Effects of gestation age and of birth weight in the concentration of carnitine in the umbilical plasma

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Summary

To investigate the factors which affect the concentrations of the total, the free, and the acylcarnitine in neonates, blood was taken from the umbilical cord of 49 newborn infants ranging in gestation age (g. a.) from 32-40 weeks (mean g. a.: 36.8 ± 2.6 weeks) and in birth weight (b. w.) from 1300 gr. - 4300 gr. (mean b. w.: 2299 ± 457 gr.).

The carnitine and its fractions were studied in plasma. Twenty-eight of the neonates studied were premature (g. a. ≤ 37 weeks) and 21 were full-term (g. a. > 37 weeks).

The concentration of the total, free, and acylcarnitine in premature neonates was 28.0 ± 2.3 μmol/L, 15.9 ± 1.3 μmol/L, and 12.0 ± 1.3 μmol/L, respectively.

For the full-term neonates the respective concentrations were: 25.2 ± 2.2 μmol/L, 14.6 ± 1.5 μmol/L, and 10.7 ± 1.5 μmol/L. These differences in concentrations between premature and full-term infants were statistically significant. For the total number of neonates studied the concentration of total, free, and of acylcarnitine was 26.8 ± 2.6 μmol/L, 15.3 ± 1.9 μmol/L, 11.5 ± 1.5 μmol/L respectively.

The calculation of the correlation coefficients for the total number of neonates showed the existence of a statistically significant negative correlation between the total, free and acetyl carnitine in terms of gestation age and birth weight.

The comparative analysis of the correlation coefficients showed greater coefficient values between the total and the acylcarnitine in terms of birth weight. The latter finding, combined with the low rate of acylcarnitine decline, are indirect indications that the fetus uses carnitine as a source of energy, which affects the levels of total and acylcarnitine in the plasma.

Introduction

The presence of carnitine in tissues and cells of the human organism in related to vital functions, as for example the transfer of long-chain fatty acids and their subsequent ß-oxidation, through the inner mitochondrial membrane, for the production of energy [1]. In this way L-carnitine contributes to the production of energy by burning fatty acids and economising on the glucose available.

We know, that fatty acids constitute the main source of energy during the period immediately after birth since glucose levels are low or drop fast [2]. Thus the presence of sufficient amounts of carnitine is of great importance for the maintenance of a normal level of metabolism.

Nevertheless, little is known about the transfer of carnitine through the placenta and the ability of the embryo to synthesise carnitine [3].

The present study was undertaken to investigate the degree to which such basic embryo factors as, gestation age, birth weight, or others relate to the levels of the total, free, and acylcarnitine in the serum of neonates.

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Materials and Methods

Forty-nine neonates, 28 premature (gestation age ≤ 37 weeks) and 21 full-term neonates (gestation > 37 weeks), were included in this study. In the total number of neonates gestation age ranged from 32 to 40 weeks while birth weight ranged from 1300 gr. to 4300 gr. (Table 1).

The course of pregnancy, the delivery, as well as the perinatal period for all neonates studied were without any complications. The mothers included in this study did not receive any medication, which otherwise would have affected the metabolism of carnitine [4]. The delivery period ranged from 4 to 20 hours. Sodium chloride (0.9%) was administered intravenously (drops) to a few parturients while others received dextrose (5%) solution during delivery. In general, all neonates were delivered without complications and none presented with hypoxia or other problems during the delivery (Apgar score > 9 during the first 3 minutes). The blood samples were taken from vessels of the umbilical cord following delivery.

Serum carnitine (separated and frozen within the first hour of collection) was determined in the supernatant after acid precipitation of serum proteins. The fraction of acylcarnitines was determined by subtracting free carnitine from the amount of total carnitine which was determined after alkaline hydrolysis of all carnitine esters. The routinely used method was based on that described by McGarry and Foster [5].

The statistical analysis of the results, as regards the difference in concentrations of carnitine and its fractions between the premature and the full-term neonates, was carried out with the application of the Student's t test [6]. Furthermore, to investi-

Table 1. — *Clinical characteristics of neonates studied*

	Number	Range	Mean±SD
Age of mothers (years)	49	19-36	25±7
Sex: Male	26		
Female	23		
Premature neonates	28		
Gestation age (weeks)		32-37	35.1±1.5
Birth Weight (grams)		1300-3350	2299±457
Full-term	21		
Gestation age (weeks)		38-40	39.2±0.8
Birth weight (grams)		2800-4300	3414±412
Total neonates	49		
Gestational age (weeks)		32-40	36.9±2.4
Birth Weight (grams)		1300-4300	2777±707

Table 2. — *Variations in total, free and acylcarnitine in the plasma of neonates studied*

