Deficiency of carnitine and its amino acids precursors in newborn infants with intrapartum hypoxia

Deficiencia de carnitina y de sus aminoácidos precursores en recién nacidos afectos de hipoxia intraparto

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ABSTRACT

The myocardial hypoxia ischemia is related to an accumulation of long chain acylcarnitines (LC) in the myocites sarcolemma improving profound alterations in the gap junctional conductance. Similar alterations are also observed in newborn infants with intrapartum asphyxia showing important derangements of the electrophysiological conductance during the neonatal period. We analyze markers of "insufficiency" and "deficiency" of carnitine (C) and its amino acids precursors [lysine (LYS) and methionine (MET)] in newborn infants at the first days of life, 39 with intrapartum hypoxia (G-I) and 35 normal neonates (control group) (G-II). Free (FC) and total (TC) carnitine and short chain (SC) and LC acylcarnitines were measured using a radioisotopic method; Amino acids LYS and MET were obtained by HPLC. SC, LC and TC plasma concentrations and LC/FC and SC+LC/FC ratios were significantly higher in G-I than in G-II. FC/TC was lower in G-I than in G-II. The 59% of G-I in front of 31% of G-II ($p<0.05$) had a "C-deficiency" (FC/TC<0.7). The SC+LC/FC ratio was >0.7 ("C-insufficiency") in 59% of G-I and 28% in G-II ($p<0.01$). In G-II, MET was correlated with FC/TC (r:0.50) and SC+LC/FC (r:-0.45) were demonstrated. There were significant inverse correlation between FC, SC, LC, TC, LC/FC, SC+LC/FC and pHua (n:74) (r:-0.26-$p<0.05$; r:-0.43-$p<0.01$; r:-0.34-$p<0.01$; r:-0.42-$p<0.01$; r:-0.30-$p<0.05$; r:-0.40-$p<0.01$, respectively). FC/TC and MET were directly correlated with pHua (n:74) (r:0.33-$p<0.01$; r:0.24-$p<0.05$, respectively). So, we conclude that: 1) an important percentage of hypoxic neonates have during the first days of life a "C-deficiency" and "C-insufficiency", and this fact is related to MET plasma levels; 2)The degree of "C-deficiency" and "C-insufficiency" is determined by the acidosis degree.

Key Words: Acylcarnitines. "Carnitine deficiency". "Carnitine insufficiency". Newborn infant. Intrapartum hypoxia.

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RESUMEN

La isquemia hipóxica de miocardio se relaciona con la acumulación de acil carnitinas de larga cadena (LC) en el sarcolema de los miocitos provocando profundas alteraciones en la conductancia de las intercomunicaciones celulares. Alteraciones similares se observan en recién nacidos con asfixia intraparto que muestran importantes desarreglos en la conductancia elecrofisiológica durante el período neonatal. Analizamos marcadores de "insuficiencia" y "deficiencia" de carnitina (C) y de sus precursores aminoácidos (lisina (LYS) y metionina (MET) en recién nacidos de un día, 39 con hipoxia intraparto (G-I) y 35 normales (Control) (G-II). Se midieron la carnitina libre (FC) y total (TC) y las acilcarnitinas de cadena corta (SC) y larga (LC) usando un método radioisotópico. Los aminoácidos LYS y MET se determinaron por hplc. Las concentraciones plasmáticas de SC, LC y TC así como los cocientes LC/FC y SC+LC/FC fueron mayores en G-I que en G-II mientras que FC/TC fue menor. El 59% de G-I frente al 31% de G-II ($p<0.05$) tuvo una deficiencia de carnitina (FC/TC <0.7). El cociente SC+LC/TC fue >0.7 (insuficiencia) en el 59% de G-I y 28% de G-II ($p<0.01$). En G-II, MET se correlacionó con FC/TC ($r=0.5$) y SC+LC/TC ($r=0.45$). Hubo una correlación negativa entre FC,SC,LC,LC/TC, SC+LC/TC y pHua ($n=74$) ($r=-0.26$, $p<0.05$; $r=-0.43$, $p<0.01$; $r=-0.34$, $p<0.01$; $r=-0.42$, $p<0.01$; $r=-0.30$, $p<0.05$; $r=-0.40$, $p<0.01$) respectivamente).FC/TC y MET se correlacionan con pHua ($n=74$) ($r=0.33$, $p<0.01$; $r=0.24$,$p<0.05$ respectivamente). Por tanto, concluimos que: 1) un porcentaje importante de neonatos hipóxicos tiene durante el primer día de vida deficiencia e insuficiencia de carnitina, y este hecho se correlaciona con los niveles plasmáticos de MET; 2) El grado de deficiencia e insuficiencia viene determinado por el grado de acidosis.

