Int. J. Vitam. Nutr. Res., 76 (3), 2006, 117–123

Plasma Free Carnitine Levels in 0–12-Month-Old Infants in Relation to Feeding Styles

Selda Hizel[1], Fatma Kaya Kiliç[2], Cihat Şanli[3], Turgay Coşkun[4], Saniye Önal[5]

[1] Associate Professor, Kırıkkale University Faculty of Medicine, Department of Pediatrics
[2] Assistant Doctor, Kırıkkale University Faculty of Medicine, Department of Pediatrics
[3] Assistant Professor, Kırıkkale University Faculty of Medicine, Department of Pediatrics
[4] Professor, Hacettepe University Faculty of Medicine, Department of Pediatrics
[5] Laboratory Technician, Nutrition and Metabolic Diseases Unit, Hacettepe University Faculty of Medicine, Department of Pediatrics

Received for publication: November 16, 2005; Accepted for publication: February 16, 2006

Abstract: This study was planned to determine the blood carnitine levels of children aged 0–1 year in Kırıkkale. Blood samples were taken on Guthrie cards and plasma free carnitine levels were analyzed by tandem mass spectrometry. The mean free carnitine level was 25.12 ± 10.1 μmol/L (10.1–49.5 μmol/L). To assess the plasma free carnitine levels according to feeding style, babies were grouped as exclusively breastfed (n = 67, 49.3%), artificially fed (n = 24, 17.7%), and mixed fed (n = 45, 33%). The exclusively breastfed infants displayed the lowest free carnitine levels ($p < 0.05$). However, when this was analyzed in accordance with age, no significant change was observed in plasma free carnitine levels according to feeding style. Results of this study are important as they reveal an indication about the normal values of plasma free carnitine in Turkish infants and their relationship to feeding styles.

Key words: Children, carnitine

Introduction

Carnitine is a quarternary amine in the structure of 3-hydroxy-4-N-trimethylamino-butyrate. In biological materials, it is found as free carnitine and acyl-carnitine. Major sources of carnitine are meat and dairy products. Apart from foods, it is also synthesized in the body from the methylated lysyl residues produced by the decomposition of lysosomal proteins. Some other compounds, such as lysine, methionine, vitamin C as cofactor, nicotinic acid, vitamin B6, and Fe++ are also required for its synthesis. Approximately 95% of carnitine is found in skeletal muscles, 4% in the liver and kidney, and 1% in extracellular fluid [1-3]. Carnitine has numerous functions, including β-oxidation of long- and middle-chain fatty acids in mitochondria, acetyl storage and transfer between cells and tissues, transfer of activated middle- and short-chain fatty acids from peroxisomes to mitochondria, and transport of potentially toxic activated fatty acids out of mitochondria in the presence of free CoA [1, 4, 5].

Determining blood carnitine levels in newborns and infants would be helpful for monitoring and treating carnitine-related inherited metabolic disorders, such as carnitine metabolism disorders, fatty acid oxidation defects, and dilated cardiomyopathy. Clinical studies have demonstrated that plasma free carnitine levels vary according to the state of hunger, with respect to age and gender, and between societies. Therefore, whether the carnitine level

DOI 10.1024/0300-9831.76.3.117

is pathologic or not can only be determined if the normal values of healthy individuals in that population are known [6–12].

Breast milk, and especially colostrum, is abundant in carnitine, whereas cow's milk- and soy-based infant formulas contain very little carnitine. For this reason, L-carnitine should be added to the diets of infants deprived of breast milk and to the nutritional substrate of preterm newborns receiving total parenteral nutrition [6, 7, 9].

The objectives of our study were to determine the normal values of plasma free carnitine levels in infants aged 0–1 year, and to show the relation between carnitine levels and feeding styles.

Materials and Methods

To determine the blood carnitine levels in infants 0–1 year of age, this cross-sectional study including 136 infants [72 girls (52.9%), 64 boys (47.1%)] was undertaken. Children between 0 and 1 year of age who were sent to the outpatient clinics of the Pediatrics Department of Kırıkkale University Medical School with any complaint between January and June 2004 were included in the study. The study group consisted of healthy children who came to the outpatient clinics for periodic monthly check-ups as well as those who were seen for disorders unrelated to primary or secondary carnitine deficiencies. Preterm newborns were not included in the study, as they are at risk for secondary carnitine deficiency. All families were informed about the study and written parental permission was attained. Information on the demographic characteristics of the family as well as gestational age, birth weight, delivery mode, feeding style, and height and weight percentiles of the baby were recorded, and all children were examined in detail for signs of primary and/or secondary carnitine deficiency. The mean age of patients was 90 days (range: 1 day to 1 year). The mean gestational age of infants was 39.49 ± 1.06 weeks (range: 37 weeks to 42 weeks), and the mean birth weight was 3221.91 ± 374.20 grams (range: 2500–4200 g). All cases were grouped according to age: one day of life; 2–7 days; 8–28 days; 2–3 months; 4–6 months; 7–9 months; and 10–12 months.

