endogenous content (the carnitine content of Robébi A was found to be 77.2 (68.1-80.2) nmol/ml; values are mean and range of four separate measurements, ref 26). 13 infants served as controls (Table I). There were no statistically significant differences in the infants' weight and postnatal age at the start of study.

The plasma acid soluble and urinary carnitines were determined as previously described /12/. Plasma beta hydroxybutyrate /31/ and ammonia /13/ were measured by enzymatic methods, the urea and glucose by enzymatic kits (Reanal, Hungary; Boehringer, Mannheim, (F.R.G.). The plasma free amino acids were measured by Biotronic LC 2000 analyser using fluorescent detection method (L.S.). Creatinine was determined by the Jaffé's picric acid method following the administration of Fuller's earth to remove the interfering chromogenics /11/. The urinary alpha amino nitrogen and plasma and urinary phosphate were measured by colorimetric methods /7,25/. The clearance and relative reabsorption rates were

calculated with the usual formula as described previously /15/. For statistical analysis the Student's test for paired samples was employed using a standard computer package. The $2p < 0.05$ level was taken as the level of statistical significance.

RESULTS

Neither acute nor late adverse effects were observed during the study and in the postexamination period. The weight gain was similar in the two groups (Table I).

In the supplemented group (Table II) the plasma acid soluble carnitines were found to be elevated by the 7th day of study as compared to the initial values. At the last day of observations the plasma level of carnitines returned to the starting value. In the control group the levels of carnitines in the plasma remained unchanged. There were no statistically significant changes in the plasma levels of glucose, beta hydroxybutyrate and creatinine in either group (Table II)

The amounts of urinary carnitines are shown in Fig. 1. The elevated dietary intake resulted in increased excretion of carnitines. At the 7th day of study the daily excreted total carnitine amounted to approximately 55 per cent of extra intake. The increase of daily excretion rate was higher for free carnitine than for carnitine esters when compared the results of day 7 vs. day 0 (4.1 times and 2.2 times, respectively). In the supplemented group the daily excretion of total carnitine remained elevated at the last day of study due to the elevated excretion of carnitine esters, whereas the excretion of free fraction returned nearly to the initial value. In the control group the excretion of total carnitine remained unchanged during the study period, by contrast, excretion of free carnitine decreased by the last day of the study as compared to the starting value (Fig. 1).

The renal clearance rates of creatinine and carnitines are shown in Table III. The clearance rate of creatinine showed a trend of increase in both groups. In the presupplementary control day (day 0) the clearance rate of carnitine esters was

TABLE II

Changes of plasma metabolite levels during the study period (means ± SEM)

	Patients (n)	Day 0	Day 7	Day 14
Supplemented group				
Carnitine, µmol/l	16			
total		31.4 ± 1.98	43.8 ± 2.43[a]	32.2 ± 1.33[b]
free		21.7 ± 2.39	28.8 ± 1.92[a]	21.0 ± 1.22[b]
acyl		9.68 ± 1.04	15.0 ± 1.17[a]	11.1 ± 1.14[b]
acyl/free ratio		0.45 ± 0.05	0.55 ± 0.05	0.57 ± 0.07
Glucose, mmol/l	13	3.71 ± 0.22	3.57 ± 0.19	3.49 ± 0.18
β Hydroxybutyrate, µmol/l	9	29.5 ± 2.73	31.1 ± 2.95	31.4 ± 3.03
Creatinine, µmol/l	10	59.6 ± 5.50	56.6 ± 5.07	54.1 ± 6.82
Control group				
Carnitine	11			
total		34.8 ± 1.31	33.6 ± 1.44	34.0 ± 1.36
free		23.7 ± 1.05	22.1 ± 0.84	23.6 ± 1.15
acyl		11.1 ± 1.21	11.4 ± 0.88	10.5 ± 0.79
acyl/free ratio		0.48 ± 0.07	0.51 ± 0.03	0.46 ± 0.04
Glucose	5	4.88 ± 0.47	4.10 ± 0.52	4.59 ± 0.43
β hydroxybutyrate	7	30.1 ± 3.52	27.8 ± 2.18	25.8 ± 3.39
Creatinine	9	50.0 ± 3.83	47.1 ± 2.86	47.7 ± 4.07