Palabras clave: Acilcarnitina. Deficiencia de carnitina. Insuficiencia de carnitina. Neonatos. Hipoxia intraparto.

Recibido:28-11-96
Aceptado:19-12-96
BIBLID [0004-2927(1996) 37:4; 1019-1027]

INTRODUCTION

Carnitine has been recently described as a conditional "essential" nutrient for human. Carnitine (C) is synthesized from the essential amino acids lysine (LYS), using also methionine (MET) as a methyl donner (1). C has a physiological role introducing long chain fatty acids accross the inner mithochondrial membrane (2-3); C also facilitates removal from mitochondria of short-chain 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 been also described (4).

At delivery there is a sudden interruption of the maternal-fetal transfer of C as well as the amino acids lysine and methionine (2-3). At the early neonatal period (ENP), energetic substrata changes (from carbohydrates to

lipids) and there is a fasting period and then a slow and progressive introduction of feeding. These fact determine the "essenciality of carnitine" for newborn infants (1,3).

Fatty acids are essential metabolic fuel under prolonged fasting or stress situations. Therefore, defective oxidation may produce pathological situations such as Sudden Infants Death Syndrome, Reye-like episodes, Hypoketotic Hypoglycaemic Coma, Muscle Ureakness and profound Cardiological Dysfunction (5).

Actually, the evaluation of the nutritional state of C measuring free (FC) and total (TC) carnitine seems to be insufficient. It has been proposed the simultaneous valoration of short chain carnitine esters (SC) and long chain carnitine esters (LC). Some researches have demonstrate that the myocardial hypoxia ischemia is related to an accumulation of long chain acylcarnitines (LC) (five times higher than the normal values) in the myocites sarcolemma, improving profound alterations in the gap junctional conductance (6-7). Similar phenomena are also observed in newborn infants with intrapartum asphyxia, showing important derangements of electrophysiological conductance during the neonatal period (8).

The ratio FC/TC is considered a good marker of "C-deficiency", being abnormal values below 0.7 (9-SEWELL, 1995). Plasma concentrations of FC<20 nmol/ml are also considered marker of carnitine deficiency (10-LARGUILLIERI, 1993). LC/FC and SC+LC/FC ratios are markers of "C-insufficiency" and all values higher than 0.4 are abnormal (11-BÖHLES, 1994).

The aim of the present study is to analyze the markers of "insufficiency" and "deficiency" of carnitine and its amino acids precursors in newborns with intrapartum hypoxia.

METHODS

Subjects

Seventy four newborn infants, with birth weight (BW) appropiate for gestational age (GA), were studied at 0-7 days of life (ENP). The subjects were subdivided into two groups; Group I comprised 35 newborn infants (control group), with a GA of 39.7±0.2 weeks (mean±SEM), BW of 3270±92 g, pHua (pH of the umbilical artery) of 7.24±0.007, hours of life (H): 34.2±4.3 h. Group II consisted of 39 neonates with intrapartum asphyxia, with a GA of 39.9±0.2 weeks, BW of 3510±67 g, pHua of 7.12±0.01 and H:35.0±8.2 h.

The samples were obtained when other neccesary biochemical analysis were done, because the presence of neonatal jaundice, poliglobulias or anaemia.

The inclusion criteria considered in the study for hypoxic and normal neonates were a maternal age between 21 and 35 years, no medical illness in the mother, normal pregnancy and well controled, delivery at the Universitary Hospital of Granada and no associated pathology in the neonatal period.