	Number	Range	Mean±SD
Premature neonates	28		
Total carnitine		23.7-32.2	28.0±2.3
Free carnitine		9.5±14.1	15.9±1.3
Acylcarnitine		9.5±14.1	12.0±1.3
Full-term neonates	21		
Total carnitine		22.0-29.3	25.2±2.2
Free carnitine		11.9-17.5	14.6±1.5
Acylcarnitine		8.9-14.1	10.7±1.5
Total neonates	49		
Total carnitine		22.0-32.5	26.8±2.6
Free carnitine		11.9-21.3	15.3±1.9
Acylcarnitine		8.9-14.1	11.5±1.5

Table 3. — *Comparing the concentration of plasma carnitine and its fractions between premature and full-term neonates*

	Number	t	p
Total carnitine	49	4.164	=0.0013
Free carnitine	49	2.634	=0.0114
Acylcarnitine	49	3.265	=0.002

Table 4a. — *Correlation coefficient values in terms of gestation age and carnitine (plasma & fractions) concentration in the umbilical plasma of neonates*

	r	p	LRA
Gestation age (weeks)			
Premature neonates			
Total carnitine	–0.468	=0.012	y=52.29-0.694x
Free carnitine	–0.408	=0.031	y=33.90-0.513x
Acylcarnitine	–0.254	>0.05*	
Full-term neonates			
Total carnitine	–0.102	>0.05*	
Free carnitine	–0.210	>0.05*	
Acylcarnitine	–0.06	>0.05*	
Total neonates			
Total carnitine	–0.602	<0.001	y=50.46-0.642x
Free carnitine	–0.480	<0.001	y=28.90-0.369x
Acylcarnitine	–0.438	=0.002	y=21.35-0.268x

Table 4b. — *Correlation coefficient values in terms of birth weight and carnitine (plasma & fractions) concentration in the umbilical plasma of neonates*

	r	p	LRA
Birth weight (grams)			
Premature neonates			
Total carnitine	–0.456	=0.015	y=33.20-0.002x
Free carnitine	–0.458	=0.014	y=20.35-0.002x
Acylcarnitine	–0.161	>0.05*	
Full-term neonates			
Total carnitine	–0.804	<0.001	y=39.75-0.004x
Free carnitine	–0.423	=0.031	y=20.43-0.002x
Acylcarnitine	–0.716	<0.01	y=19.38-0.003x
Total neonates			
Total carnitine	–0.720	<0.001	y=34.13-0.003x
Free carnitine	–0.548	<0.001	y=19.34-0.001x
Acylcarnitine	–0.560	<0.001	y=14.75-0.001x

LRA: Linear Regresion Analysis
* : Not statistically significant

Table 5. — *Comparison of correlation coefficients in terms of the parameters studied*

	Total carnitine		Free carnitine		Acylcarnitine	
	r	p	r	p	r	p
Gestational age	–0.6022	<0.001	–0.4797	<0.001	–0.4385	<0.0016
Birth weight	–0.7203	<0.001	–0.5477	<0.001	–0.5605	<0.001

gate the correlation between, on the one hand, the values of total carnitine and those of its fractions in the umbilical cord, and gestation age or birth weight, on the other hand, we applied the linear regression analysis method (y=a+bx). Finally, to compare the coefficients of various parameters we applied multivariable regression analysis [7].

Results

For an in depth examination of the parameters affecting the levels of the total, free, and acylcarnitine in the blood of the umbilical cord, the 49 neonates included in the study were subdivided into groups, the premature and the full-term group. The same parameters were studied also in terms of the total number of neonates.

For each group, as well as for the total number of neonates, the mean values and the standard deviation of the values for the total, free, and acylcarnitine of the plasma in the umbilical cord are given in Table 2.

For the premature group the concentration of the total, free, and acylcarnitine was 28.0 ± 2.3 μmol/L, 15.9 ± 1.3 μmol/L, and 12.0 ± 1.3 μmol/L, respectively. For the full-term group the respective concentrations were comparatively lower: 25.1 ± 2.6 μmol/L, 14.6 ± 1.5 μmol/L, and 10.7 ± 1.5 μmol/L. The differences were statistically significant, both for the total and for the fractions of carnitine.

The full-term neonates, in comparison to their full-term counterparts, exhibited lower carnitine concentrations in the umbilical cord, i. e. with the progress of pregnancy the concentration of total, free, and acylcarnitine is significantly reduced (Table 3).

For the total number of neonates studied (premature and full-term), the concentrations of carnitine were: 26.8 ± 2.6 μmol/L, 15.3 ± 1.9 μmol/L, and 8.9 ± 14.1 μmol/L for the total, free, and acylcarnitine, respectively.