[a] $p < 0.05$ vs day 0

[b] $p < 0.05$ vs day 7

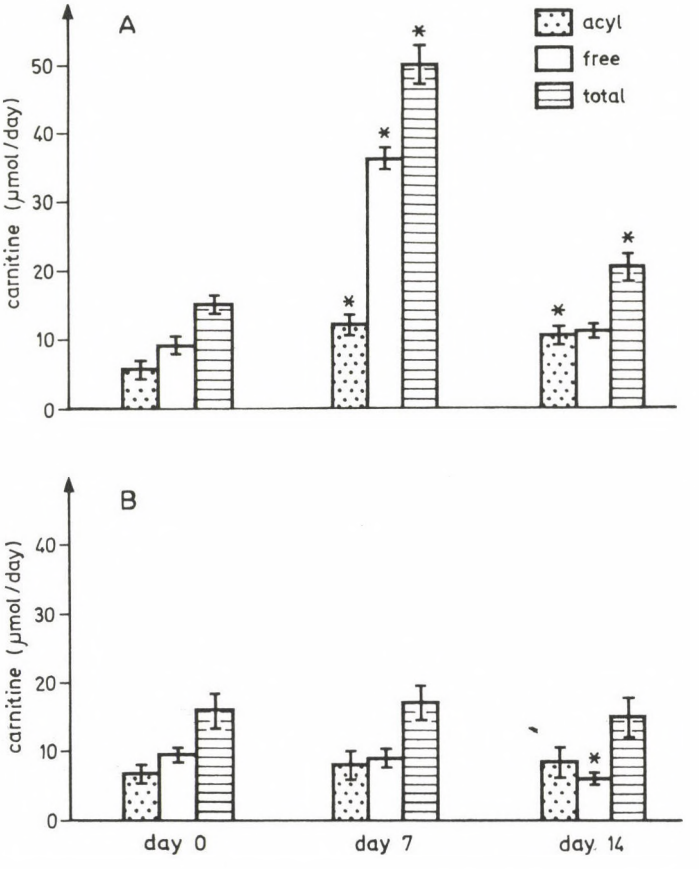

Fig. 1. Urinary excretion of carnitines in the supplemented (A; n=11) and in the control (B; n=9) group of infants.
* The differences are significant for at least $p < 0.05$ vs day 0

higher than that of free carnitines in both groups. The elevated urinary excretion of carnitines (day 7) was followed by an increase of clearance values in the supplemented group. Since the level of acylcarnitines in the supplemented group returned to the initial value by the last day of study, whereas the daily excretion of acylcarnitines remained elevated, the clearance rate of carnitine esters showed a further increase at the end of study. In the control group the fall of free

carnitine excretion was accompanied by a decrease of its clearance rate (day 14 vs 0, Table III.).

Changes of plasma and urinary carnitines were associated with changes of relative reabsorption rates of carnitines (Table IV.). The elevated urinary excretion caused decreased reabsorption in both fractions examined. To exclude the changes of tubular functions, the relative reabsorption rate of phosphate was also determined in both groups. Its value remained unchanged during the study period (not shown) suggesting, that the changes of reabsorption rates of carnitines were not associated to the age related changes of tubular functions.

The daily excreted amounts of ammonia, urea, alpha amino nitrogen and creatinine are depicted in Fig. 2. A moderate, but statistically significant decrease (nadir) was found in the daily excreted ammonia (1.07 ± 0.15, 0.87 ± 0.13 and 1.42 ± 0.20, means $\pm$ SEM; day 0,7 and 14 respectively, $p < 0.05$ as compared the results of day 7 vs 0 and 14 together) and in excreted urea (2.60 ± 0.23, 2.25 ± 0.18 and 2.31 ± 0.21; $p<0.05$ on day 7 vs day 0 and 14 together) within the supplemented group. In the control group the daily excreted amount of ammonia remained constant, whereas the excreted amount of urea exhibited a trend to decrease. The daily excreted amounts of alpha amino nitrogen and creatinine remained unchanged in both groups.

The plasma levels of free amino acids in the supplemented group are shown in Table V. The levels of alanine and glutamine were the lowest at the 7th day of study ($p < 0.05$ on day 7 vs day 0 and 14 together).

DISCUSSION

One of the regulatory mechanisms by which the higher organisms are normally capable of influencing their carnitine status is the renal handling of carnitines. A relatively excessive carnitine load causes enhanced renal eliminitation of carnitines predominantly as free carnitine /10/. By contrast, as the body is depleted of carnitine, the total amount of

TABLE III

Renal clearance rates (ml/min) of creatinine and carnitines (means with ranges in parantheses).

