Plasma and Urine Carnitine Levels During Development

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Summary

Plasma and urine free carnitine concentration during human development was evaluated.

A positive correlation was found between plasma level and body weight in premature neonates weighing between 1.15 and 1.80 kg.

In full terms newborns, the free carnitine concentration (mean $\pm$ S.E., 31.2 ± 2.5 nmoles/ml) is similar to that of premature babies with 33 to 36 wk of gestational age (37.5 ± 3.1 nmoles/ml) but significantly lower than that of premature babies aged 30 to 33 wk of gestation (43.0 ± 5.6 nmoles/ml).

In the first 2 wk of life, free carnitine level showed a good correlation with age in the full-term newborns.

Adult levels are reached by the end of the first 6 months. No sex related difference was observed in any of the different groups during growth.

Urinary excretion of free carnitine per day is significantly lower in infants 0 to 3 years old (mean $\pm$ S.E., 15.5 ± 1.8 μ moles/24 hr) and in children 3 to 10 years old (115.3 ± 11.4 μ moles/24 hr) than in subjects ranging in age from 22 to 70 years (216.9 ± 20.6 μ moles/24 hr).

We found no difference between day- and nighttime urinary excretion in newborns and infants.

Speculation

The present observations suggest that fetal free carnitine storage mainly occurs during the 30th to 33rd wk of life. Because the fatty acids are the main energy fuel in the postnatal period and their β -oxidation is carnitine dependent, an exogenous source of this substance seems useful to maintain plasma levels similar to those found in the fetus.

The lower free carnitine excretion observed in our children group compared to adult may be related to a smaller muscle mass, different kidney excretion, or reduced endogenous synthesis.

Carnitine (3-hydroxy-4-*N*-trimethyl-amino butyrate) has been measured in tissues during development in animals (1, 11) and man (12, 13, 19). Its synthesis occurs in liver and testis (23), and also in kidney of the cat, rabbit, and man (7, 8).

The role of carnitine in tissues is to facilitate the transport of long-chain fatty acid esters through the mitochondrial membranes (9). The oxidation of short-chain fatty acids and possibly of branched keto-acids (20, 24) is also enhanced by the intramitochondrial pool of carnitine. Some data are available regarding the carnitine content of fetal and neonatal blood (18, 19). The level is higher in cord than in maternal blood and is usually greater in the umbilical artery than vein (12), thus indicating an active synthesis of this substance by the fetus. Breast-fed newborns or those fed with cow's milk showed higher levels of carnitine than those fed on a soy-based formula (18, 25), which is essentially carnitine free. Little data are available on free carnitine plasma and urine levels in man at various stages of development. The purpose of the

present investigation is to compare the free carnitine levels at different ages and to determine the time at which normal adult values are reached. The excretion of free carnitine in urine has been studied in various age groups to establish a pattern during development.

MATERIALS AND METHODS

The free carnitine concentration during development was evaluated by a cross-sectional approach. For this purpose, 231 children from birth (minimum, 28 wk gestation) to 10 years of age were studied. All were apparently free from neuromuscular, hepatic, cardiac, and kidney diseases. Children under 6 months of age were fed the same "humanized" cow's milk-based formula. Older children were on standard hospital diet, normocaloric for the age; the calories were supplied in this percentage: carbohydrates, 50%; proteins, 12 to 15%; lipids, 35 to 38%. Fifteen percent of the total calories were supplied by meat.

Routine laboratory examinations were normal in all cases. Blood for free carnitine determination was withdrawn in the morning after a night of fasting in older age groups and before the morning feeding in the case of premature and full-term neonates. The heparinized blood was immediately centrifuged and stored at -20°C .

Plasma levels were studied in 142 children: 29 premature newborns appropriate for gestational age, all in catabolic state (<5 days old; mean gestational age, 32.6 wk; range, 27 to 36; mean birth weight, 1861 g; range, 1150 to 2890), 15 full-term healthy neonates of various postnatal age (Fig. 2), 49 infants (aged 1 to 6 months), and 49 children (6 months to 6 years of age). These values were compared with 18 healthy adults (23 to 66 years of age).