The correlation between gestation age, as well as birth weight, in terms of plasma carnitine and its fractions, was studied separately for the premature, full-term, and total number of neonates (Tables 4a, 4b).

As regards gestation age, a statistically significant negative correlation in the concentration of the total and free carnitine was observed, but not for acylcarnitine, in the premature neonates. As for the group of full-term neonates, a significant correlation, in the concentration of total carnitine and its fractions, was not found. However, in the total group of neonates studied, a negative correlation, statistically very significant was observed between gestation age and the values of the total, free, and acylcarnitine of the plasma.

Regarding birth weight for the premature group, the results are equivalent to the correlations found between the values of carnitine in terms of gestation age. For example, a statistically significant negative correlation was established between the values of total and free carnitine, but not acylcarnitine. For the full-term group and also for the total number of neonates studied, a statistically very significant negative correlation was established for the total, free and acylcarnitine.

Comparison of the correlation coefficients for the parameters studied (Table 5) showed that, for the total number of neonates, the highest coefficient is observed in the total carnitine in terms of gestation age and birth weight. Additionally, the most statistically significant correlation found was the value of total carnitine in terms of birth weight (r=0.7203) as compared to gestation age (r=0.6022).

Discussion

The concentrations of the total, free, and acylcarnitine in the plasma of the umbilical cord described in this paper are similar to those cited by other authors [8, 9, 10]. Another similarity is the fluctuation of carnitine level with respect to gestational age [11].

Today the gradual decrease of (plasma) carnitine in mothers and foetuses has been established [10, 12]. In the present study the carnitine was measured in the blood of the umbilical cord in order to estimate the degree by which gestation age and birth weight affect carnitine concentrations as well as its fractions in the blood of neonates during birth.

A number of factors have been reported to affect the levels of carnitine in the blood of the umbilical cord. Firstly, the increased concentration of carnitine in the premature is likely due to the increased production of carnitine in the embryo from the onset of pregnancy. Nevertheless, experiments have shown that for the premature, the production of endogenous carnitine is limited. This is supported by the fact that while the liver is the most important organ for the production of carnitine, the action of special hydroxylase in the liver of premature infants is only 12% that of a normal mature adult [13].

Increased carnitine levels could also be attributed to the increased supply of carnitine through the maternal placenta during the initial stages of pregnancy. Research with regard to mother-embryo carnitine relations, and those of its metabolists has shown that increased transport of carnitine through the placenta covers most of the embryo needs for carnitine [10, 14].

Sachan *et al.* [11] found that in foetuses of birth weight less than 950 gr. or of gestation age ranging between 24 to 31 weeks, there is a positive correlation between gestation age or birth weight and the levels of total and free carnitine in the embryo. Following this, carnitine stabilises at 20 μmol/L at the age of 34 weeks to gradually drop until birth. These findings support the view that there is an increased supply of carnitine to the embryo through the placenta during the first stages of gestation.

This mechanism possibly counterbalances the relative inefficiency of the embryonal tissues, mainly of the muscles and the heart, to take up and store carnitine. This results in increased levels of carnitine in the plasma. Measurements have confirmed low level carnitine concentrations, analogous to carnitine deficiency, in the muscles, the heart and the liver in premature embryos [9, 15]. Possibly, the inefficiency of the take up and storage

mechanisms is responsible for high level carnitine concentrations. However, the placental transport of the carnitine mechanism is not quite known since it differs from one animal to another. In sheep, for example, the transport of carnitine through the placenta is low in comparison to that of guinea pigs [16].

Up to the present day there are few indications concerning the mode of transport of carnitine through the placenta in humans. At least three mechanisms have been reported, e.g. passive diffusion through the extracellular space, cellular intake and, finally, intra-cellular deconstruction, and diffusion of the metabolists.

The findings of this paper, as well as those of other authors [17, 18] sustain the view that passive diffusion is the main factor. This derives from the observed high correlation of total carnitine in the blood of the umbilical cord with gestation age in premature neonates borne normally and complication-free during the perinatal period.

To-date, the possible effect of embryo metabolism has not been reported to affect the levels of carnitine or those of its fractions. Indications point to the view that the embryos use certain amounts of energy to oxidise fat [19]. In such cases increased levels of carnitine are observed, mainly of free and acylcarnitine in the maternal umbilical plasma [20].

In the present study, lower correlation coefficients were observed in the case of acylcarnitine. This observation, combined with its slow reduction rate, as compared to total carnitine, particularly with regard to birth weight, constitutes indirect evidence to the fact that the embryo uses carnitine for the production of energy. This affects total and acylcarnitine levels in the plasma. The rate of carnitine metabolism in the embryo must be included in the factors regulating the levels of the substance (carnitine) in the embryo.

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