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Role of intrapartum hypoxia in carnitine nutritional status during the early neonatal period

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Abstract

We analyze markers of carnitine insufficiency and deficiency, lysine (LYS) and methionine (MET), in 39 neonates with intrapartum hypoxia (selection criteria: umbilical artery pH <7.20, lactate >1.8 mmol/l and PaO_2 <25 mm Hg), and in 35 healthy newborn infants (control group) in the early neonatal period (1–7 days of life). Free (FC), total (TC) carnitine and acylcarnitines (AC = short-chain + long-chain acylcarnitines) were measured using a radioisotopic micromethod; LYS and MET were determined by high-pressure liquid chromatography. AC and TC plasma concentrations and AC/FC ratio were higher while FC/TC ratio was lower in the hypoxic neonates than in the control group. Hypoxic newborn infants (59%) presented "carnitine deficiency" (FC/TC <0.7) and 60% of them "carnitine insufficiency" (AC/FC ratio >0.4) vs. 31% and 28%, respectively, for the neonates of the control group ($p<0.05$). In the healthy neonates group, MET correlated with FC/TC and the AC/FC ratio. FC, TC, AC, AC/FC and umbilical artery pH (pHua) were inversely correlated. FC/TC and MET correlated with pHua. We conclude that: (1) an important percentage of newborn infants with intrapartum hypoxia suffer carnitine deficiency and carnitine insufficiency in the early neonatal period, related to MET plasma levels; (2) the carnitine deficiency or insufficiency in the neonate is determined by the degree of intrapartum acidosis.

Keywords: Acylcarnitines; Carnitine deficiency; Carnitine insufficiency; Newborn infant; Intrapartum hypoxia

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1. Introduction

Carnitine is synthesized from the essential amino acid lysine, using methionine as a methyl donner [1]. Carnitine plays a physiological role, transporting long-chain fatty acids through the inner mithochondrial membrane [2,3]; it also facilitates the removal from the mithochondria of the short- and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism. Its role in the metabolism of branched chain amino acids and ketone bodies has also been described [4].

At delivery, there is a sudden interruption of the maternal–fetal transfer of carnitine, as well as of the amino acids lysine and methionine [2,3]. Just after birth, the energetic substrata change from carbohydrates to lipids [5–8], and there is a fasting period, followed by a slow, progressive introduction of feeding. The low supplies of these elements, together with the immaturity of the liver against enzymatic reactions, suggest that carnitine may be essential for newborn infants [1,3].

Fatty acids are an essential metabolic fuel under situations of prolonged fasting or stress [5–8]. Therefore, the defective oxidation of fatty acids may produce pathological situations such as Sudden Infant Death Syndrome, Reye-like episodes, Hypoketotic Hypoglycaemic Coma, Muscle Ureakness and profound Cardiological Dysfunction [9].

The evaluation of the nutritional state of carnitine by measuring free carnitine (FC) and total carnitine (TC) is generally considered to be insufficient [10]. The simultaneous evaluation of acylcarnitines (AC = short-chain + long-chain acylcarnitines) and the ratios AC/FC and FC/TC are also necessary [10–14]. Plasma FC concentrations under 20 nmol/ml are considered a marker of carnitine deficiency [13]. The FC/TC ratio is also considered a good marker when abnormal values are detected (<0.7) [11,12,14]. AC/FC ratio is a marker of carnitine insufficiency and all values >0.4 are abnormal [12–14].

The aim of the present study is to determine whether intrapartum hypoxia plays a role in the development of carnitine deficiency or insufficiency during the early neonatal period, which markers are modified and which can be considered the best for evaluating these situations in the neonate.

2. Methods

2.1. Subjects

Seventy four newborn infants, with birth weight appropriate for gestational age, were studied in the early neonatal period (1–7 days of life). The subjects were subdivided into two groups, homogeneous for gestational age, birth weight and hours of life. Group I (G-I) consisted of 39 neonates with intrapartum asphyxia, had a mean gestational age of 39.9 ± 0.2 weeks (mean ± S.E.M.), birth weight of 3510 ± 67 g, Apgar score range at the first minute of 2–6 and at 5 min 5–9, umbilical artery pH (pHua) of 7.11 ± 0.01, lactate in umbilical artery of 2.8 ± 0.1 mmol/L, PaO_2 in umbilical artery of 20.5 ± 3.7 mm Hg and a mean of 35.0 ± 8.2 h of life. Group II (G-II) comprised 35 healthy newborn infants (control group), with a gestational age of 39.7 ± 0.2 weeks, birth weight of 3270 ± 92 g, Apgar score range at the first minute of 7–10, pHua of 7.24 ± 0.007,

lactate in umbilical artery of 1.6 ± 0.01 mmol/l, PaO_2 in umbilical artery of 31.9 ± 2.5 mm Hg and 34.2 ± 4.3 h of life.