	Creatinine[a]	Free carnitine	Acylated carnitine
Supplemented group (n=11)			
day 0	2.38 (1.10-5.36)	0.29 (0.19-0.51)	0.39 (0.09-1.16)
day 7	2.31 (1.39-3.44)	0.87 (0.61-1.23)*	0.64 (0.17-1.36)*
day 14	3.48 (1.31-6.27)	0.39 (0.22-0.51)	0.81 (0.16-1.88)*
Control group (n=9)			
day 0	2.37 (1.62-3.08)	0.30 (0.18-0.43)	0.50 (0.13-2.19)
day 7	2.72 (1.65-3.19)	0.29 (0.16-0.48)	0.44 (0.03-1.25)
day 14	2.86 (1.95-4.16)	0.19 (0.11-0.28)*	0.46 (0.14-1.13)

*the differences are significant for at least $p < 0.05$ vs day 0; [a]n=10 in the supplemented group

TABLE IV

Relative reabsorption rates (per cent) of carnitines during the study period (means and ranges in parentheses).

	Free carnitine	Esterified carnitine
Supplemented group (n=10)		
day 0	86.1 (77.5-95.7)	79.1 (52.2-95.4)
day 7	58.7 (38.3-77.1)*	69.3 (10.1-94.0)*
day 14	85.2 (70.9-96.4)*	68.3 (47.7-97.4)*
Control group (n=9)		
day 0	87.2 (79.7-82.8)	83.1 (47.4-95.4)
day 7	89.8 (83.6-94.5)	81.3 (48.3-98.9)
day 14	92.4 (87.0-97.4)*	80.9 (48.6-94.7)

*differences are significant for at least $p < 0.05$ vs day 0

TABLE V

Plasma free amino acids in the supplemented group of children (μmol/l, n=10, means ± SEM).

	Day 0	Day 7	Day 14
Taurine	69.7 ± 13.5	124.5 ± 21.0	118.3 ± 27.2
Aspartate	12.3 ± 0.5	17.9 ± 2.5	16.0 ± 1.4
Threonine	180.5 ± 15.3	172.5 ± 17.7	200.9 ± 13.5
Serine	150.5 ± 9.1	139.0 ± 10.9	149.1 ± 10.8
Asparagine + glutamate	114.0 ± 21.0	129.3 ± 23.9	127.1 ± 17.3
Glutamine	372.7 ± 50.0	317.6 ± 47.1*	415.8 ± 45.7
Glycine	233.9 ± 16.0	230.1 ± 18.0	225.0 ± 15.5
Alanine	211.5 ± 23.3	184.2 ± 26.2*	231.5 ± 16.3
Valine	153.6 ± 12.8	143.7 ± 10.7	175.9 ± 11.2
Methionine	24.0 ± 2.2	25.9 ± 1.8	33.9 ± 2.0
Isoleucine	39.3 ± 1.8	40.1 ± 2.0	48.6 ± 3.6
Leucine	73.2 ± 4.5	70.9 ± 4.5	84.3 ± 6.1
Tyrosine	96.9 ± 11.9	95.7 ± 9.2	124.4 ± 8.1
Phenylalanine	50.8 ± 3.9	45.9 ± 2.5	55.4 ± 3.8
Ornitine	63.5 ± 5.9	58.8 ± 4.9	65.0 ± 4.5
Lysine	142.2 ± 16.0	143.3 ± 14.3	159.2 ± 12.4
Histidine	60.1 ± 2.8	59.1 ± 2.8	67.7 ± 2.8
Tryptophan	43.4 ± 6.2	52.9 ± 3.1	57.8 ± 3.3
Arginine	79.8 ± 9.5	75.7 ± 10.4	90.2 ± 7.5

