Comparative study of plasma carnitine: determination in the neonate and in normal delivery

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Summary

Free total plasma carnitine and acylcarnitine were determined in 20 mothers with normal deliveries and their neonates. Women of reproductive age and children constituted the comparative control groups. The mothers had lower total and free carnitine concentrations as compared to the neonates and the differences were statistically significant. The acylcarnitine values in the mothers were higher but the differences were not statistically significant.

The women had higher total and free carnitine concentrations as compared to the children, while the values of acylcarnitine were higher in the children and the differences were statistically significant. The women had higher total and free carnitine concentrations as compared to the mothers and the differences were statistically very significant. In contrast the concentration of acylcarnitine was higher in the mothers than in the women and the difference was statistically significant. The children had higher total and free carnitine concentrations as compared to the neonates. On the other hand the concentration of acylcarnitine was higher in the neonates than in the children. The difference was statistically significant. It appears that transfer of maternal carnitine to the fetus constitutes the main factor of determining carnitine concentration in the neonate.

Key words: Plasma carnitine; Neonate; Normal delivery.

Introduction

The presence of carnitine in tissues and cells of the human organism is related to vital functions, such as that of transfer of long chain fatty acids for the internal mitochondrial membrane, so that their ß-oxidation might constitute the production of energy [4,20]. A portion of carnitine in the human body is derived from food and another portion is synthesised endogenously from lysine and methionine [8].

Immediately after birth lipids constitute the main source of energy, in which a more precise oxidation requires the presence of an ample concentration of carnitine [9,14,16].

Very little is known about the transportation of carnitine through the placenta and the ability of the embryo or the neonate to synthesize carnitine [13]. Carnitine measurements in the mother and the umbilical plasma, as well as in the experimental data following in vitro carnitine infusion in a section of placenta have suggested that carnitine passes the placental barrier [14,20].

The present study was undertaken in an effort to contribute to the relationship between maternal and neonate plasma carnitine concentration, when pregnancy and the perinatal period are without complications and, also, to compare these concentrations with those of other population groups.

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Patients and Method

Twenty mothers and their neonates constitute the material of this study. The mothers were free of any chronic illness and had not received drugs during pregnancy, except iron, folic acid and vitamin supplements. Their mean age was 24.5 years (18-35 years) and gestational age at delivery was between 37 and 40 weeks (mean duration of pregnancy 38.8 weeks). Pregnancy developed without any complications and delivery had a normal outcome in all cases.

The newborn infants constituted the second group of the study. All neonates were fullterm and of normal birthweight. None of the infants presented with any signs of serious hypoxia during delivery. Their Apgar scores were 8-9 at the first minute of life. Nine of the neonates studied were males and there were 11 females. Nineteen non-pregnant, fertile and of reproductive age women, without any evident problems of health, who were not under any drug treatment, constituted the control group. Their mean age was 25 years (19-35 yrs), they were mature and had delivered at least one child during the last three years.

A second control group constituted 15 children of school age ranging from 8-11 years (mean 9.5 yrs), apparently healthy, a free personal history and were not taking any drugs.

Maternal blood was taken from a vein in the arm at delivery. Immediately after delivery of the placenta about 4 ml of blood was collected from the separated umbilical cord for carnitine assay.

Serum carnitine (separated and frozen within 1 hr of collection) was determined in 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 [11]. Statistical analysis of the results, as regards the comparison of two samples, was carried out by the parametric method [5], while the investigation of the existence of a correlation between the concentrations of the mother and neonate was carried out by the regression analysis method [23].

Results

The findings for the mothers and neonates and those of the non-pregnant women and the older children are described separately:

a) Mothers-neonates

Table 1 shows the mean values, the standard deviations as well as the highest and lowest value of carnitine concentration in the mothers' and neonates' serum. In the mothers the total carnitine values rose to 22.7±2.6 μmol/L with the highest and lowest value ranging from 18.2 to 26.1 μmol/L. The values were higher in the neonates, namely 26.7±3.5 μmol/L, with the highest and lowest values ranging from 19.9 to 32.3 μmol/L. The differences were statistically significant ($p<0.001$). Table 1 also shows the values of free and acylcarnitine.