Gestational age was calculated from the first day of the mother's last menstrual period or, when dates were uncertain, by the method of Dubowitz et al. [15].

The study protocol was reviewed and approved by the Bioethical Committee on Research Involving Human Subjects of the University of Granada, and consent was obtained from one or both parents, after the nature and purpose of the study had been explained and was fully understood.

2.2. Clinical status of the newborn infants

The criteria considered for inclusion in the study, for both hypoxic and healthy neonates, were a maternal age of between 21 and 35, no medical illness in the mother, normal and monitored pregnancy; for the healthy neonates, no infant had delivery complications, evidence of intrauterine malnutrition, congenital malformations or metabolic abnormalities and no relevant associated pathology in the neonatal period was showed; all mothers had controlled delivery at the "San Cecilio" University Hospital of Granada (Spain). With respect to the markers of intrapartum hypoxia, umbilical artery pH <7.2, Apgar score lower than 7 in the first minute, PaO_2 <25 mm Hg and lactate >1.8 mmol/l were jointly considered pathological and clearly indicative of asphyxia.

For the control group, samples were obtained at the same time as other necessary biochemical analyses were carried out, for example, because of the presence of neonatal jaundice, polycythemia or the risk of anaemia. All samples were taken between 9:00 and 10:00 a.m. because analytical requests are performed at this time in the neonatal unit.

2.3. Procedures

Blood samples (3 ml) were obtained by venipuncture from each infant and transferred into tubes containing ethylenediaminetetraacetate (EDTA-K_3). The blood samples were stored on ice and centrifuged within 15 min at 3500 rpm to obtain the plasma; the plasma supernatant was immediately separated into aliquots and frozen at -70 °C until the analysis was made.

Plasma concentrations of FC, TC and AC (short-chain + long-chain ACs) were measured in nmol/ml using a modified method based on the radioisotopic techniques of McGarry and Foster [16] in 1976, Di Donato et al. [17] and Arenas et al. [18]. Serum carnitine is determined in the supernatant after acidic precipitation of serum proteins using perchloric acid 0.5 N. The fraction of ACs was determined by subtraction of FC from the amount of TC which is determined after alkaline hydrolysis of all carnitine esters. FC and short-chain carnitine esters were thus determined in the acidic supernatant and long-chain acyl esters in the precipitate. Acetyl-CoA ^{14}C was used for radiochemical dosage in a Beckman β-Centelleum Counter.

The amino acids methionine (Met) and Lysine (Lys) were determined in μmol/dl using the high-pressure liquid chromatographic method (HPLC) described by Peinado et al. [19].

Table 1
Plasma concentrations of carnitines, their esters and the ratios obtained, and the amino acid precursors lysine and methionine, in neonates with intrapartum asphyxia (G-I) and in healthy neonates (control group, G-II)

	G-I (n: 39) ($x \pm$ S.E.M.)	G-II (n: 35) ($x \pm$ S.E.M.)	p
FC (nmol/ml)	35.5 ± 2.7	30.6 ± 1.6	NS
SC (nmol/ml)	15.7 ± 2.6	6.8 ± 1.1	< 0.01
LC (nmol/ml)	6.2 ± 0.7	3.1 ± 0.2	< 0.001
TC (nmol/ml)	53.5 ± 4.8	41.2 ± 2.0	< 0.05
FC/TC	0.67 ± 0.02	0.75 ± 0.02	< 0.05
LC/FC	0.18 ± 0.02	0.10 ± 0.01	< 0.01
AC/FC	0.60 ± 0.006	0.36 ± 0.05	< 0.01
MET (μmol/dl)	2.9 ± 0.2	3.0 ± 0.2	NS
LYS (μmol/dl)	21.8 ± 1.8	23.4 ± 2.1	NS

n: number of cases; x: mean; S.E.M.: standard error of the mean; p: level of significance; NS: non significant; FC and TC: free and total carnitines; SC and LC: short- and long-chain acylcarnitines; AC: acylcarnitines (SC + LC); MET: methionine; LYS: lysine.