Gestational age was calculated from the first day of mother´s last menstrual period or, when dates were uncertain, by the Dubowitz et al.´s method (12). The study protocol was reviewed and approved by the University of Granada Committee on Research Involving Human Subjects, and written consent was obtained from one or both parents, after the nature and purpose of the study were explained and fully understood.

Procedures

Blood samples were obtained by venous punction from all infants and transferred into tubes containing EDTA-K3. The samples were centrifuged during 10 minutes at 3500 r.p.m. to obtain the plasma. The plasma was separated in aliquots and they were frozen at -70ºC until the moment of the analysis.

Plasma concentrations of FC, TC, SC and LC were measured in nmol/ml using a modified method based on McGarry and Foster´s radioisotopic tecnique (13), using to separate FC, SC and LC perchloric acid 0.5 N; Acetyl-CoA **14**C was used to radioisotopic dosification in a Beckman ß-Centelleum Counter. Amino acids methionine (MET) and lysine (LYS) were determined using a high pressure liquid chromatographic method (HPLC) described by Peinado et al. (14).

Statistical methods

The comparison of plasma carnitine concentrations between the two groups using the Student´s/Welch "t" test for unpaired data, were done. Fisher exact test to compare two proportions was also done. A correlation analysis and fixing curves were also realized. A "p" value <0.05 was considered significant.

RESULTS

Plasma concentrations of FC,TC,SC,LC and LYS and MET are shown in Table I. Table I also contained the mean values of FC/TC, LC/FC and SC+LC/FC ratios in both groups.

At the ENP, FC, TC and LC plasma levels and LC/FC and SC+LC/FC

TABLE I.—Plasma concentrations of carnitines, its esters and the ratios obtained, and its amino acids precursors Lysine and Methionine, in normal neonates (control group - G-I) and in neonates with intrapartum asphyxia (G-II).

	G-I (n:35) (x±SEM)	*G-II (n:39) (x±SEM)*	*p*
FC (nmol/ml)	30.6±1.6	35.5±2.7	NS
SC (nmol/ml)	6.8±1.1	15.7±2.6	<0.01
LC (nmol/ml)	3.1±6.2	6.2±0.7	<0.001
TC (nmol/ml)	41.2±2.0	53.5±4.8	<0.05
FC/TC	0.75±0.02	0.67±0.02	<0.05
LC/FC	0.10±0.01	0.18±0.02	<0.01
SC+LC/FC	0.36±0.05	0.60±0.06	<0.01
MET (mmol/dl)	3.0±0.2	2.9±0.2	NS
LYS (mmol/dl)	23.4±2.1	21.8±1.8	NS

n: number of cases; x: mean; SEM:standar error of mean; FC and TC: free and total carnitines; SC and LC: short and long chain acylcarnitines; MET: methionine; LYS: Lysine; p: level of significance.

ratios were significantly higher in neonates with intrapartum hypoxia than those of control group. FC/TC was significantly lower in group II than in group I. No differences between the mean values of FC, MET and LYS were observed between the two groups.

Fisher´s exact test showed the following results: G-II vs G-I: FC<20 nmol/ml ("C-deficiency"): 12.82% (5/39) vs 8.57% (3/35), p:NS; FC/TC<0.7 ("C-deficiency"): 58.97% (23/39) vs 31.43% (11/35), p<0.05; LC/FC>0.4 ("C-insufficiency"): 12.82% (5/39) vs 0% (0/35), p<0.05; SC+LC/FC>0.4 ("C- insufficiency"): 58.97% (23/39) vs 28.57% (10/35), p<0.01. Furthermore, the same babies of group II (59%) with FC/TC<0.7, also have the SC+LC/FC ratio >0.4. Five newborn infants of group II have FC plasma levels below 20 nmol/ml, and four of them have FC/TC ratio below 0.7. There were no neonates of group I with plasma levels of FC lower than 20 nmol/ml.

Table II show the significant correlations found in group II and the correlations which demonstrate the influence of pHua on carnitines, acylcarnitines and MET plasma levels, and the pHua influence on the ratios markers of "C-deficiency" and "C-insufficiency".