Cases were also assessed in three groups according to feeding style, including exclusively breastfed infants (49.3%) (infants receiving breast milk and herbal teas only), artificially fed infants (17.7%) (infants fed only cow's milk and/or formulas), and mixed fed infants (33%) (infants receiving both breast milk and cow's milk and/or formulas). Once written consent was obtained from the family, blood samples were taken on Guthrie cards from the heels of newborns and from the fingertips of older infants, and serum free carnitine levels were analyzed by tandem mass spectrometry in the Nutrition and Metabolic Diseases Unit of the Pediatrics Department at Hacettepe University Medical School. This study was approved by the local Ethics Committee.

All collected data were evaluated by means of a computerized SPSS program. ANOVA was employed in the comparison of free carnitine levels with demographic parameters, feeding style, and age groups; Tukey's method was used to detect from which group the discrepancy emanated; and Pearson's chi-square test was utilized for determination of the relationship between gender and free carnitine levels. The significance limit was set as $p < 0.05$.

Results

The study group consisted of 136 infants: 72 girls (52.9%) and 64 boys (47.1%) in the 0 to 1 year age group. A high proportion of mothers (46.3%) and fathers (83.2%) of the infants had schooling of eight years or more, and 97% of the families had at least one kind of social insurance (Table I). The mean plasma free carnitine level of the whole group was 25.09 ± 10.06 μmol/L (10.1–49.5 μmol/L).

The mean plasma free carnitine level in the one-day-old group (15.66 ± 1.11 μmol/L) was significantly higher than that of the second group (11.39 ± 1.09 μmol/L; $p = 0.01$) (Table II). The plasma free carnitine levels of the

Table I: Demographic characteristics of the study group.

Demographic features		n	%
Mother's employment	Unemployed	109	80.1
	Employed	27	19.9
Father's employment	Unemployed	4	2.9
	Employed	132	97.1
Mother's education	Illiterate	3	2.2
	Literate	2	1.5
	Primary School	68	50
	Elementary school	23	16.9
	High school	27	19.9
	University	14	9.6
Father's education	Illiterate	1	0.7
	Literate	1	0.7
	Primary School	21	15.4
	Elementary school	20	14.7
	High school	59	43.4
	University	34	25
Social insurance	No insurance	3	2.2
	Retirement fund	72	52.9
	Social Security Ins.	14	10.3
	Bağ-kur	31	22.8
	Green-card	16	11.8

Table II: Free carnitine levels with respect to age and gender.

Group	Age	Sex	n	Free carnitine levels (µmol/L) mean ± SD	(min–max)
1	1 day	Total	16	15.66 ± 1.11	(14.5–18.6)
		Female	10	15.38 ± 0.85	(14.5–16.9)
		Male	6	16.13 ± 1.39	(14.5–18.6)
2	2-7 days	Total	13	11.39 ± 1.09	(10.1–13.8)
		Female	9	11.43 ± 1.27	(10.1–13.8)
		Male	4	11.30 ± 0.66	(15.1–18.6)
3	8-28 days	Total	18	17.23 ± 1.14	(15.1–19.1)
		Female	8	17.66 ± 1.17	(16.2–19.1)
		Male	10	16.88 ± 1.03	(15.1–18.6)
4	2-3 months	Total	22	21.14 ± 1.44	(18.6–23.8)
		Female	12	20.57 ± 1.05	(18.6–22.2)
		Male	10	21.81 ± 1.61	(19.2–23.8)
5	4-6 months	Total	23	24.85 ± 1.75	(20.1–28.2)
		Female	8	24.85 ± 2.27	(20.1–27.6)
		Male	15	24.84 ± 1.50	(23.2–28.2)
6	7-9 months	Total	20	36.72 ± 3.95	(29.5–44.2)
		Female	11	35.92 ± 3.77	(29.5–40.6)
		Male	9	37.70 ± 4.16	(30.7–44.2)
7	10-12 months	Total	24	38.89 ± 5.17	(30.1–49.5)
		Female	14	38.54 ± 4.15	(32.1–49.5)
		Male	10	39.38 ± 6.55	(30.1–49.2)
	1 day-12 months	Total	72	25.09 ± 10.06	(10.1–49.5)
		Female	64	24.70 ± 10.32	(10.1–49.5)
Total		Male	136	25.54 ± 9.83	(10.5–49.2)