2.4. Statistical methods

The comparison between the two groups of plasma carnitine concentrations and the amino acids analyzed was made using the Student's t-test for unpaired data. The Fisher's exact test to compare two proportions was also performed. Correlation and fixing curves analysis was also carried out. A value of $p < 0.05$ was considered significant. The SPSS version 10.0 programme was used for the statistical analysis.

3. Results

Table 1 gives the mean values of the plasma concentrations of free and total carnitine, and of the esters, lysine and methionine. During the early neonatal period, the plasma levels of free, total and long-chain carnitine esters, and the AC/FC ratio were significantly higher in neonates with intrapartum hypoxia than those of the control group. The FC/TC

Table 2
The percentage of normal and hypoxic newborn infants with carnitine deficiency or insufficiency

	Group I (n: 39)	Group II (n: 35)	p
%FC < 20 nmol/ml (carnitine deficiency)	12.82 (5/39)	8.57 (3/35)	NS
%FC/TC < 0.7 (carnitine deficiency)	58.97 (23/39)	31.43 (11/35)	< 0.05
%AC/FC > 0.4 (carnitine insufficiency)	58.97 (23.39)	28.57 (10/35)	< 0.01

Fisher's exact test was performed to analyze the differences between the hypoxic neonates (Group I) and the healthy newborn infants (Group II).
n: number of cases; p: level of significance; NS: non significant; FC and TC: free and total carnitines; AC: short-chain + long-chain acylcarnitines.

Table 3
Significant correlations found in the hypoxic neonates group (G-I) and the analysis of the influence of acidosis on carnitine, acylcarnitine and methionine (MET) plasma concentrations, and the influence of pHua on the marker ratios of carnitine deficiency and insufficiency (control group + hypoxic neonates = Group I + Group II)

	r	*p*
Group I (n = 39)		
FC/TC – MET	0.50	< 0.01
LC/FC – MET	0.40	< 0.05
(SC + LC)/FC – MET	– 0.45	< 0.01
Group I + Group II (n = 74)		
FC – pHua	– 0.26	< 0.05
TC – pHua	– 0.42	< 0.01
SC – pHua	– 0.43	< 0.01
LC – pHua	– 0.34	< 0.01
FC/TC – pHua	0.33	< 0.01
LC/FC – pHua	– 0.30	< 0.05
AC/FC – pHua	– 0.40	< 0.01
MET – pHua	0.24	< 0.05

n: number of cases; *r*: correlation coefficient; *p*: level of significance; FC and TC: free and total carnitines; SC and LC: short- and long-chain acylcarnitines; AC: acylcarnitines (SC + LC); MET: methionine; pHua: umbilical artery pH.

ratio was significantly lower in G-I than in G-II. No differences in the mean values of free carnitine, methionine and lysine were observed between the two groups.

Table 2 shows the Fisher's exact test results; there are significant differences between G-I and G-II in the FC and the ratios of carnitine deficiency or insufficiency. Furthermore, the same babies in G-I (59%) with FC/TC < 0.7, also had an AC/FC ratio > 0.4. Five neonates in G-I had FC plasma levels below 20 nmol/ml, and four of them had an FC/TC ratio below 0.7. There were no newborn infants in G-II with FC plasma levels lower than 20 nmol/ml.

Table 3 shows the significant correlations found in G-I and G-II, which demonstrate the influence of pHua on plasma levels of carnitines, acylcarnitines and methionine, as well as on the ratios that are markers of carnitine deficiency and insufficiency.

4. Discussion

One extremely important function of carnitine is the transport across membranes of carboxylic acids that have been activated to the coenzyme A (CoA) level. Thus, the ability of carnitine to confer "transportability" to high-energy carboxylic acid means that it can facilitate the delivery of a needed substrate, the elimination of toxins, the "trapping" of acylated compounds of exogenous or endogenous origin, and the transport of high energy from one subcellular or cellular location to another [3,20]. These acylated compounds also inhibit numerous cellular enzymatic activities so that carnitine, by preventing their accumulation, provides protection against metabolic acidosis [3,21]. This function of carnitine affects many different metabolic pathways.