In the first concentrations the values rose to 11.6±1.6 μmol/L and 15.7±2.8 μmol/L correspondingly with regard to the mothers and neonates. The differences in these cases as well were statistically significant ($t=5.6$, $p<0.001$). Finally as regards the acylcarnitine concentrations, they were 11.0±1.3 μmol/L and 10.9±1.8 μmol/L correspondingly. The corresponding highest and lowest concentration values were 9.3 and 13.5 μmol/L and 7.1-14.3 μmol/L. The differences were not statistically significant ($p>0.05$).

The existence of a correlation between the concentration values in the parturient and the neonate was also investigated. The results showed the existence of a positive linear correlation between the maternal and neonate values both as concerns total and free carnitine; however this was not shown for the acylcarnitine form ($r=0.487$, $p=0.0294$, $r=0.470$, p.0.0365 and $r=0.284$, $p>0.05$, correspondingly).

b) Adult non-pregnant women and older children

Table 2 shows serum carnitine concentrations in the adult non-pregnant women and the older children. The values are compared with those of the mothers and neonates. In the women, the total serum carnitine concentrations were 39.6±2.3 μmol/L, with the lowest and highest value 36.1 and 44.3 μmol/L. The values in the children were still higher with a mean value of 38.9±2.8 μmol/L, with the lowest 34.3 and highest 43.2 μmol/L values. The differences were not statistically significant ($p>0.05$). As concerns free carnitine the corresponding values were 31.6±1.9 μmol/L, ranging from 29.0 to 36.1 μmol/L for the non-pregnant women and 29.7±2.4 μmol/L for the children. These differences were found to be statistically significant ($p=0.013$).

Finally as regards acylcarnitine, the values in the women were 7.9±1.3 μmol/L, with the lowest value 6.0 and the highest 10.3 μmol/L and for the children 9.2±1.9 μmol/L with the lowest and highest values 5.7 and 12.5 μmol/L, correspondingly. These differences were statistically significant ($p=0.0366$). The comparison between the non-pregnant women and the corresponding values of the parturients showed that in the latter, excluding acylcarnitine, the concentrations were about 50% to those of the non-pregnant women of reproductive age; that is, for total carnitine 39.5±2.3 μmol/L as opposed to 22.7±21.6 μmol/L. The difference which was noted was statistically very significant ($p<0.001$).

The findings for free carnitine were analogous, with corresponding values of 31.6±1.9 as opposed to 11.6±1.6 μmol/L ($p<0.001$). Finally as regards acylcarnitine, the opposite was observed; that is, the maternal concentration was higher as compared to the concentrations in the non-pregnant women, namely 11.0±1.3 μmol/L in the former and 7.9±1.3 μmol/L in the latter. The difference was also statistically significant ($p<0.001$). The comparison of the carnitine values of the older children as compared to the values found in the neonates showed that in the fullterm neonates the values of total carnitine were distinctly lower to those of the former, that is 26.7±3.5 μmol/L as opposed to 38.9±2.8 μmol/L. The differences were found to be statistically significant ($p<0.001$).

The findings for free carnitine were also similar, in which the concentration in the children was 29.7±2.4 μmol/L and 15.7±2.8 μmol/L in the neonates ($p<0.001$). Finally, as regards acylcarnitine, it was observed that as with the non-pregnant women and the parturients the concentrations in the neonates were higher when compared to those of the older children namely, 10.9±1.8 μmol/L. The difference was found to be statistically significant ($p<0.01$).