$p > 0.05$

first group were observed to be significantly lower than those of fourth, fifth, sixth, and seventh groups ($p < 0.05$), but this was not observed between the first and the third groups ($p = 0.694$). An increase in plasma free carnitine levels was observed starting with the seventh day of life until six months of age. No significant relationship between the free carnitine levels and age was detected among infants aged 6 and 12 months (Table III).

Table III: Differences in free carnitine levels with respect to age groups.

Group	Age	n	Free carnitine levels (µmol/L) mean ± SD	(min–max)
1	1 day	16	15.66 ± 1.11	(14.5–18.6)
2	2–7 days	13	11.39 ± 1.09	(10.1–13.8)
1 and 2			p = 0.001	
3	8–28 days	18	17.23 ± 1.13	(15.1–19.1)
2 and 3			p = 0.001	
4	2–3 months	22	21.13 ± 1.44	(18.6–23.8)
3 and 4			p = 0.001	
5	4–6 months	23	24.85 ± 1.75	(20.1–28.2)
4 and 5			p = 0.001	
6	7–9 months	20	36.72 ± 3.95	(29.5–44.2)
5 and 6			p = 0.001	
7	10–12 months	24	38.89 ± 5.17	(30.1–49,5)
6 and 7			p = 0.169	
Total		136	25.09 ± 10.06	(10.1–49.5)

Mean plasma free carnitine levels were calculated as 24.70 ± 10.32 µmol/L in girls and 25.54 ± 9.83 µmol/L in boys, with no significant difference between the two genders ($p > 0.05$). Plasma free carnitine levels also did not vary significantly according to age or gender, as shown in Table II.

Our results revealed that 93.6% of the infants in the 0–1 month age group were exclusively breastfed and the rest were mixed fed (Table IV). However, the exclusively breastfed-infant rate dropped to 49.3% after six months of life. In the 6–12-month-age group, 52.2% of the infants were having supplementary foods in addition to breastfeeding, whereas the rest (47.8%) were on artificial feeding. When we examined the plasma free carnitine levels with respect to feeding styles, the levels were lower in exclusively breastfed infants (17.21 ± 3.94 µmol/L) compared with the infants having mixed (37.43 ± 6.6 µmol/L) or artificial feeding (30.26 ± 7.72 µmol/L; $p < 0.05$). However, when mean free carnitine levels were examined with respect to age and feeding styles, this difference became insignificant (Table IV).

Table IV: Free carnitine levels with respect to age and feeding styles.

Age (months)	Exclusively breastfed infants		Mixed fed infants		Artificially fed infants		Total	p
	n	FCL*	n	FCL*	n	FCL*		
0–1	44	14.95 ± 2.6	3	16.98 ± 0.8	0	–	47	p = 0.01
2–6	23	21.53 ± 1.73	19	24.40 ± 2.2	3	25.84 ± 0.65	45	p = 0.412
7–12	0	–	23	36.82 ± 4.0	21	39.09 ± 5.20	44	p = 0.035
Total	67	17.21 ± 3.94	45	37.43 ± 6.6	24	30.26 ± 7.72	136	p = 0.01

* Free carnitine levels (μmol/L)

Discussion

Children, particularly preterm newborns, constitute the highest risk group for carnitine deficiency, and variations in serum carnitine levels in children can be observed by age, as demonstrated in various studies published in the literature [6, 7, 13–15]. Clinical studies reported that plasma free carnitine levels may vary with respect to age, gender, and race. Moreover, mean free carnitine levels differ between communities. It has been postulated that these differences could be the result of variability in inclusion criteria of the study groups (e.g., different ages and genders), usage of different samples (e.g., blood, serum) and analysis methods for blood carnitine levels and the dietary habits of the societies [6–12]. Battistella et al showed that mean free carnitine levels were lower in the early months of life, reach adulthood levels at the end of six months of age, and were not affected by gender in children [16]. Similar to these results, Schmidt et al also measured carnitine levels in 353 healthy children and demonstrated that carnitine levels were not affected by sex, gradually increased with age, and neither total nor free carnitine levels changed according to age, after the second year of life, until adulthood period (Table V) [12].