DISCUSION

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

TABLE II.—The table recopiles the significant correlations found in the hypoxic neonates group (G-II) and the analysis of the influence of acidosis on carnitines, acylcarnitines and methionine (MET) plasma concentrations, and the pHua influence on the ratios markers of "carnitine deficiency" and "carnitine insufficiency" (control group + hypoxic neonates = Group I + Group II).

GROUP II (n:39)	*r*	*p*
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
SC+LC/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; MET: methionine; pHua: umbilical artery pH.

substrate, the elimination of toxins, "trapping" acylated compounds of exogenous or endogenous origin, and the transport of high energy from one subcellular or cellular location to another (3, 15). These acylated compounds also inhibit numerous cellular enzymatic activities, so that carnitine by preventing their accumulation provides protection against metabolic acidosis (3). This function of carnitine impacts many different metabolic pathways.

It has been described that high plasma levels of LC determine a risk of acyl-CoA accumulation in the myocardium (7). Recent studies in cultured human myocites have demonstrated that long-chain carnitines directly activate the calcium channel allowing an increased influx of calcium ions eventually producing arrythmia (16). Our data in this study shows a higher plasma concentrations of LC in the hypoxic neonates than in the normal babies, so probably these newborns could have an accumulation of acyl-CoA compounds in the myocardium. These fact should explain the electrophysiological alterations in the myocardium conductance found usually in newborn infants with intrapartum hypoxia during the neonatal period (8).

In a homogenizated rat heart under hypoxia conditions, the addition of carnitine corrected the profound depression of the piruvate-dehidrogenase complex and the mitochondrial respiratory function (17). Karaev et al. (18) have demonstrated that L-carnitine administration to hypoxic rats prolonged its life. Our results demonstrate linear inverse correlations between FC and

SC+LC/FC ratio with pHua (FC-pHua: r:-0.26; SC+LC/FC-pHua: r:-0.40), indicating the relationship between acidosis and the degree of "C-insufficiency". Perhaps, the hypoxic neonates can be beneficiated with carnitine treatment or supplementation during the very early neonatal period. The efficient and neccesary dose of carnitine to supply must be define (15).

Böhles et al. (11) have described that any state of disturber intermediary metabolism leading to an intramitochondrial accumulation of abnormal acyl-CoAs is reflected in the appearance of unusual acylcarnitines (AC). Their accumulation leads to a decreased availability of free carnitine which is reflected in an increase in the serum AC/FC ratio. This effect may probably explain the higher SC+LC/FC ratio in the hypoxic neonates compared to normal babies, despite the similar levels of FC in plasma in both groups. 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 is decreased, however just representing a state of a decreased carnitine availability (carnitine insufficiency)(11,19). Physiological serum acylcarnitine concentrations are 5-10 mmol/L and can increase several fold in patients with metabolic disease (20). Near a 60% of the hypoxic newborn infants of this study had a SC+LC/FC ratio higher than 0.4, and the same babies had a FC/TC ratio <0.7, so a high percentage of neonates with intrapartum asphyxia develop a "C-insufficiency" and a "C-deficiency" during the first days of life, meaning that these babies have a evident decreased carnitine availability; moreover, this ratios are correlated with pHua (SC+LC/FC-pHua: r:-0.40; FC/TC-pHua: r:0.33), showing that the pHua can indicate the degree of "C-deficiency" that the newborn could develop at the early neonatal period.

L-carnitine is considered to be an essential nutrient for newborn infants. The capacity to synthesize carnitine is less than adequate to meet the needs of growth in infants and children (1,21). It has been reported that the administration of carnitine spares the methyl-groups that are needed for carnitine synthesis (1,22); this is probably the explanation of the relationship demonstrated with the correlations between "C-deficiency" and "C-insufficiency" ratios and MET found in the hypoxic group. These correlations made possible to think that MET is also a marker of the diminution of carnitine availability in the neonate.

ACKNOLEDGEMENTS

This work was supported by the Spahish Ministry of Industry, Center of Industrial Development and Tecnological Approach (CDTI) and Laboratories ORDESA (Grant n.º: 920083).

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