The data obtained in this study show higher plasma concentrations of long-chain AC in the hypoxic neonates than in the healthy babies. The deletereum effect of these results should be studied in further prospective clinical trials. It has been shown that high plasma levels of long-chain AC produce a risk of acyl-CoA accumulation in the myocardium [22,23]; furthermore, myocardial hypoxia ischemia is related to an accumulation of long-chain acylcarnitines (five times higher than the normal values) in myocytes sarcolemma, producing profound alterations in gap junctional conductance [22,24]. Recent studies in cultured human myocytes have demonstrated that long-chain acylcarnitines directly activate the calcium channel, allowing an increased influx of calcium ions and potentially leading to arrythmia [25,26]. These facts would explain the electrophysiological alterations in the myocardium conductance usually found in newborn infants with intrapartum hypoxia during the neonatal period [27].

In a homogenized rat heart under hypoxia conditions, the addition of carnitine corrects the profound depression of the piruvate–dehydrogenase complex and the mitochondrial respiratory function [28]. Karaev et al. [29] have demonstrated that L-carnitine administration to hypoxic rats prolongs their lives. Our results demonstrate inverse linear correlations between FC and the AC/FC ratio with pHua (FC–pHua, r: -0.26; AC/FC–pHua, r: -0.40), indicating the relationship between acidosis and the degree of carnitine insufficiency. Hypoxic neonates might benefit from carnitine treatment or supplementation during the very early neonatal period. The efficient and necessary dosage of carnitine to be supplied has yet to be defined [20,30].

Böhles et al. [14] have reported that any disturbance of the intermediary metabolism leading to an intramitochondrial accumulation of abnormal acyl-CoAs is reflected in the appearance of unusual acylcarnitines. Such an accumulation leads to a decreased availability of free carnitine, which is reflected in an increase in the serum AC/FC ratio. This effect might explain the higher AC/FC ratio in the hypoxic neonates of the present study, compared to the healthy babies, despite the similar levels of FC in plasma of both groups. These results agree with those reported by Meyburg et al. [31]. Nevertheless, the relationship between serum acylcarnitine and free carnitine is highly sensitive to intramitochondrial metabolic alterations. An AC/FC increase occurs long before the total serum carnitine concentration falls, although this just represents a state of decreased carnitine availability (carnitine insufficiency) [14,32]. Physiological serum acylcarnitine concentrations are 5–10 mmol/l and can increase several fold in patients with metabolic disease [33]. Almost 60% of the hypoxic newborn infants in this study had an AC/FC ratio >0.4, and the same babies had an FC/TC ratio <0.7, so a high percentage of neonates with intrapartum asphyxia develop carnitine insufficiency or deficiency during the first days of life, suggesting that these babies have an evident decreased carnitine availability; moreover, these ratios are correlated with pHua (AC/FC–pHua, r: -0.40; FC/TC–pHua, r: 0.33), showing that pHua can indicate the degree of carnitine deficiency developing in the newborn in the early neonatal period. Our results agree with those published recently by Meyburg et al. [31], who found that lower umbilical artery pH caused accumulation of mainly long-chain acylcarnitines, and so, long-chain acylcarnitines could serve as a parameter of perinatal asphyxia in neonates.

L-Carnitine is considered an essential nutrient for newborn infants. The capacity to synthesize carnitine is less than adequate to meet the growth needs of infants and children

[1,34]. It has been reported that the administration of carnitine releases the methyl-groups that are needed for carnitine synthesis [1,35]; this is probably the explanation for the relationship demonstrated by the correlations between the carnitine deficiency and carnitine insufficiency ratios and the methionine found in the hypoxic group. These correlations suggest that methionine may also be a marker of the diminution of carnitine availability in the neonate.

In conclusion, the data reported in this study show that the determination of FC and TC plasma levels is not sufficient to evaluate carnitine insufficiency or deficiency in neonates, for which acylcarnitines are necessary. There is no relationship between carnitine or acylcarnitine plasma levels with lysine, but there are lineal correlations between the FC/TC and AC/FC ratios and methionine, suggesting the utility of methionine to approximate the degree of carnitine deficiency or insufficiency in neonates. The hypoxic neonate has a higher risk of developing carnitine deficiency and/or insufficiency during the first days of life. Carnitine can be considered an essential nutrient in newborn infants with intrapartum hypoxia as is suggested by Milana and Mazzone [30] and Meyburg et al. [31]. Whether hypoxic neonates may benefit from carnitine supplements (quantity?) and/or methionine is a question that remains to be clarified in future prospective studies.

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