In the study of Güneral and colleagues, the mean plasma free carnitine level in children aged six months to two years was found to be 35.02 ± 9.2 μmol/L. This value did not vary according to gender and increased with age, reaching 52.8 ± 11.1 μmol/L at age 6–10 years and remaining unchanged until the adulthood period [17]. Similar to Güneral's study, Aydın and colleagues also demonstrated no significant difference in free and total carnitine levels and acyl carnitine/free carnitine ratios between males and females, and free carnitine levels were determined to be 34.72 ± 8.68 μmol/L in 0–1-year-old children (Table V) [9]. However, in contrast to the aforementioned studies, Özyürek *et al* showed no change in free carnitine levels by age [6]. Unlike other studies, the results of our study revealed relatively lower free carnitine levels in the first year of life (25.09 ± 10.06 μmol/L). The mean free carnitine level in the one-day-old group (15.66 ± 1.11 μmol/L) was significantly higher than in the second group (11.39 ± 1.09 μmol/L, p = 0.01), but was significantly lower than those of the fourth, fifth, sixth, and seventh groups ($p < 0.05$). An increase in the plasma free carnitine level was observed starting with the seventh day of life until six months of age, but no significant relationship was detected between free carnitine levels and age, after the age of six months (Table II).

The activity of the γ-butyrobetaine hydroxylase enzyme, which hydroxylates γ-butyrobetaine, a carnitine precursor, to carnitine is only 12 % of the adult level during the first month of life [7, 10, 13]. Therefore, carnitine synthesis is limited in newborns, especially in preterms. The low carnitine level at birth increases with age. Supporting this, various studies have demonstrated low free and total carnitine levels in newborns and infants until the sixth month of life [10, 13]. Winter et al analyzed free carnitine levels in 145 healthy infants and found levels of 20.2 ± 5.1 μmol/L in the first five days of life, rising to 40.2 ± 6.1 μmol/L at the age of one week to one year [18]. On the other hand, Meyburg and colleagues detected the total and free carnitine levels in 70 term newborns as 55.7 ± 1.6 μmol/L and 30.6 ± 10.6 μmol/L respectively, on the fifth day of life [19]. The results of the two studies were quite different from each other. Deufel and co-workers' study comprising 75 newborns showed that plasma free and total carnitine levels steadily increased in the first three weeks of life and reached adulthood levels after three months [11]. The researchers of this study stated that low carnitine levels could be related to low enzyme activity and insufficient exogenous carnitine intake, as the total carnitine level increased in older children with increased intake of exogenous carnitine by diet [11]. In Schmidt's study, mean carnitine levels were lower in term newborns compared to other age groups (20.1 ± 6.7 μmol/L on the first day, 14.9 ± 3.0 μmol/L between the second and the seventh days, and 27.6±9.7 μmol/L between the eighth and 28th days). They claimed that plasma carnitine levels increase within the first day, then dropped by the end of the first day, and reached adulthood levels at the end of the second year with a progressive increase after the first week of life [12]. In our study the same but less dramatic pattern of change was observed in the first month of life

Table V: Free carnitine levels in various studies.

Researchers (R)	Year	Total sample size	Age group	Average free carnitine level (µmol/L) mean ± SD
Schmidt *et al* (12)	1988		First day	20.1 ± 6.7
			2–7 days	14.9 ± 3.0 L
			8–28 days	27.6 ± 9.7
		353	1 month–1 year	35.5 ± 6.5
			1–6 years	41.7 ± 7.9
			6–10 years	41.4 ± 10.0
			10–17 years	39.4 ± 8.7
		40	Umbilical cord	22.0
Güneral *et al* (17)	1995		6 months–2 years	35.02 ± 9.2
(Ankara)		68	3–5 years	43.63 ± 11.9
			6–10 years	52.8 ± 11.1
			20–35 years	52.2 ± 11.9
Aydın *et al* (9)	1996		0–1 years	34.72 ± 8.68
(Istanbul)		100	2–5 years	46.48 ± 18.73
			6–9 years	44.83 ± 12.22
			10–12 years	49.97 ± 13.87
Özyürek *et al* (6)	1998		1–6 months	42.89 ± 12.68
(Izmir)		80	7 days–2 years	43.22 ± 15.81
			3–6 years	45.60 ± 14.35
Tanzer *et al* (13)	1994	33	0–1 month (term)	51.1 ± 0.9
(Sivas)				
Winter *et al* (18)	1992	145	Fifth day	20.2 ± 5.1
			7 day–1 year	40.2 ± 6.1
Deufel *et al* (11)	1986	75	0–1 month	30.1 ± 6.3
Chace *et al* (10)	2003	24	0–1 month (term)	72.42 ± 20.75
		50	Umbilical cord	31.27 ± 10.50
Novak *et al* (24)	1981	17	Umbilical cord	30.0
Bargen *et al* (25)	1981	21	Umbilical cord	23.0
Hızel *et al*	2004		First day	15.66 ± 1.11
(Kırıkkale)			2–7 days	11.39 ± 1.09
			8–28 days	17.23 ± 1.13
		136	2–3 months	21.13 ± 1.44
			4–6 months	24.85 ± 1.75
			7–9 months	36.72 ± 3.95
			10–12 months	38.89 ± 5.17