* $p < 0.05$ vs day 0 and day 14

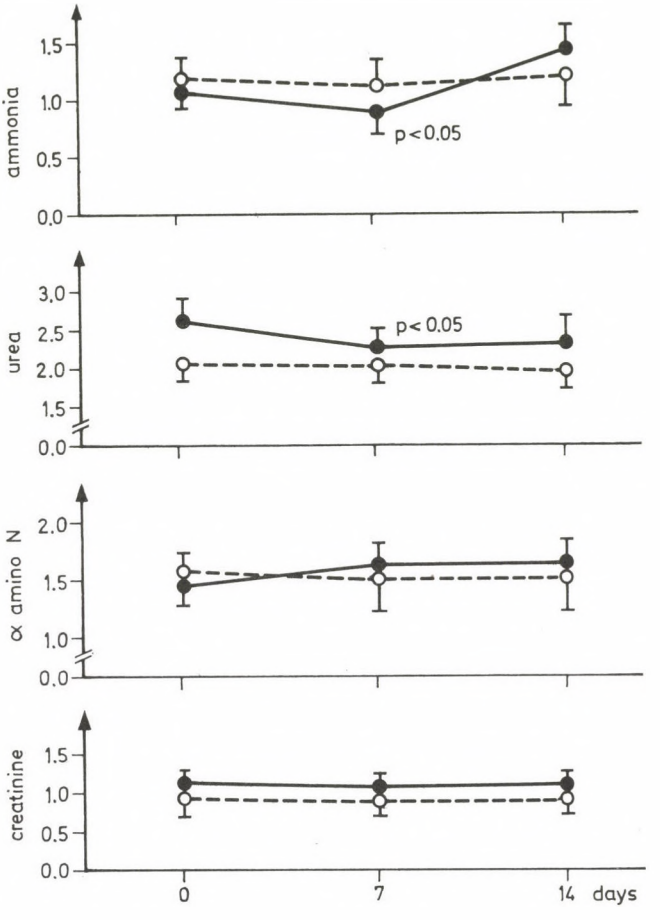

Fig. 2. Urinary excretion of ammonia, urea, alpha amino nitrogen and creatinine (mmol/kg/day) in the supplemented (—●—, n=11) and in the control (--O--, n=9) infants. Significance is shown between day 7 vs day 0 and 14 within the supplemented infants.

carnitine excreted may decrease, although the high ratio of acyl to free carnitine in the urine would be maintained /6,16,28/. In the present study the daily excreted amount of total carnitine was found to be higher than in a previous one /17/, where a similar cohort of preterm infants maintained on human milk was monitored. Moreover, in the present work the excreted amount of free carnitine was found to be higher than

the amount of carnitine esters (Fig. 1, day 0), whilst in the previous one the daily excretion of acylcarnitines exceeded that of free fraction in the presupplementary period /25/. This indicates that the carnitine reserves of the infants monitorized in the present work were larger in the control period than those of maintained on pooled human milk /25/.

The elevated dietary intake resulted in increased plasma carnitine levels and increased urinary excretion rates in the supplemented group. The elevation of plasma carnitines was proportional (no change in the ratio of acyl/free), whereas in the urine the relative partition of free fraction became higher (Fig. 1, day 7). This was reflected in clearance rates of carnitines. The increase of clearance rate of free carnitine was more pronounced (approximately 3 times, vs acylcarnitine increased approximately 1.6 times, when day 7 was compared with day 0). It is of interest that despite the elevated urinary elimination of carnitines during enhanced intake, a considerably high relative reabsorption rate was observed for both fractions examined (Table IV), similarly to our previous observation in infants fed pooled milk /18/. The marked increase of daily excreted carnitines suggests, on the other hand, that the dose of administered carnitine was higher than the daily demand, but was certainly sufficent to reach higher carnitine levels in the carnitine pools of the body /22/.

The main function of carnitine is the transport of long chain fatty acids into the mitochondrial matrix space /5/. Carnitine insufficiency may exist when there is insufficient free carnitine to buffer the intracellular acyl-CoA compounds /15,28/, leading to impaired fatty acid oxidation and ketogenesis. In the present work no changes in beta hydroxybutyrate levels were observed in either group, showing, that the initial carnitine status of infants was adequate in respect of the ketogenic process. On the other hand, the elevated dietary intake was associated with increased formation of acylcarnitines without increase of ketone body production (4).

It seems that by an unkwown mechanism, the carnitine may participate in the intermediary metabolism of some nitrogen

containing metabolites, moreover, under certain circumstances, it may alter the nitrogen balance of the whole organism /1,17,18,20,21/. In the present work a moderate but statistically significant decrease was found in plasma alanine and glutamine levels (Table V) with a concomitant moderate decrease of urinary excreted ammonia and urea at the 7th day of carnitine administration (Fig. 2). These changes strongly suggest altered protein and/or amino acid metabolism during elevated carnitine intake, however, at present there is no evidence for a direct function of carnitine in the protein and/or amino acid metabolism.Nevertheless,a simple theoretical explanation for decreased amino acid catabolism comes from the primary role of carnitine: the elevated tissue carnitine availability may lead to promoted fatty acid oxidation, thereby sparing other metabolic fuels /18,19/. This hypothesis, however, requires further experimental support considering the limited nature of the data.

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