Table 1. — *Mean values ± SD of total carnitine, Free carnitine and acylearnitine concentration in the mothers' and neonates' serum.*

	Total carnitine μmol/L	Free carnitine μmol/L	Acylcarnitine μmol/L
Mothers (20)	22.7±2.6 (18.2-26.1)	11.6±1.6 (9.4-13.6)	11.0±1.3 (9.3-13.5)
Neonates (20)	26.7±3.5 (19.9-32.3)	15.7±2.8 (13.2-17.1)	10.9±1.8 (7.1-14.3)

Table 2. — *Mean values ± SD of total carnitine, free carnitine and acylearnitine levels in the adult non-pregnant women and the older children.*

	Total carnitine μmol/L	Free carnitine μmol/L	Acylcarnitine μmol/L
Women (19)	39.6±2.3 (36.1-44.3)	31.6±1.9 (29.0-36.1)	7.9±1.3 (6.0-10.3)
Children (15)	38.9±2.8 (34.3-43.2)	29.7±2.4 (27.2-33.1)	9.2±1.9 (5.7-12.5)

Discussion

Up to the present date, quite a few writers have reported on the gradual decrease of free as well as total plasma carnitine with progressing pregnancy [1,9]. The plasma carnitine levels of mothers at delivery are about half of those found in non-pregnant women of reproductive age [6]. It is worth while mentioning that the decrease in plasma carnitine concentration is higher in the beginning of pregnancy, where the fetal weight is minimal. This means that other factors, besides the requirements of the fetus, contribute to the decrease observed.

Cederblad *et al.* (1985) [8] have defined the total plasma carnitine values in women who were subjected to caesarian section at the end of their pregnancy. The carnitine concentration found in these cases rose to 17.4±12.5 μmol/L.

The results of our study are in agreement with those cited in the literature, as regards the low levels of carnitine in pregnancy. The concentrations of total plasma carnitine were 22.7±2.6 μmol/L at the end of pregnancy as opposed to 39.6±2.3 μmol/L in non-pregnant fertile women of reproductive age. In order to achieve a more reliable approach to the matter we checked not only the total but also the carnitine fractions, that is free carnitine and acylcarnitine which have not been reported in the literature up to the present date. We observed that free plasma carnitine values were also significantly lower as compared to those of non-pregnant women (11.6±1.6 μmol/L over 31.6±1.9 μmol/L).

On the other hand the acylcarnitine value was slightly higher in the pregnant women (11.0±1.3 μmol/L over 7.9±1.3 μmol/L).

The present investigation was aimed at directing attention to a vigorous selection of cases so that the measurements could refer to healthy women, with normal pregnancies and deliveries, without complications as well as full-term apparently healthy neonates. In previous reports, the births were both by vaginal delivery as well as caesarian section, at different ages of pregnancy, while the perinatal period was not always without complications.

Bargen *et al.* (1981) [1] have reported that women with complicated pregnancies have considerably different carnitine levels. It should be noted however that the plasma carnitine levels do not correspond to the total carnitine reserves of the body, since no correlation between the concentrations in the plasma and the skeletal muscles, where their highest reserves exist, have been found. On the other hand, no decrease in carnitine levels in the muscles has been found at the end of pregnancy [6,8].

Quite a good number of factors seem to contribute to the decrease of carnitine levels in the maternal plasma. Nevertheless, in the 20th week of gestation, in which the plasma volume increase is relatively low, plasma carnitine has already decreased significantly. The increased renal clearance may be another factor. It is known that the kidneys are involved in the regulation of carnitine rejection, since in cases of renal failure its value in plasma increases [8,10]. During pregnancy, there is increased carnitine clearance and this is deduced from the fact that its section from the urine differs between pregnant and non-pregnant women, despite its lower concentration in the plasma of pregnant women [8]. The hormonal environment of pregnancy probably negatively influences the concentration of the substance in the plasma [3]. Carnitine seems to facilitate remóval of the potentially surplus toxic acyl groups from the cells; the latter are secreted as acylcarnitine in the urine [7]. There may be an increased need for carnitine in the tissues during pregnancy in order to carry out these metabolic functions; a fact which leads to a decrease of the levels of free carnitine in the plasma and a relative increase in the presence of acylcarnitine. The corroboration of the investigated cases, that the concentrations of acylcarnitine in the plasma of pregnant women was 11.0±1.3 over 7.9±1.3 μmol/L in the non-pregnant women supports this view. This difference was statistically significant.