The urine was collected in plastic bags for a period of 24 hr. An aliquot sample was stored at -20°C . Free carnitine excretion in urine was measured in a different group of 58 children (aged 1 day to 3 years). In 30 of these cases, the data were subdivided into day/night period. The free carnitine levels were then compared with those of 28 healthy children (3 to 10 years) and 29 normal adults (22 to 70 years old).

The radiochemical method of Cederblad and Lindstedt (4) was used for free plasma and urine carnitine determination. The free carnitine urine level was expressed per day and per kg body weight.

All data were statistically analyzed using the Mann-Whitney test (22).

RESULTS

PLASMA

A correlation between the plasmatic concentration and body weight was observed in a group of 15 premature neonates weighing less than 1.8 kg (Fig. 1). This correlation was not found for the 14 premature babies weighing more than 1.8 kg and for the other

Fig. 1. Regression line depicting plasma free carnitine concentration in 15 premature newborns plotted against body weight.

Fig. 2. Plasma free carnitine concentration in 29 premature newborns and in 15 full-term newborns from birth to 15 days of life. The regression line indicates the correlation between plasma free carnitine level and postnatal age.

groups. For this reason, it appeared worthless to express the plasma free carnitine values as nmol/kg. In fact, although the weight was increased, the free carnitine concentration (mean $\pm$ S.E. = 37.0 ± 2.7 nmol/ml) was not significantly different from that of premature babies weighing less than 1.8 kg. In full-term newborns, the free carnitine concentration is similar than that of premature babies with 33 to 36 wk of gestational age, but significantly lower than that of premature babies aged 30 to 33 wk of gestation (Fig. 2). However, the free carnitine level in the full-term newborns showed a good correlation with age during the first 2 wk of life (Fig. 2). On the other hand, no correlation was observed between the free carnitine level and gestational age in the group of 29 premature neonates considered as a whole.

Table 1 schematically summarizes the overall plasma values during growth. In particular, all the reported values were found significantly different from children and adult concentrations. No sex-related difference was observed in any of the different groups during growth. In addition, the level in the nine adult males (mean $\pm$ S.E. = 47.6 ± 1.9 nmol/ml) and in the nine adult females (40.8 ± 1.3 nmol/ml) was not found significantly different.

Table 1. Content of plasma free carnitine in groups of prematures, newborns, children, and adults

Age groups	N	nmol/ml	
		Mean $\pm$ S.E.	Range
Premature newborns (27-36 wk)	29	34.9 ± 2.6^1	10.9-62.9
Full-term newborns (0- $\frac{1}{2}$ mo)	15	31.2 ± 2.5^1	20.1-52.4
Infants ($\frac{1}{2}$ mo- $\frac{1}{2}$ yr)	49	36.6 ± 2.0^1	12.8-77.5
Children ($\frac{1}{2}$ -6 yrs)	49	44.1 ± 1.7	20.7-78.3
Adults (23-66 yrs)	18	44.7 ± 1.7	35.1-62.6

¹ $P < 0.05$ compared with children and adults.

URINE

Table 2 shows free carnitine excretion in 86 subjects during growth compared with an age group from 22 to 70 years. These values are low in the first 3 years of life; they subsequently increase but do not attain the adult values. On the contrary, when the values are expressed per kg body weight, the higher excretions of free carnitine were found in the group from 3 to 10 years. Adult males excretion values (mean $\pm$ S.E. = 228.9 ± 23.7 μ mol/24 hr) were higher but not significantly from adult females (204.1 ± 35.0 μ mol/24 hr).

Table 3 summarizes the urinary excretion patterns observed in 30 samples collected separately in 12-hr day- and nighttime periods; no circadian variations were observed.

DISCUSSION

Aside from the growing number of published reports relating to the syndrome of carnitine deficiency (type I lipid storage myopathy) (6), interest in carnitine metabolism in man is rapidly increasing.

The purpose of the present study has been to investigate plasma and urinary free carnitine levels in the course of human growth up to adult age. It is well known that free carnitine is the major component of the carnitine pool. However, in some metabolic conditions, like fasting state, the amount of acylcarnitines increases at a high extent (10). Because the plasma and urine concentrations are not constant, we have analyzed the collected data grouping them in small age classes and applied Mann-Whitney nonparametric statistics. In most of the age classes, the homogeneity hypothesis was verified except for a few groups in which a slight "deviation" was found. This deviation is, however, acceptable because of the rigorous selection of our sample collection. On the basis of this statistical analysis, we could therefore identify definite critical age steps in which plasma and urinary free carnitine levels rise during development.