(free carnitine levels were found to be 15.66 ± 1.11 µmol/L on the first day of life, 11.39 ± 1.09 µmol/L in infants ages two to seven days, and 17.23 ± 1.13 µmol/L in the infants of eight to 28 days of life). The free carnitine levels were noticed to be significantly higher on day one than on the second to seventh day of life ($p = 0.01$). Free carnitine levels were relatively lower than the findings of other studies cited above. However our results could still be accepted as important in showing that the changes in the newborn and infancy period may be the most common clinical manifestations of carnitine-related metabolic disorders that begin early in infancy. Therefore, knowing the normal values of plasma free carnitine levels in the newborn and infancy period will help clinicians in the diagnosis of these rare metabolic disorders.

Ohtani *et al* demonstrated that the mean total carnitine level in breast milk was 53.5 ± 5.6 nmol/mL during the first month of life [22]. Similarly, Sandor *et al* measured the total carnitine concentration in breast milk as 62.9 nmol/mL (minimum = 56.0; maximum = 69.8) during the first 21 days of life. In this study, breast milk was emphasized to be the main source of carnitine in infancy, and they claimed that breastfed newborns had higher plasma carnitine levels than those fed with soy-based formulas [23]. When we take into account the feeding styles of our study group, the mean free carnitine level of infants exclusively breastfed was found to be lowest when compared to artificially fed and mixed fed infants ($p < 0.05$). However, this significant difference vanished when the cases were grouped in accordance with age and feeding style

(Table V). We believe that this was a natural result, as usage of breast milk decreases and usage of additional foods in the diet of an infant increases with age; thus blood carnitine levels increase with age. Therefore, the differences in blood carnitine levels in infants with different feeding styles should be discussed within the same age groups.

In almost all studies presented in the literature, blood carnitine concentrations were reported to be low in newborns and infants until the age of two years and varied between societies [11, 12, 17]. As breast milk is the major nutritional source in the first six months of life and as breastfeeding and infant nourishing patterns change according to the traditions, habits, and customs of a community, variations in blood carnitine levels within communities are expected. Even among infants fed exclusively with breast milk, low blood carnitine levels can still be encountered due to the influence of other factors, such as low enzyme activity, decreased synthesis capacity, and limited tissue reservoirs in these ages; thus arrival to adulthood levels may take longer. However, as emphasized by many researchers, it should not be forgotten that breast milk is an accelerating factor in the achievement of adulthood levels [10, 11, 22, 24].

In conclusion, although the sampling group of this study was relatively small, we still believe that our efforts light the way for researchers for further studies on normal plasma free carnitine levels in Turkish infants. Furthermore, the results of our study also demonstrated variations in blood carnitine levels according to feeding styles. Therefore, the feeding style of an infant should also be taken into account in the estimation of normal blood carnitine levels in the infancy period. There is no debate that breast milk is the main source of feeding in the first six months of life, and it must also be emphasized that supplementary foods should be started after the sixth month of life so as to increase plasma free carnitine levels. In order to anticipate at which age plasma free carnitine levels reach adulthood levels, a cohort-type prospective study with a larger sampling size is necessary. All previous studies established in Turkey were with small sample sizes and were regional and do not allow us to make a generalization for Turkish children and create Turkish normograms for infant blood carnitine levels (Table V). Because of all these factors, multi-centered research equipped with a sampling method capable of more fully representing the Turkish society, with more cases in each age group, is needed in order to determine the normal blood carnitine levels of Turkish children.

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Dr. Selda Hizel

Konrad Adenauer Caddesi 48/9 Yildiz
Gankaya/Ankara/TURKEY
Tel. + 90 312 491 85 32
Fax + 90 318 225 28 19
E-mail: sfbulbul@yahoo.com