The role of maternal carnitine supply to fetal circulation via the placenta, as perhaps the main factor in the production of the low levels of carnitine in the pregnant mother, must be pointed out. It appears that carnitine passes the placental barrier, as the measurements in the maternal and umbilical plasma by Schmidt-Sommerfeld *et al.* (1981) [18] have shown. This view has been corroborated by experimental results in fullterm human placenta in vitro [20]. However it is not known as to what degree the supplying of maternal carnitine for the needs of the embryo can be covered via the placenta.

The present study showed that the plasma carnitine values, during the beginning of neonatal life, are definitely decreased as regards both children of school age and a group of adult women (26.7±3.5 μmol/L, over 29.7±2.4 μmol/L and 31.6±1.9 μmol/L correspondingly).

It has been reported that the carnitine levels in the embryo decrease during pregnancy [21]. Other writers report that premature infants are born with smaller reserves of carnitine as compared to fullterm infants [14]. Nevertheless, the supplying of maternal carnitine appears to be the most significant regulatory factor for the concentration of carnitine in the plasma in the beginning of neonatal life, as shown by the positive correlation which existed in our measurements among the concentrations of maternal-umbilical plasma during pregnancy. Cederblad *et al.* (1985) [8] reached a similar conclusion. In spite of all these speculations it is almost sure that other factors also come into play in determining the concentration of the substance in the plasma during embryonal life. Rebouche *et al.* (1980) [15] have supported the view of decreased activity of the enzymatic systems of the embryo which are responsible for the production of the substance (g-butyric hydrocylase). The fact that some writers have observed a poor oxidation of fatty acids and disturbances in ketogenesis, a situation which was corrected with the addition of carnitine in the nutrition solutions [19] in premature infants and neonates under parenteral nutrition, constitutes an indirect acceptation. In certain cases, lysine, methionine and vitamin C were administered exogenously to these neonates without the existence of a satisfactory carnitine synthesis [14]

Nevertheless, despite the lower concentration of carnitine in the maternal and embryonal plasma [21] and amniotic fluid [2], the fullterm neonates seem to have accumulated carnitine in their muscular tissue so that during delivery the levels are the same as those of the adults [16]. It is most possible however that this does not apply to premature infants [22]. Measurements in carnitine in the muscles and liver of premature neonates have shown that the reserves per kilogram of body weight amount to approximately 60% to that of the fullterm neonates. It is even lower (about 40%) in premature infants under 30 weeks of gestation. It appears that in the premature, as compared to the fullterm neonate, the rhythm of carnitine biosynthesis in the liver during fetal life is lower, transfer via the placenta is decreased and, still more, the ability of the tissues to take up and store the substance is decreased [14]. Under discussion is the case of how necessary the supplementation of carnitine is to premature infants to cover their energy needs [12]. Is decreased carnitine a pathological phenomenon that needs correction or just a physiological response of the organism at a given situation? It is possible for premature newborns to need fat for other functions such as membrane formation.

In conclusion it appears that the transfer of maternal carnitine to the embryo constitutes the main factor of determining the concentration of carnitine in plasma during the beginning of fetal life, while its synthesis in the fetal liver is insufficient to a lesser or greater extent. The relatively low concentration in the umbilical plasma during the beginning of neonatal life is probably not related to insufficient coverage of the needs of the neonate, since the concentration in the tissues, no matter how much it has been studied, appears to exist in values similar to those of the adult. Nevertheless, several authors suggest the indispensable transfer of maternal or humanized milk which contains quantities of carnitine, so that the requirements of the neonate are met directly after discontinuation of maternal transfer via the placenta [12]. Maternal milk contains 60 μmol/L of carnitine [17], and appears to be a significant source for the neonate which, as known, produces energy mainly from the consumption of fats to cover its needs.

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