Plasmatic data show a low concentration in the premature and full-term newborns (Table 1), probably caused by an immature hepatic synthesis (19) and/or reduced oral intake of carnitine (17). However, the free carnitine concentrations are higher in premature (30 to 33 wk of gestation) than in full-term newborns (Fig. 2) as also found by Novak *et al.* (18) for the acetylcarnitine determined in the mixed cord blood (Ref. 18; Fig. 5). A positive correlation was found between plasma level and body weight in premature neonates weighing between 1.15 and 1.80 kg (Fig. 1). This correlation was not found in premature newborns weighing more than 1.8 kg and in full-term neonates. These data seem to suggest that fetal carnitine storage mainly occurs in the first 30 to 33 wk of gestation (Fig. 2). Carnitine dietary intake is probably of greater importance during the anabolic state. In fact, we found a positive correlation between plasma level and age during the first 2 wk of life, a period in which the ratio amount of milk/kg body weight is increasing. However, during the first wk of postnatal age, caloric

Table 2. Excretion of free carnitine during growth in man¹

Age (yr)	N	Urinary carnitine			
		$\mu\text{moles}/24 \text{ hr}$		$\mu\text{moles}/24 \text{ hr/kg}$	
		Mean $\pm$ S.E.	Range	Mean $\pm$ S.E.	Range
0-3	58	15.5 $\pm$ 1.8	0.5-78.5	2.1 $\pm$ 0.0	0.1-8.5
3-10	28	115.3 $\pm$ 11.4	14.1-251.7	5.1 $\pm$ 0.4	0.5-14.0
22-70	29	216.9 $\pm$ 20.6	85.6-515.4	3.1 $\pm$ 0.0	1.4-7.3

¹ All the values are significantly different at $P < 0.05$ level.

Table 3. Excretion of free carnitine in 30 children separately in the 12 day- and nighttime hours

Age (mos.)	N	Urinary carnitine ($\mu\text{moles}/12 \text{ hr}$)			
		Day		Night	
		Mean	Range	Mean	Range
0-2	6	6.2	1.1-14.9	6.2	2.3-10.1
2-4	4	7.2	0.9-20.0	7.5	1.2-17.0
4-6	10	5.0	0.1-26.1	4.5	0.4-11.2
6-8	3	6.3	1.0-11.8	4.9	1.0-9.6
8-12	3	22.7	2.2-49.6	26.8	19.7-31.7
12-30	4	14.2	12.0-15.3	18.3	7.8-25.6

intake is needed to compensate the energy expenditure, and only in the second wk is an anabolic phase beginning. These considerations could explain the lack of relationship between free carnitine concentration and body weight during the first 15 days of life. A similar rise in free and bound plasma carnitine content with age was found by Secombe *et al.* (21) in the rat. Free carnitine plasma level becomes constant after the first months of life. In this age, the daily ratio amount of milk/body weight diminishes. Adult levels are reached by the end of the first 6 months, possibly as a consequence of increased hepatic synthesis and/or of increased carnitine intake associated with a diet containing meat.

Urinary excretion of free carnitine per day is significantly lower in full-term newborns and children under 10 years of age than in subjects ranging in age from 22 to 70 years ($P < 0.05$). On the other hand, excretion per kg body weight is highest in the age group from 3 to 10 years. We emphasize that in malnutrition (14), inborn errors of carnitine metabolism, or muscular dystrophy (5, 16), the free carnitine excretion should also be expressed per kg body weight.

In the literature, a sex-related difference in the urinary free carnitine excretion pattern is reported after adolescence, and a hormonal factor was suggested (15). In addition, in the adult, a day/night differentiation in free carnitine excretion was observed (3) and explained as being due either to endocrine factors or to a quantitatively different muscular activity (2). In the other hand, we found no difference between day- and nighttime urinary excretion in newborns and infants.

In the first weeks, besides the lower free carnitine plasma level, there is a reduced urinary excretion of carnitine. It is possible that during the first months of life the dietary carnitine intake in the form of human or cow's milk is necessary to maintain normal plasma carnitine levels because of an immature endogenous synthesis. Balance and compartmentation studies are needed to decide if carnitine is a semiessential component during the first age of life.

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